



## FUNCTIONAL AND NUTRITIONAL COMPOSITION OF ACTIVATED CHARCOAL OF Terminalia Catappa

## \*1Rosephine Enadeghe, <sup>1</sup>Samuel I. Ojeaburu and <sup>1,2,3</sup>Olusanya Olasehinde

<sup>1</sup>Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria <sup>2</sup>Laboratory of Protein and Peptide Pharmaceuticals & Proteomics Laboratory, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China.

<sup>3</sup>University of Chinese Academy of Sciences, Beijing 100049, China.

\*Corresponding authors' email: rosephine.enadeghe@uniben.edu Phone: +2348065241266

## ABSTRACT

Terminalia catappa (T. catappa) is a folklore plant whose leaves, roots and stem bark treat various infectious diseases such as dermatosis and hepatitis. However, herbalists often transform some parts, like the stem, via pyrolysis to charcoal to achieve a different therapeutic outcome. Charcoal has been known as an anti-poisonous substance with no therapeutic or nutritional relevance. This study transformed the stem bark of T. catappa to charcoal, evaluated its proximate minerals and phytochemical compounds using standard methods, including inductive coupled plasma atomic emission spectroscopy (ICP AES) and Gas chromatography-mass spectroscopy (GC-MS). Stem bark were gotten from tree, dried in the sun, and then subjected to a Thermofitcher muffle furnace at 1450 °C without oxygen for about an hour to activated charcoal. Subsequently. The charcoal was pulverised to smooth powder and then subjected to analysis. Proximate analysis reveals that T catappa charcoal is rich in primary nutrients such as carbohydrates (65.20±4.34), proteins (5.68±0.21), crude fibre  $(8.31\pm1.10)$ , ash  $(13.20\pm2.10)$  and moisture content  $(7.50\pm0.10)$  %. Mineral analysis of the extract revealed the presence of essential minerals such as calcium (128.26±11.09), copper (0.83±0.001), magnesium (77.80±9.08), iron (24.51±5.08), manganese (1.83±0.05), and zinc (4.59±0.12) mg/100g as the most predominant, which are crucial for numerous cellular processes. Two (2) phytochemical compounds were detected by GC-MS, including methyl-6-0 beta-galatose (93.24%) and stigmastan-3, 5-diene (6.76%). The study revealed that charcoal is contains nutrients and functional compounds that may be included in feed and a potential therapeutic drug target against hypercholesterol and cancer.

Keywords: Terminalia ivorensis, ICP-AES, Hypercholesterol, Cancer, Proximate

## INTRODUCTION

A variety of medicinal plants have become famous for the cure of both human and animal diseases over the years (Abayomi et al., 2013). Cultural and economic factors have increased the acceptance of traditional medicines over orthodox medicines (Abayomi, 2006). Terminalia catappa (T. catappa) is a folklore plant whose leaves and stem bark are used to treat infectious diseases. The plant extracts exhibit anthelmintic and biological activities, including antiinflammatory antioxidants and pre-biotic potential (Enadeghe et al., 2024). In India, a plaster of T. catappa leaves is employed in treating leprosy wounds, scabies and other skin infections (Enadeghe, et al 2024). However, some parts of Terminalia catappa are often transformed via pyrolysis to charcoal and ashes to achieve a different broader therapeutic outcome. Activated charcoal is a fine odourless, black and tasteless powder with free bonding sites, such that one tea spoon of activated charcoal has about same surface area within 2,800-3,500 m<sup>2</sup>/g. Activated charcoal is similar to common charcoal, but produced with limited amount of oxygen with reduced pore size and increased surface area unlike the common charcoal. WHO approved the use of AC for emergency treatment of unknown poisoinigs or overdoses. Over the years, charcoal has been known as an anti-poisonous substance with little or no nutritional activity.Activated charcoal is available worldwide under different names: carbon, activated carbon, animal, coconut shell and charcoal. It helps to decrease flatulence (internal gas), reduce cholesterol levels, uric acid, avert hangovers, and treat cholestasis (bile flow problems) in women during pregnancy. Heating regular charcoal with limited oxygen makes it develop many pores/ internal spores, expanding its surface

area like a sponge and effectively activating it to trap chemicals.

One gram of activated carbon has a surface area like that of a football field and thus can adsorp to its surface toxins. The carbon molecules mop free radicals wih its electronic bond of resonance and neutralize them (Enadeghe, et al 2024). Charcoal has been known as a robust anti-poisonous substance with no scientific validated therapeutic or nutritional relevance. This study transformed the stem bark of *T. catappa* to charcoal and evaluated its nutritional, minerals and functional composition that may be responsible for its therapeutic activity.

## MATERIALS AND METHODS

## **Collection of plant materials**

We collected the stem barks of *Terminalia catappa* from trees that had fallen in the open area of the Students Affairs Division, University of Benin, Benin City, Nigeria. Subsequently, the trees were cut down on January 25, 2021, and assembled the wooden logs on February 12, 2021. A reputable taxonomist from the Department of Plant Biology and Biotechnology at the University of Benin, Benin City, Edo State, examined and identified them. The specimen labelled with voucher number UBHS260 was placed in the same department, while its stem barks were extracted and subjected to a 24-hour sun-drying process.

## **Preparation of charcoal**

Pyrolysis of stem bark was conducted in the laboratory using a Thermofitcher muffle furnace at 1450 °C for about one hour. This procedure intended to facilitate the conversion of carbon into charcoal, which resulted in the generation of numerous spores.

In addition to the Agilent 720-AES with megapixel C.C.D. detector, the Agilent SPS3 autosampler was also used to introduce samples. Data acquisition and instrument control were performed using Agilent Expert II Software. A calibration solution and quality control solution (Q.C.) were prepared using the reference material below.

## **Proximate Analysis**

This procedures allow for a comprehensive understanding of the sample's nutritional composition. A method of association of official analytical chemists was employed to analyse the sample (AOAC, 1990).

## Ash Content

After moisture determination, take the dried sample and heat it in a muffle for three hours at 600 °C. The process was based on a method established by AOAC (1990) method, which involved loading 2g of the sample into an empty, pre-weighed crucible (w0), which was then heated in a muffle furnace set at the above temperature for three hours.

#### Percentage ash was obtained by

$$\% Ash = \frac{W_{1-}W_2}{W_{1-}W_0} x \, 100 \tag{1}$$

Where: w<sub>0</sub>=Weight of the empty crucible, g w<sub>1</sub>= Weight of crucible + sample, g w<sub>2</sub>= Weight of crucible + ashed sample, g

#### **Crude fibre determination**

Place a 1 liter conical flask (w<sub>0</sub>) was filled with 2 g of the samples. The flask was filled with 200 ml of 1.25% H2SO4 acid, which was then allowed to boil for 30 minutes while being constantly monitored by cooling fingers and then filtered using a poplin cloth. Using a spatula, the residue was transferred back to the flask, and 200 ml of 1.25% NaOH (produced by dissolving 100g of NaOH pellets in 1 liter of distilled water) was added. The mixture was then allowed to boil for an additional 30 minutes while being constantly agitated with cooling fingers. A poplin cloth was used to filter the material, and the residue was properly cleaned with hot distilled water before being thoroughly rinsed twice with industrial methylated spirits, acetone, or ethanol. Rinse three times with petroleum ether (B.P. 40-60 °C) to finish. Heat dry overnight at 105°C in the oven, allow it to drain completely and scrape the residue into a crucible. Once removed, it was chilled in a desiccator. In a muffle furnace, the weighed sample (w1) was ashed for 90 minutes at 550 °C. In the end, it was dried in a desiccator and weighed once more (w<sub>2</sub>) (AOAC, 1990).

The percentage crude fibre was calculated thus; Where:

% of crude fibre = 
$$\frac{W_1 - W_2}{W_0} \times 100$$
 (2)

 $W_0$  = Weight of sample, g  $W_1$  = Weight of dried sample, g

 $W_2$  = weight of ash sample, g

## Crude protein determination

The AOAC (1997), a modified version of the micro-Kjeldahl method, was employed to determine crude protein.

This procedure is intended to streamline the transformation of carbon into charcoal, leading to the generation of multiple spores.

A minute quantity of anti-bumping granules was introduced into a micro-Kjeldahl digestion flask, along with three grams of each of the defatted samples that were individually weighed on pre-weighed scales. Each flask was provided with a 2g catalyst solution (CuSO4: Na2SO4: SeO2, 5:1:02 w/w) and 10 ml of concentrated H2SO4 free of nitrogen. The flasks were arranged on a heating mantle in a fume cupboard at an inclined position. Once the foaming subsided, the temperature was increased to 50°C for an additional 30 minutes and then further raised to the maximum heating level of 100°C until a transparent solution was obtained. To ensure complete digestion and conversion of nitrogen to ammonium sulphate, the mixture was simmered for another 30 minutes, keeping the temperature below the boiling point. After completion of the digestion process, the samples were washed and cooled to room temperature before being placed accurately into 100 mL volumetric flasks. Volumes were adjusted to the appropriate levels using distilled water.

A 10 ml pipette was utilised to transfer precisely 10 ml of the digest filtrate into a 25 ml standard flask. A 2 ml solution of alkaline phenate was added, and the liquid was vigorously stirred to achieve thorough mixing. After thorough agitation, 2ml of sodium hypochlorite was introduced, followed by 6ml of sodium potassium tartrate. The absorbance of the solution at 630 nm was measured using a U.V./visible spectrophotometer after dilution with distilled water up to the 25 ml mark. The sample and the nitrogen standards were treated identically (AOAC, 1997).

## Calculation % Nitrogen =

nstrument.Reading.x Slope Reciprocal x Color Vol.x Digest Vol Weight of Sample x Aliquot Taken X 10000 (3)

% Crude Protein =% Nitrogen  $\times 6.25$ 

#### Crude Fat

Extract the fat using the soxhlet extraction method from a known weight of the sample using a solvent (such as ether) as described by AOAC in 1973. has almost evaporated entirely (AOAC, 1973).

Calculation:

Weight of empty porous thimble = W<sub>0</sub> Weight of empty thimble + ground sample = W<sub>1</sub> Weight of ground sample = W<sub>1</sub>-W<sub>0</sub>

Weight of empty extraction flask = W<sub>2</sub>

Weight of empty extraction  $flask + ether = W_3$ 

Weight of ether (fat or oil) =  $W_3 - W_2$ 

$$\% Fat = \frac{W_3 - W_2}{(W_1 - W_0)} x \ 100 \tag{4}$$

## Moisture content

Weigh a known quantity of the sample and place it in a drying oven at 105°C until a constant weight is achieved. (AOAC, 1997).

The percentage of moisture was calculated using the equation below:

% moisture = 
$$\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$
 (5)

## Determination of soluble carbohydrate (nitrogen-free extractive) Test method:

The nitrogen-free extractive (N.F.E.), also referred to as soluble carbohydrate, is calculated by subtracting the combined amount of ash, protein, crude fat, and crude fiber from the total crude protein, rather than being directly measured.

N. F. E. 100 – (%Ash + % crude fibre + % crude fat + crude protein) The word N.F.E. encompasses all starches and sugars, varying amounts of lignin, and a tiny bit of hemicellulose. It makes reference to no particular chemical or group of chemicals.

#### **Elemental analysis**

Certain trace minerals were analysed by means of nitricperchloric acid digestion using the Atomic Absorption Spectroscopy (A.A.S.) method. Weighing about 50 mg of dried methanol leaf extract of *Terminalia catappa*, we added 5 ml of a mixture of nitric and perchloric acid to a 250 ml conical flask and left it for 24 hours. Additionally, known elemental and blank samples were prepared following the digestion of the dry extract. Absorbance values were obtained by running the digested samples and blank on the A.A.S. Elements in the samples had their concentrations determined using the standard (AOAC, 1997).

#### **GC-MS Analysis**

After fine filteration, about 100 g of activated charcoal of *Terminalia catappa* that had been dissolved in 100 mL of distilled water and then further diluted with water 1:1. Using a Perkin-Elmer G.C. Clarus 500 system and a gas chromatograph connected to a Mass Spectrometer (GC-MS) fitted with an Elite-I fused silica capillary column (30 mm x

## **RESULTS AND DISCUSSION**

Table 1: Physical properties of *Terminalia catappa* stem back charcoal after heating

Sample	Color	Feel	State	рН
Activated Charcoal	Fine Black	Coarse powder	Solid amorphous	5.5

#### **Proximate analysis**

Analysis of the samples showed that moisture content was very low (7.05 %), suggesting a very good shelf life of the

samples, carbohydrates (65.20 %), crude fibre (8.31 %). Protein (5.68 %), and ash content of (13.20 %) were obtained.

#### Table 2: Nutritional composition of activated charcoal of Terminalia catappa

Parameters	Moisture	Protein	Crude fat	Crude fibre	Ash	carbohydrate
Concentrations (%)	$7.50\pm0.10$	$5.68 \pm 0.21$	$0.00\pm0.0$	8.31±1.10	13.20±2.10	65.20±4.34

Activated charcoal is a source of primary nutrients. The percentage of proteins (5.68 %) is high bearing in mind that this is a charcoal sample (meat and fish possess between 15

and 20 g of proteins for each 100 g). crude fat of 0.00 % with high amount of carbohydrate and varying amounts of hydrogen and oxygen (65.20 %).

Table 3: Elemental composition (macro and trace) of pulverised stem bark charcoal of Terminalia catappa using ICP	-
AES	-

Elements Name	Wavelengths	Concentration G/100g	
Calcium (Ca)	396.847	128.26	
Iron (Fe)	238.204	24.51	
Potassium (K)	766.491	29.9	
Magnesium (Mg)	279.553	46.7	
Sodium (Na)	589.592	39.44	
Indium (In)	230.606	0.17	
Bismuth (Bi)	223.061	0.41	
Lithium (Li)	670.783	0.69	
Aluminum (Al)	167.019	0.03	
Asernic (As)	188.98	0.02	
Barium (Ba)	455.403	0.22	
Cupper (Cu)	327.395	0.83	
Manganese (Mn)	257.61	1.83	
Thallium (Tl)	190.794	0.51	
Thorium (Th)	283.7	1.08	
Uranium (U)	385.957	0.25	
Zinc (Zn)	213.857	4.59	
Boron (B)	249.772	0.7	
Silicon (Si)	251.611	29.1	

0.25 mm 1D x 1 µMdf, 100% Dimethylpolysiloxane), Terminalia catappa was GC-MS analysed. An electron ionisation device using 70 eV of ionising energy in a singlephase ion mode was employed for GC-MS detection. The carrier gas was 99.999% helium gas flowing at a constant rate of 1 mL/min; the injection volume was 0.50 ml (split ratio of 10:1); the injector temperature was 250°C; and the ion-source temperature was 280°C. From 110°C (isothermal for 2 minutes), the oven temperature was scheduled to rise by 10°C/min to 200°C, then by 5°C/min to 280°C, and to finish with an isothermal of 9 minutes at 280°C. Fragments ranging from 45 to 450 Da were scanned at 0.5-second intervals in the 70 eV mass spectra. The G.C. ran for twenty-seven minutes in all. Comparing the average peak area to the total areas yielded the relative % quantity of each component. Turbo mass is software that was modified to manage chromatograms and mass spectra.

## Identification of components

Interpretation using the National Institute of Standards and Technology (NIST) database, which contains more than 62,000 patterns, was used to analyse the mass spectrum from GC-MS.

Strontium (Sr)	407.771	1.03	
Titanium (Ti)	336.122	0.7	

Mineral analysis of *activated charcoal* reveals the composition of diverse essential minerals. They also contain significant divalent ions, including magnesium, calcium, iron and zinc. The calcium content is (128.26 mg/100) compared to diary and vegetables sources, although the amounts of extract taken are usually less than that of milk or dairy products. These ions play important roles in various chemical reactions, biological processes and industrial applications due

to their ability to form stable compounds and participate in ionic bonding.

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# Gas chromatogram of Activated charcoal of Terminalia catappa samples

As presented in Figure 1, the results revealed two peaks identified in the activated charcoal of *Terminalia catapppa* whose spectra were matched with standard compounds of the National Institute of Standard and Technology (NIST) library.

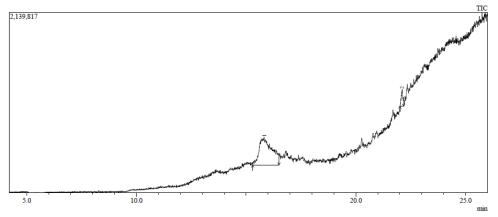


Figure 1: Gas chromatogram of activated charcoal of Terminalia catappa

The peaks represent unique compound. Percentage area covered by each peak corresponds with the intensities of ions at specific mass to charge m/z values, corresponding to that compound's relative concentration. Total time for GC-MS elution was 25 minutes.

# Phytochemicals identified in activated charcoal of *Terminalia catappa* by GC-MS

Shows compounds identified with their retention time, areato-height ratio, and area percentage  $(\%)_{\pm}$  which corresponds to the relative abundance of a compound. Methyl 6- beta galactopyranoside and Stigmastan 3,5-diene (93.24, 6.76 %) were identified as the most predominant compounds.

Table 4: Functional compounds detected in activated charcoal of Terminalia catappa

Peak	Retention Time	Area	Area%	Height	Height%	A/H	Name
1	15.818	13099460	93.24	331628	62.11	39.5	Methyl 6- beta galactopyranoside
2	22.108	950118	6.76	202277	37.81	4.7	Stigmastan 3,5-diene
		14049575	100	533905			

The table shows compounds identified, including methyl 6-beta galactopyranoside a carbohydrate ester and Stigmastan 3,5diene a plant sterol with reported numerous medicinal activity.

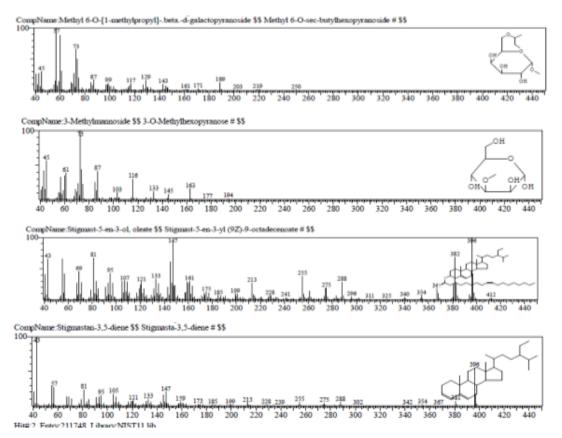


Figure 2: Single ion recording (S.I.R.) chromatogram of structures of some identified compounds in *Terminalia catappa* charcoal

Table 5: Biological activity	y of activated charcoal of <i>Terminalia cat</i>	appa
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Compound name	Chemical structure	Molecular formula/weight	<b>Biological activity</b>	Reference	Concentration (Area %)	
Methyl 6- beta galactopyranoside	OH OH OH	C11H22O6 250	Anticancer, antiviral and anti-diabetes		93.24	
Stigmastan 3,5- diene	HOT H	C <sub>47</sub> H <sub>82</sub> O <sub>2</sub> 396	Anti-diabetic potential. Anti-osteoarthritis, anticancer, anti- inflammatory effect.	Nualkaew <i>et a</i> l., 2015. Dong <i>et al.</i> , 2021	6.76	

## Discussion

In addition to nutrients found in all food, two types of compounds that are not found in foods of animal origin include antioxidants (specific vitamins and minerals) and phytochemicals with curative properties. The proximate analysis technique provides fundamental characteristics and properties of a substance, which can be valuable in nutrition and energy utilisation (Ilouno *et. al.*, 2018). The diet's protein content and amino acid composition affect animal growth by regulating body protein synthesis and mean body mass that primarily composes organs, red muscle, and role as enzymes for numerous cellular processes (Enadeghe *et al.*, 2023). The percentage of proteins in activated charcoal of *T. catappa* is  $5.68\pm0.21$  % (Table 2) is high, bearing in mind that this is a charcoal sample compared to meat and fish that possess between 15 and 20 g of proteins for each 100 g. This is also

in comparison to Piper guineensis (Benin pepper or uziza pepper), whose protein content was reported to be 8.75%, and Morinda lucida (Brimstone tree), whose protein content was reported to be 2.28%). Despite being higher than Morinda lucida, the protein content of activated charcoal is still much lower than that of Moringa oleifera leaves (22.99%) (Asaolu et al., 2012), Ageratum convzoides leaves (18.62%), and Anthocleista djalonensis leaves (17.28%) (Nduche et al., 2015). This result suggest charcoal as a source of protein that can be used as protein supplement for feeds. Ash content provides relevant information about a sample's mineral or non-combustible material. It is also a valuable indicator for assessing the purity and quality of food samples. The ash content of activated charcoal of T. catappa is 13.30%, suggesting the charcoal consists of inorganic residues or minerals that were not volatilised during the activation

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process (pyrolysis). Low ash content in charcoal of Terminalia catappa (less than 15%) indicates high quality sample with no impurities that could potentially interfare with its adsorption potential. Crude fibre content refers to the indigestible portion of a sample, and the results reveal it contains 8.31% (as shown in table 2). Studies have shown that high crude fibre potentially impacts the activated charcoal's purity and efficiency. Its low content of 8.31 will contribute to its high surface area adsorption properties. According to Enadeghe and Omoregie (2023), fibre in human diets is known to facilitate the flow of waste through the colon, acting as a potent anti-constipating agent. Analysis of the samples showed that moisture content was very low (7.05 %) suggesting a very good shelf life of the sample preventing its microbial deterioration. Plant extracts are highly hygroscopic, such that they have low moisture content and keep the humidity levels under control, which is crucial to sample stability.

Certain minerals are essential for our normal metabolic reactions even in trace amounts. The human body on its own cannot produce these minerals and, thus are required for various physiological processes including calcium, iron, magnesium, potassium, sodium, phosphorus, zinc, selenium, copper, and iodine. The calcium content is the highest (128.26 mg/100 as shown in table 3) compared to other detected minerals and diary/vegetables sources. Although the amounts of extract taken are usually less than that of dairy or vegetables. Calcium plays a crucial role in the development and stability of cell walls as well as the preservation of membrane permeability and structure (Enadeghe and Edosa 2023a). Magnessium is a divalent ion (whose concentration is 46.70 as shown in table 3) involved in the transmission of metabolic functions and irritability, cramps and spasms. No single mineral comes anywhere close to the role magnesium plays in optimising our health, for proper muscle and nerve function, heart health, transportation of other vital minerals throughout the body, cells use it to make energy, the brain needs it to regulate sleep cycle. It participate in over 600 chemical reactions in the body. Its relative abundance in AC. suggest its usage as potential supplement.

These minerals play important roles in various chemical reactions, biological processes and industrial applications due to their ability to form stable compounds and participate in ionic bonding. These minerals can also form complexes with lipophilic compounds especially poisonous compounds deterring them from absorption or movement into various cells. These could be a possible mechanism charcoal uses to adsorp poisonous substances from bioaccumulating into cells in addition to its internal spores cum surface area like sponge that can effectively trap chemicals. The iron and potassium concentration (24.51 and 29.9) as shown in table 3 is higher than the reported methanol leaf extract of Terminalia ivorensis by Enadeghe and Omoregie et al., 2023. This also suggest that the char samples of the plant seems to be richer in essential minerals like iron, zinc and potassium needed for hemoglobin synthesis and insulin exocytosis. Two compounds were detected via GC-MS including methyl 6- β galactopyranoside as the most predominant compound with percentage area of 93.24% suggesting its nearness to purity. Methyl 6- ß galactopyranoside is a carbohydrate esters of galactose derivative found in various foods and used in the pharmaceuticals for carbohydrate metabolism. It can act as a carrier molecule by improving the bioavailability and efficacy of therapeutic compounds. It is widely used in glycosylation processes crucial for developing treatments for cancer, diabetes and autoimmune disorders. This compound relatively dominate the pulverised charcoal with its large

surface area and can be conjugated and functionalised with other anticancer agent to enhance permeability into target tumor cells to release the anticancer agent for optimal therapeutic activity. Certain galactose derivatives have also shown potential antiviral activity against viruses such as herpes simplex virus. Studies have reported that Methyl 6-  $\beta$ galactopyranoside is a potential inhibitor for SARS CoV-2 papain-like protease (SARS-CoV-2 PLpro c) enzyme (Amin et al., 2022). An essential coronavirus enzyme required for viral replication and spread. SARS-CoV-2 PLpro c) is used to control host interferon (IFN) and NF-KB pathways. Upon infection, SCoV2-PLpro contributes to the cleavage of ISG15 from interferon responsive factor 3 (IRF3) and attenuates type I interferon responses (Amin et al., 2022). Inhibition of SCoV2-PLpro with activated charcoal impairs the virusinduced cytopathogenic effect, fosters the antiviral interferon pathway and reduces viral replication in infected cells and thus may attenuate variant mutation (Enadeghe and Omoregie 2023b). Methyl 6-  $\beta$  galactopyranoside is a precursor to many potential antiviral compounds including mannose. Mannose interfares with glucose metabolism of tumor cells. These cells have high demand and affinity for glucose (Enadeghe and Omoregie, 2023b). Based on the fact that mannose and glucose are introduced into cells by the same transporters (Thorens and Mucker, 2010). Mannose accumulates in the form of mannose-6-phosphate (M6P) and the accumulation of M6P suppresses glucose phosphate isomerase (GPI) and other enzymes related to glucose metabolism, which impairs the further metabolism of glucose in glycolysis, triacarboxylic acid cycle, pentose phosphate pathway and glycan synthesis (Gonzalez et al 2018). Studies have shown that mannose combined with conventional therapy such as cisplatin and adriamycin down regulate MCL and Bcl-XL protein levels and enhance apoptosis in tumor cells. It is also known as a potent methyl donor in epigenetics(DNA methylation) for development, ageing and diseases. Stigmastan 3, 5-diene detected in charcoal (6.76%) as shown in table 4 is a plant sterol found in plant based-foods and some dietary supplements. This compound is structurally similar to cholesterol known to inhibit the absorption of cholesterol in the intestines, potentially reducing LDL cholesterol levels. Studies have also shown this compound also possess antiinflammatory effect suppressing inflammation, hv suppressing glycolysis, promoting TGF-ß activation and inducing Treg cell (Zhang et al., 2020).

Stigmastan 3, 5-diene is currently enlisted by the European union as a food additives under the number E499, to boost phytosterol levels in the production of foods to improve lowdensity lipoprotein cholesterol (LDL-Cholesterol) (Ashraf and Bhatti 20221). Animal studies hane demonstrated that Stigmastan 3, 5-diene possess anti-diabetic potential by activating the glucose transporter type-4 (GLUT-4) translocation and insulin resistance and potentiating  $\beta$  cell regeneration. Like other phytosterols, it also exhibit various anti-osteoarthritis properties by relieving cartilage degradation in rodent model of osteoarthritis (Antwi et al., 2018). A molecular docking analysis demonstrated an antiinflammatory and analgesic role. It reduce the release of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) nitric oxide (NO) and proinflamatory cytokines as well as inhibition of cyclooxygenase-2 (COX-2) thus alleviating pain and inflammation.

## CONCLUSION

The results of this study demonstrate that activated charcoal contains nutrients, minerals and functional compounds that can be used as feed/food supplements and as potential

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