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Effects of Some Plant Leaf Methanol Extracts on Fungi Isolated from Seeds of a Selected Landrace of *Sorghum bicolor* (L.) Moench

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ABSTRACT

The antifungal properties of methanol extracts of selected plant (*Adansonia digitata, Ambrosia maritiama, Moringa oleifera, Ocimum basilicum, Ocimum canum, Ocimum gratissimum* and *Vernonia cinerea*) leaves on fungi isolated from a selected landrace of sorghum sold in Sabon Gari market, Kaduna State, Nigeria was investigated**.** Extraction and quantification of phytochemicals from the leaves, and the minimum inhibitory and fungicidal concentrations was done using standard methods. Antifungal activity of the extracts was evaluated by comparing the mycelial growth inhibition (MGI) with standard antifungal drug Mancozeb at concentrations of 50mg/ml and 20mg/ml. Nine phytochemicals were found associated with the extracts and there were significant variation in the quantities of phytochemicals, with *O. canum* having the highest concentrations of Alkaloids (59.96mg/g), Flavonoids (101.09mg/g), Saponins (62.71mg/g), Phenols (160.08mg/g), Tannins (115mg/g) and Steroids (55.09mg/g); while *A. maritiama*, *O. basilicum* and *M. oleifera* had the highest concentrations of Carbohydrates (356.98mg/g), Tritipenes (32.76mg/g) and C/glycosides (66.53mg/g) respectively. There is significant variation ($p<0.05$) in the effect of leaf extracts and Mancozeb on the MGI of isolated fungi with *O. canum* having highest efficacy against four fungi species (*Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum* and *Fusarium solani*), recording 100% MGI. Sensitivity of fungi to the extract showed that *M. oleifera* extract had minimum inhibitory concentration (MIC) on the isolates only at 50mg/ml. *O. canum* extract had its MIC against *A. flavus*, *Didymaria* sp. and *Curvularia lunata* at 25mg/ml. Its MIC against *Penicillium chrysogenum*, *F. oxysporum* and *F. solani* was further observed at 12.5mg/ml. *Ocimum basilicum* extract had its MIC against *Dydimaria* sp and *C. lunata* at 25mg/ml. Extract from *Ambrosia maritiama* had its MIC against *Dydimaria* sp and *C. lunata* at 25mg/ml and 12.5 mg/ml respectively. Extract of *O. canum* had its MFC against *P. chrysogenum* and *F. oxysporum* at concentration of 25mg/ml each. Extract from *Ocimum basilicum* had its MFC at 50mg/ml against *Dydimaria* sp and *C. lunata* whereas *A. maritiama* had its MFC against *C. lunata* at 25mg/ml. Investigations into the mechanisms by which *O. canum* extract inhibits the growth of *A. flavus*, *A. niger*, *F. oxysporum*, and *F. solani* can aid in developing new antifungal agents since it is the most active in terms of antifungal activity.

Keywords: *Ocimum canum*, Antifungal, *Sorghum bicolor*, Seeds, Postharvest, Storage, Pathogens.

Introduction

Sorghum (*Sorghum bicolor* (L) Moench) is the fifth most important world cereal after rice, wheat, corn and barley and an important native cereal in Africa (Muhammad *et al*., 2022). The largest world's sorghum producers are the USA with total annual grain production of 8.7 million tons from 5.4 million hectares, Nigeria (6.9 million tons and 2.0 million hectares), Ethiopia (5.3 million tons and 1.9 million hectares), and Sudan (3.7 million tons in 6.8 million hectares) (FAO, 2019; Muhammad *et al*., 2022). The four leading sorghum producers in Africa are Nigeria, Ethiopia, Burkina Faso and Niger (Muhammad *et al*., 2022).

Sorghum is the largest staple cereal crop accounting for 50% of the total output and occupying about 45% of the total land area devoted to cereal crops production in Nigeria (FAO, 2019; Mrema *et al*., 2020). The sorghum productivity in the country is 1.23 t ha−1, which is relatively low compared with the world average of 1.45 t ha−1 and the USA with 4.58 t ha−1 (FAO, 2019).

In Nigeria, Sorghum is grown in different ecological zones (Sudan savannah, Northern Guinea savannah and Southern Guinea savannah) due to its adaptability to a wide range of environmental conditions including particularly drought (Ajeigbe *et al*., 2018).

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Sorghum is a principal source of energy, protein, vitamins and minerals to the poorest people of the semi – arid tropics. The crop is dried stored and later used to prepare stiff porridge, thin porridge or fried dumpling (Mofokeng *et al*., 2017). It is also used in brewing local beer (Catherine *et al*., 2020). The leaves provide fodder for farm animals and the stalks are used in fencing, roofing, weaving baskets and mats and also as fuel wood (Ogbonna, 2011). It is also mixed with wheat flour for confectionaries e.g bread, cake etc. Sorghum grains are used industrially in the manufacture of wax, starch, syrup, alcoholic and non-alcoholic beverages, dextrose agar, edible oils and gluten feed (Awada, 2016).

The major biotic constraints of sorghum production are insects, parasitic weeds, birds and diseases (Mrema *et al*., 2020). Fungi are important sorghum disease causing organisms (Muui *et al*., 2020). Moulds are ubiquitously distributed in nature and their spores can be found in the atmosphere even at high altitudes, carried and disseminated by wind and air currents, as well as can be spread by insects, rodents and other animals. Food products, being organic substances and containing essential nutrient, are very suitable substrate for the mould growth (Marie *et al*., 2016). Due to their powerful arsenal of hydrolytic enzyme, moulds can cause a high degree of deterioration when present in/on foods/feeds and can be responsible for considerable economic losses (Marie *et al*., 2016).

Besides the possible food decay caused by moulds and ultimate changes in nutritional and organoleptical characters, the moldiness in food stuffs is toxicologically significant since some of the mould species growing on such products are mycotoxigenic (Marie *et al*., 2016). Mycotoxins are metabolites produced by filamentous fungi that is toxic to other animals specifically man and his animals (Grenier and Oswald, 2011). The main toxic effects are carcinogenicity, genotoxicity, terratogenicity, nephrotoxicity, hepatotoxicity, reproductive disorders and immuno suppression (Gallo *et al*., 2015; Marie *et al*., 2016). Fungi account for yield losses of up to 67% of sorghum seeds during storage by the production of hydrolytic enzyme and mycotoxins (Marie *et al*., 2016; Abdel *et al.,* 2017). A number of studies on fungi associated with sorghum seeds during storage reveals the presence of *Alternaria, Aspergillus, Cercospora, Bipolaris, Curvularia, Dreschlera, Fusarium, Penicillium, Pyricularia, Pythium, Rhizoctonia and Rhizopus* spp (Mohammed *et al*., 2015).

Many of these fungi are implicated in causing yield loss by reducing seed size and weight, seed abortion, seed rot, seed necrosis, reduction of germination capacity and seedling damage (Pushpavathi *et al*., 2017; Jayashree and Wesley, 2019). A significant portion of the agricultural produce in the country and the world become unfit for human consumption due to mycotoxins contamination of grains (Ogbonna *et al*., 2015; Pushpavathi *et al*., 2017). Even though effective and efficient control of seed borne fungi can be achieved by the use of synthetic chemical fungicides, the same cannot be applied to stored seeds for reasons of pesticide toxicity (Mohammed *et al.,* 2015; Adamu *et al*., 2020).

The use of botanical seed treatment method is considered to be the safest and the cheapest approach for controlling seed-borne diseases as well as deterioration of grains (Pushpavathi *et al.,* 2017; Jayashree and Wesley, 2019). Studies have indicated that in some plants, there are many substances with potential significant therapeutic application against fungal pathogens (Heuze *et al*., 2013; Abiona *et al*., 2015). Generally, Plant extracts act as contact fungicides by disrupting cell membrane integrity at different stages of fungal development, while others inactivate key enzymes and interfere with metabolic processes (Neela *et al*., 2014). Crops treated with plant extracts produce and accumulate elevated levels of specialized proteins and other compounds which inhibit the establishment of fungal diseases in susceptible varieties (Nguefack *et al*., 2013).

Materials and Methods

Study Area

The study was carried out in the Departments of Crop protection and Pharmacognosy, Faculty of Agriculture and Pharmaceutical sciences respectively, Ahmadu Bello University, Zaria, Nigeria. Zaria is located between latitude 11º9′N, Long 7º2′ E at an altitude of 686 meters above sea level. Its annual rainfall, average temperature and relative humidity are 1055mm, 24.55ºC, and 43.6% respectively (Meteorological unit IAR, 2009).

Source of Materials and their preparation

A local landrace of Sorghum (Kaura) was obtained from Sabon Gari market in sterile polyethene bag which was transported to Department of crop protection for mycological analysis.

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Adansonia digitata, Ambrosia maritiama, Moringa oleifera, Ocimum basilicum, Ocimum canum, Ocimum gratissimum and *Vernonia cinerea* leaves were obtained from the wild and their identity was confirmed by the Herbarium unit of Department of Botany, Ahmadu Bello University, Zaria while Methanol was obtained from chemical store. The fungal strains were obtained from research by Danazumi *et al*. (2024).

Determination and Quantification of Phytochemical Compounds in the Leaf Extract of the Selected Plants

Extraction of the phytochemical compounds was done using the maceration method of Kokate *et al*. (2002). The leaves of collected plants were washed under tap water followed by distilled water and dried in shade. The air-dried powdered leaves (100g) were extracted with the methanol solvent (70%). Powders of the leaves were mixed individually with the solvent and left overnight to allow the constituents to get dissolved in the methanol which were then filtered through muslin cloth followed by Whatman filter paper No 1. The filtrates were then evaporated at 40°C to obtain the crude extract. The processed crude extract was poured in the Erlenmeyer flask, plugged with cotton separately and heated at 50°C for 15 minutes to avoid contamination (Madavi *et al*., 2005). The extract was then stored in a refrigerator for further use in the experiment

Phytochemical Screening

The leaf extract of the selected plants was subjected to different tests to identify the phytochemical constituents present in them. Each ml of the distinct extract was dispensed separately into various test tubes with the aid of a syringe and was used for the following tests.

Test for alkaloid

To 1ml of each extract in a test tube, 2 drops of 0.5 M sulphuric acid was added and vigorously shakened. The formation of creamy precipitate indicates the presence of alkaloid (Kokate *et al*., 2008).

Test for Flavonoids

To 1ml of each extract in a test tube, 5ml of ammonia and 1ml of concentrated tetraoxosulphate (VI) acid was added. A yellow colouration that disappears on standing indicates the presence of flavonoid (Ayoola *et al*., 2008).

Test for Tannins

To 1ml of each extract, 3-5 drops of lead sub-acetate solution was added. Brown green precipitate indicates the presence of tannins (Kokate *et al*., 2002).

Test for Saponins

To 1ml each of the extract, 5ml of distilled water was added, shaken vigorously and observed for persistent froth. Then, 3 drops of olive oil were mixed and formation of emulsion indicate the presence of saponins (Kokate, 2000).

Test for steroids

To 1ml of each extract, 2ml of acetic anhydride and sulphuric acid was added gently by the side walls of the test tube one after the other. Colour change from violet to blue green indicates the presence of steroids (Kokate *et al*., 2008).

Test for phenols

To 1ml of the extract, few drops of lead acetate solution was added. A yellow colored ppt indicates phenols (Ayoola *et al*., 2008).

Test for glycosides and for Terpenoids

To 1ml of the extract, 1ml of glacial acetic acid with a trace amount of ferric chloride was added then 1ml of concentrated Tetraoxosulphate (VI) acid was also added. Formation of reddish brown colour ring at the junction of 2- layers, with upper layer turned into bluish green colour, indicates presence of glycosides (Kokate *et al*., 2002).

For Terpenoids; To 2.0 ml of acetic acid, chloroform was added to the plant crude extract. The mixture was then allowed to cool and subsequently concentrated Tetraoxosulphate (VI) acid was added. Green color indicates the presence of Terpenoids (Kokate, 2000).

Test for Carbohydrate

To 0.5g of the extract, 3ml of distilled water was dissolved (heat if necessary), and few drops of Molisch's reagent was added. Subsequently, small amount of concentrated Tetraoxosulphate (VI) acid from the side of the test tube was also added to form a lower layer. Red colour of the interfacial ring indicates that the drug is a carbohydrate (Kokate, 2000).

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Quantitative phytochemical screening

Determination of cardiac glycosides

Cardiac glycosides were quantitatively determined according to Satish *et al.* (2007) by some modifications. For determination of cardiac glycosides, 1ml solution of the extract was mixed with 10 ml freshly prepared Baljet's reagent (95 ml of 1% picric acid + 5 ml of 10% NaOH). After an hour, the mixture was diluted with 20 ml distilled water and the absorbance was measured at 495 nm UV/VIS spectrophotometer. For preparation of the standard curve, 1 ml of different concentrations (20-100 mg/l) of securidaside was prepared from the extract. Total glycosides from triple replicates were expressed as mg of securidaside/Digoxin per g of dried extracts.

Steroids

The Liebermann-Bur chard reaction method was used to detect sterols and terpenoids that give dark pink to green colour, due to the hydroxyl group reacting with acetic anhydride and Tetraoxosulphate (VI) acid. Varying concentrations of cholesterol (10-100 µg/ml) was used for standard calibration curve, which was read spectrophotometrically at 640 nm. The concentration of steroids was expressed in milligrams/gram of the crude extract (Harborne, 1973).

Total triterpenoid content

For determination of Triterpenoid content, 1.0 ml of the solution of the extract was mixed with vanillin-glacial acetic acid solution (5% w/v) and perchloric acid solution (5.0 ml). The sample solution was heated for 45 min at 60◦C and then cooled in an ice-water bath to the ambient temperature. After the addition of glacial acetic acid (2.25 ml), the absorbance was measured at 548 nm, using a UV-visible-light spectrophotometer. Ursolic acid/Cholesterol (20–100 µg/ml in methanol) was used as a standard. Results were expressed as milligram ursolic/cholesterol/linalool acid equivalents (mg ursolic acid/cholesterol/g extract) (Harborne, 1973).

Total carbohydrate

This was done using the Sulfuric Acid–UV method. 1 ml aliquot of carbohydrate solution was rapidly mixed with 3 ml of concentrated sulfuric acid in a test tube and vortexed for 30 sec. The temperature of the mixture rises rapidly within 10–15 sec after addition of sulfuric acid. Then, the solution was cooled in ice for 2 min to bring it to room temperature. Finally, UV light absorption at 715 nm was read using UV spectrophotometer. Reference solutions (glucose) were prepared following the same procedure as above, except that the carbohydrate aliquot was replaced with DDI water (DuBois *et al*., 1956).

Anthraquinone

The Bornträger-reaction was used to detect the anthraquinone substance aglycones in an extract. To 1.0ml of the solution of the extract, 1.0ml solution of 10% Tetraoxosulphate (VI) acid was added, stirred and placed in a hot water bath for 5 minutes. The insoluble parts were filtered and then the solution was allowed to cool to room temperature. The filtrate was removed and then 0.5ml of 10% Ammonia was added and shaked vigorously. The change in colour of the solution to pinkish red, indicates the presence of anthraquinone. The concentration of anthraquinone was determined by checking the dilution factor of each sample compared to the standard anthraquinone solution (Rhein/Anthracene) at 515nm (Sakulpanich and Gritsanapan, 2009).

Determination of total phenolic content (TPC)

Estimation of total phenol content was measured spectrophotometrically by Folin–Ciocalteu colorimetric method, using Gallic acid as the standard and expressing results as Gallic acid equivalent (GAE) per gram of sample. Different concentrations (0.01-0.1 mg/ml) of Gallic acid were prepared in methanol. Aliquots of 0.5 ml of the test sample and each sample of the standard solution was taken, mixed with 2 ml of Folin–Ciocalteu reagent (1:10 in deionised water) and 4 ml of saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foils and incubated at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using methanol as blank. All the samples were analyzed in three replications. The total phenol was determined with the help of standard curve prepared from pure phenolic standard (Gallic acid) (Ainsworth and Gillespie, 2007).

Determination of total flavonoid content (TFC)

The TFC was determined by aluminium chloride colorimetric assay (Zhishen *et al*., 1999). Briefly, 0.5 ml aliquots of the samples (Methanol extract and fractions) and standard solution (0.01-1.0 mg/ml) of quarcetin were added with 2 ml of distilled water.

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Subsequently, 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) solution was also added. After 6 minutes, 0.15 ml of (10% $AlCl_3$, w/v) solution was added. The solutions were allowed to stand for 6 min and after that 2 ml of sodium hydroxide (4% NaOH, w/v) solution was added to the mixture. The final volume was adjusted to 5 ml with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 min. The absorbance of each mixture was determined at 510 nm against the same mixture. TFC was determined as mg quarcetin equivalent per gram of sample with the help of calibration curve of quarcetin. All determinations were performed in triplicate.

Determination of total alkaloid content (TAC)

TAC was quantified by spectrophotometric method. This method was based on the reaction between alkaloid and bromocresol green (BCG). The plant extract and fractions (1 mg/ml) was dissolved in 2ml of hydrochloric acid and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. 1 ml of this solution was transferred to a separating funnel, and then 5 ml of BCG solution along with 5 ml of phosphate buffer was added. This was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extract was collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. TAC was determined as mg Atropine equivalent per gram of sample with the help of calibration curve of Atropine. The whole experiment was conducted in three replicates (Sharief *et al*., 2014).

Determination of tannin content (Ferric Chloride test, Bromine Water test)

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin –Ciocalteu phenol reagent, 1 ml of 35 % Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min.

A set of reference standard solutions of Gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared. Absorbance for test and standard solutions was measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract (Marinova *et al*., 2005).

Determination of saponins content (Frothsing test, Haemolysis test)

Total saponins were determined according to method described by Satish *et al.* (2007). A known quantity of freeze-dried extract was dissolved in aqueous 50% methanol and a suitable aliquot (5 mg/ml) was taken. Vanillin reagent (0.25 ml; 8%) was added followed by sulphuric acid (2.5 ml; 72% v/v). The reaction mixtures were mixed well and incubated at 60°C in a water bath for 10 min. After incubation, the reaction mixtures were cooled on ice and absorbance at 544 nm (UV visible spectrophotometer) was read against a blank that does not contain extract. The standard calibration curve was obtained from suitable aliquots of diosgenin (0.5 mg/ml in 50% aqueous methanol). The total saponins concentration was expressed as mg diosgenin equivalents (DE) per g dry weight (DW).

Effects of Different Concentrations of the Extract on Mycelial Growth Inhibition of Fungi Species

Antifungal activity of plant extracts was determined by mycelial growth inhibition assay as described by Fiori *et al*. (2000). Varying concentrations (mg/ml) of 50 and 20 of the various plants extract were prepared from the crude extract. 2ml each of varying concentrations of the extract were dispensed separately onto the 20ml PDA in Petri dishes with the help of a syringe. These were thoroughly mixed and allowed to solidify. A portion of mycelial growth from the periphery of the fungi was cut with the help of a sterilized cork borer of 5mm (0.5cm) in diameter and then placed onto the middle on each Petri dish and incubated at room temperature (25-27°C) in triplicates for 7 days. After incubation, measurement of the colony (cm) was taken directly with the help of a rule. PDA medium with Mancozeb was used as the positive control whereas the medium without methanolic extract will serve as the negative control. Effect of different concentrations of the extracts (Fungi toxicity) was recorded in terms of percentage mycelial growth inhibition using the food poison technique (Riad *et al*., 2014).

$$I = \frac{DC - DT \text{ x } 100I}{\text{DC}}$$

Where, I = Percentage inhibition
DC = Average diameter of control (Negative)
DT = Average diameter of growth with treatment

The antifungal activity of the different extracts was evaluated by comparing the percentage mycelial growth inhibition with standard antifungal drug Mancozeb (80WP) at a concentration of 50 mg/ml (Adamu *et al*., 2020) and 20mg/ml.

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Preparation of Standard Fungal Inoculum

This was done using the McFarland standard (EUCAST, 2014). The spores from the surface of the individual plates containing the various fungi species were collected with inoculating needle and suspended in 8 ml of normal saline solution each. The mixture was subsequently homogenized and 2 ml of 10% Tween 20 was added to reduce the clumps of the hyphae to the spore. The mixtures were stirred and kept for 6 hours in which the spores settled at the bottom. The supernatant was discarded and the spores were gradually decanted into distinct sterile tubes. The spore suspension was adjusted to 0.5 McFarland standards which is equivalent to the turbidity of the suspension by a spectrophotometer at a wavelength of 530 nm for moulds, based on optical density of the solution. The optical density of 1 was taken as 0.5 McFarland standard which correspond to a spore count of 1.5×10^8 CFU/ml of each test organism.

Determination of Minimum Inhibitory and Fungicidal Concentrations of the Extracts

The MIC was determined using the broth dilution method (CLSI, 2009) in which Potato dextrose broth was prepared according to manufacturer recommendation. Serial dilution of the extract was done to obtain a concentration of 50, 25, 12.5, 6.25 and 3.13 mg/ml. Already prepared Standard fungal inoculum (0.1ml) was introduced into each of the test tube containing varied concentration of the extracts dissolved in the Potato dextrose broth and incubation was done for 7 days at room temperature.

The MIC was read as the test tube having the least concentration of the extract with no sign of fungal growth. The content of the test tubes showing no visible growth from the determination of Minimum inhibitory concentration were sub cultured in freshly prepared PDA which was then incubated at room temperature (25-27°C) for 7 days. The plates with the lowest concentration of the extract that showed no growth of the test fungal organisms were taken as minimum fungicidal concentration.

Statistical Analysis

The Effects of different concentrations of the leaf methanol extract (Fungi toxicity) and quantified phytochemicals was subjected to one way analysis of variance. Duncans Multiple Range Test (DMRT) was used to separate means where significant difference is observed between the treatments. All analysis was performed using R Statistics for windows (V.4.0.3)

Results

Screened and Quantified phytochemicals associated with the selected plants extract

Result of the phytochemical screening of the various plant extracts reveals the presence of nine compounds namely Saponin, Tannin, Alkaloid, Cardiac glycosides, and Phenolic compounds, Steroids, Flavonoids, Carbohydrates and Terpenoids in all the extracts as presented in (Tables 1a and b) while Anthraquinones were not present in the extracts.

Table 1a: **Phytochemical compounds present in the methanol extract of leaves of the selected plants**

Methanol Leaf Extract	Phytochemical Constituent/Test performed)									
	Alkaloids			Cardiac Glycosides		Saponins		Phenolic compounds	Tannins	
	Mayers	Dragendorff	Wagner	Keller-Kiliani	Kadde	Frothsing	Haemolysis	Lead acetate	Ferric Chloride	Bromine Water
A. digitata	+	+	+	+	-	+	+	+	+	+
A. maritiama	+	+	+	+	-	+	+	+	+	+
V. cineria	+	+	+	+	+	+	+	+	+	+
O. canum	-	+	+	+	+	+	+	+	+	+
M. olefeira	-	+	+	+	+	+	+	+	+	+
O. basilicum	-	+	+	+	+	+	+	+	+	+
O. gratissimun	+	+	+	+	+	+	+	+	+	+

Key: + Present -Absent

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Table 1b: **Phytochemical compounds present in the methanol extract of leaves of the selected plants**

Methanol Leaf Extract	**Steroids**	**Carbohydrates**		**Flavonoids**		**Terpenoids**	**Anthraquinones**	
	Salkowiski	Molisch	Fehling	Shinoda	Alkaline	Liebermann Burchard	Bontragers	Modified Bontragers
A. digitata	+	+	+	+	+	+	-	-
A. maritiama	+	+	+	+	+	+	-	-
V. cineria	+	+	+	+	+	+	-	-
O. canum	+	+	+	+	+	+	-	-
M. olefeira	+	+	+	+	+	+	-	-
O. basilicum	+	+	+	+	+	+	-	-
O. gratissimun	+	+	+	+	+	+	-	-

(Header spanning columns: Phytochemical Constituent/Test performed))

Key: + Present -Absent

All the screened phytochemicals were quantified and *Occimum canum* was found to have highest concentration of alkaloids (59.96 mg/g), flavonoids (101.09 mg/g), saponins (62.71 mg/g), phenols (160.08 mg/g), tannins (115mg/g) as well as steroids (55.09mg/g) whereas highest concentration of carbohydrate (356.98mg/g) was recorded by *Ambrossia maritiama. Occimum basilicum* recorded the highest concentration of triterpenes (32.76mg/g) and cardiac glycoside (66.53mg/g) was highest in *Moringa oleifera* (Table 2). Statistically, the quantified phytochemicals were significantly different with respect to the distinct plant extract at p>0.05

Table 3 revealed the effect of same concentration of the extract and Mancozeb (50mg/ml) on mycelial growth inhibition of the isolates. It was observed that four plant extracts namely *Ambrosia maritiama, Ocimum basilicum, Ocimum canum* and *Moringa oleifera* were found to possess 100% growth inhibition in all the isolates while in some instances even performing better than the positive control. In case of *Aspergillus flavus*, the four plant extracts recorded 100% growth inhibition, whereas the positive control recorded 75.53%. It was also observed that in the case of *Fusarium solani*, the four plant extracts recorded 100% whereas the positive control recorded 62.53%. The effect of the plant extract and Mancozeb at same concentration shows that there is significant variation in mycelial growth inhibition of the isolates at ($P < 0.05$).

Table 4 shows the effect of same concentration of Plant extract and Mancozeb (20mg/ml) on mycelial growth inhibition of the isolated fungi species. It was however observed that *Ocimum canum* had 100% mycelial growth inhibition against four isolates namely *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum* and *Fusarium solani* performing better than the positive control. The effect of same concentration of the selected plant extract and Mancozeb shows that there is significant variation in the mycelial growth inhibition of the isolates at ($P < 0.05$).

The four most effective plants extracts interms of the mycelial growth inhibition namely *Amrosia maritiama*, *Ocimum basilicum*, *Ocimum canum* and *Moringa oleifera* were selected for the determination of Minimum inhibitory and fungicidal concentrations. The minimum inhibitory concentration (Table 5) of fungi sensitive to the extract showed that *Moringa oleifera* extract had no any growth inhibition at lower concentrations. However, growth inhibition was only observed on all the fungal isolates at the highest concentration (50mg/ml). *Ocimum canum* extract had its MIC against *Aspergillus flavus*, *Didymaria* sp. and *Curvularia lunata* at 25mg/ml. It was further observed to have its MIC against *Penicillium chrysogenum*, *Fusarium oxysporum* and *Fusarium solani* at 12.5mg/ml. However, *Ocimum basilicum* had its MIC against *Dydimaria* sp and *Curvularia lunata* at 25mg/ml. The extract from *Ambrosia maritiama* had its MIC against *Dydimaria* sp and *Curvularia lunata* at 25mg/ml and 12.5 mg/ml respectively.

The minimum fungicidal concentration (Table 6) was not determined for the *M. oleifera* extract beacause it was only the highest concentration (50mg/ml) that inhibited the growth of all the fungal isolates. However, the extract from *O. canum* had its MFC against *P. chrysogenum* and *F. oxysporum* at concentration of 25mg/ml each. The extract from *O. basilicum* had its MFC at 50mg/ml against *Dydimaria* sp and *C. lunata* whereas *Ambrosia maritiama* had its MFC against *Curvularia lunata* at 25mg/ml.

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Table 2: **Quantified Phytochemical compounds (mg/g) present in the leaf methanol extract of the selected plants**

Plant Species	Alkaloids	Flavonoids	Saponins	Phenols	Tannins	Carbohydrates	Steroids	Triterpenes	C/glycosides
Adansonia digitata	24.18±0.06d	54.25±0.10e	54.53±0.10b	150.82±4.21c	68.79±0.11d	239.56±0.14e	33.92±2.07d	20.69±0.00c	47.61±0.00c
Ambrosia maritiama	19.81±0.16de	69.74±0.00d	53.71±0.10b	114.66±2.22e	59.46±0.11e	356.98±6.41a	15.35±1.00f	29.27±0.04b	36.94±0.00e
Venonia cinerea	40.96±0.00b	84.83±0.10c	24.22±0.10d	97.32±2.14f	80.34±0.11c	256.37±1.00d	36.59±3.07c	32.29±0.05a	51.51±0.00b
Moringa oleifera	43.63±0.06b	47.58±0.10f	49.68±0.20c	155.24±3.08b	100.01±3.11b	317.39±0.00b	27.58±2.07e	21.03±0.00c	66.53±0.03a
Occimum basilicum	37.45±0.12c	42.77±0.10g	54.81±0.27b	158.66±3.08ab	103.68±0.11b	197.39±0.00f	44.24±4.13b	32.76±0.02a	51.86±0.03b
Occimum canum	59.96±0.00a	101.09±0.00a	62.71±0.30a	160.08±6.00a	115±3.00a	154.46±0.14g	55.09±1.07a	17.27±0.02d	47.13±0.00c
Occimum grattissimum	18.60±0.00e	92.31±0.10b	18.87±0.10e	127.08±4.00d	42.23±1.06f	288.14±67.70c	34.7±2.07cd	18.14±1.39d	40.94±0.00d
p value	0.000	0.000	0.000	0.000	0.004	0.001	0.000	0.000	0.000

Mean ± S.E with different letters (a-g) along the same column were significantly different ($P < 0.05$)

Table 3: Effect of Plant Extract at 50mg/ml and Mancozeb at 50 mg/ml on Mycelial Growth Inhibition of Fungi Isolated from Sorghum Seeds

Fungi Species	Mancozeb	Neg control	*A. digitata*	*A. maritiama*	*O. basilicum*	*O. canum*	*O. grattissimum*	*V. cinerea*	*M. oleifera*	p-value
Aspergillus flavus	75.53±0.00b	6.25±0.00c	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	98.70±0.00a	100.00±0.00a	100.00±0.00a	0.033
Aspergillus niger	100.00±0.00a	6.25±0.00c	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	77.37±10.67b	100.00±0.00a	100.00±0.00a	0.025
Fusarium oxysporum	100.00±0.00a	6.25±0.00b	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	0.034
Fusarium solani	62.53±3.61c	0.00±0.00d	87.50±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	77.13±4.17b	87.5.00±0.00a	100.00±0.00a	0.023
P. chrysogenum	100.00±0.00a	0±0.00b	81.30±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	81.30±0.00a	100.00±0.00a	100.00±0.00a	0.022
Didymaria sp.	100.00±0.00a	6.25±0.00c	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	77.80±5.88b	100.00±0.00a	100.00±0.00a	0.034
Curvularia lunata	100.00±0.00a	16.70±2.10b	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	82.90±1.40a	100.00±0.00a	100.00±0.00a	0.034

Mean ± S.E with different letters (a-g) along the same column were significantly different ($P < 0.05$)

Table 4: Effect of Plant Extract at 20 mg/ml and Mancozeb at 20 mg/ml on Mycelial Growth Inhibition of Fungi Isolated from Sorghum Seeds

Species	Mancozeb	Neg control	*A. maritiama*	*O. basilicum*	*O. canum*	*M. oleifera*	p-value
Aspergillus flavus	45.80±0.90b	6.25±0.00c	2.67±0.00e	4.00±0.00d	100.00±0.00a	2.67±0.00e	0.011
Aspergillus niger	40.00±0.00b	6.25±0.00c	4.00±1.33d	4.00±1.33d	100.00±0.00a	2.67±0.00e	0.002
Fusarium oxysporum	100.00±0.00a	6.25±0.00b	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	0.022
Fusarium solani	0.00±.00d	0.00±0.0d	20.87±2.07c	100.00±0.00a	100.00±00a	66.70±2.10b	0.013
P. chrysogenum	62.50±0.00a	0.00±0.0e	37.50±0.00d	43.77±6.27c	49.17±0.83b	41.67±8.33cd	0.010
Didymaria sp.	53.33±6.67b	6.25±0.00d	57.77±2.23a	48.47±1.77c	60.00±0.00a	44.47±2.23c	0.010
Curvularia lunata	54.90±1.10c	16.7±0.00d	100.00±0.00a	13.87±5.82e	61.00±0.50b	53.47±0.33c	0.021

Mean ± S.E with different letters (a-d) along the same row were significantly different ($P < 0.05$)

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Table 5: Minimum Inhibitory Concentrations (M.I C) of Methanol Leaf Extract of Selected Plants on Fungal Isolates

Methanol Plant Extract	Concentrations (mg/ml)	Test organisms						
		A. flavus	*A. niger*	*P. chrysogenum*	*Didymaria* sp	*F. oxysporum*	*F. solani*	*C. lunata*
Moringa oleifera	50	MIC	MIC	MIC	MIC	MIC	MIC	MIC
	25	+	+	+	+	+	+	+
	12.5	+	+	+	+	+	+	+
	6.25	+	+	+	+	+	+	+
	3.0625	+	+	+	+	+	+	+
Ocimum canum	50	-	MIC	-	-	-	-	-
	25	MIC	+	-	MIC	-	-	MIC
	12.5	+	+	MIC	+	MIC	MIC	+
	6.25	+	+	+	+	+	+	+
	3.0625	+	+	+	+	+	+	+
Ocimum basilicum	50	MIC	MIC	MIC	-	MIC	MIC	-
	25	+	+	+	MIC	+	+	MIC
	12.5	+	+	+	+	+	+	+
	6.25	+	+	+	+	+	+	+
	3.0625	+	+	+	+	+	+	+
Ambrosia maritiama	50	MIC	MIC	MIC	-	MIC	MIC	-
	25	+	+	+	MIC	+	+	-
	12.5	+	+	+	+	+	+	MIC
	6.25	+	+	+	+	+	+	+
	3.0625	+	+	+	+	+	+	+

Key: + = Growth, - = No growth

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Table 6: Minimum Fungicidal Concentration (M.F.C.) of the Methanol Extract of Selected Plants on Fungal Isolates

Methanol Plant Extract	Concentration (mg/ml)	Test organisms						
		A. flavus	*A. niger*	*P. chrysogenum*	*Didymaria* sp.	*F. oxysporum*	*F. solani*	*C. lunata*
Ocimum canum	50	MFC	ND	-	MFC	-	MFC	MFC
	25	+	ND	MFC	+	MFC	+	+
	12.5	+	ND	+	+	+	+	+
	6.25	+	ND	+	+	+	+	+
	3.0625	+	ND	+	+	+	+	+
Ocimum basilicum	50	ND	ND	ND	MFC	ND	ND	MFC
	25	ND	ND	ND	+	ND	ND	+
	12.5	ND	ND	ND	+	ND	ND	+
	6.25	ND	ND	ND	+	ND	ND	+
	3.0625	ND	ND	ND	+	ND	ND	+
Ambrosia maritiama	50	+	+	ND	MFC	ND	ND	-
	25	+	+	ND	+	ND	ND	MFC
	12.5	+	+	ND	+	ND	ND	+
	6.25	+	+	ND	+	ND	ND	+
	3.0625	+	+	ND	+	ND	ND	+

Key: + = Growth, - = No growth; ND = Not determine for M.F.C; Sample A was not determined for M.F.C. Therefore, the extract was fungistatic against the tested Organisms.

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Discussion

Microorganisms especially fungi are known to be the major cause of market and field losses of crops (Jayashree and Wesely, 2019). Different fungal genera such as *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium solani*, *Curvularia lunata*, *Didymaria sp.* and *penicillium chrysogenum* were found to contaminate the *Sorghum bicolor* landrace. These fungi might have colonized the seeds during production in the field, transportation or storage (Sultan *et al*., 2022), and many are facultative parasites or saprophytic fungi, as they contribute to pre- and post-harvest deterioration of grains (Pushpavathi *et al*., 2017).

Nine phytochemical compounds namely Tannins, saponins, alkaloids, phenols, flavonoids, Terpenoids, Carbohydrates, steroids and cardiac glycosides were found associated with these plants extract. Phytochemicals have been reported to possess antifungal activities (Ana *et al*., 2023; Tripathi *et al*., 2023), and their mechanism of action is that they inhibit cell membrane integrity at different stages of the fungal development, while others inactivate key enzymes and interfere with metabolic activities (Neela *et al*., 2014). Saponins had a great potential in resulting to toxicity to broad range of microorganism and also it could serve as chemotheraphy of mycotic infections on plant pathogenic microbes. This is also in line with the findings of Sodipo *et al*. (1991) whom reported that saponins foams and has a great hemolytic effect and can also bind to cholesterol sites and causes anti-mycotic effects on plants pathogens. Saponin is known to cause lysis of the cell and cell wall constituent of microorganism as reported by (Sodipo *et al*., 1991; Ladan *et al*., 2014). The sapogenin content could also bind to cholesterol sites there by inactivating key enzymatic reactions between the cell wall and the nutrient medium there by serving as substrate deprivation, also the reaction between the spores of this fungi species with the phenolic compound present in the extract could result in the formation of complex which could inactivate enzyme formation (Ladan *et al*., 2014).

Alkaloid had been known to exert a broad spectrum of biological activities. Ladan *et al*. (2011) reported that alkaloids such as cytisine, lupinine, anargynin and sparteinin showed antifungal activity on *Aspergillus* species from plant pathogenic microbes.

Phenols are a group of secondary metabolites distributed in plants that are used as antimicrobial agents due to their potential to damage membrane structural integrity in a nonspecific way and to inhibit certain electron transport enzymes (Bhattacharya *et al*., 2010). They had also been known to bind to adhesion complex on fungal cell wall leading to inactivation of key enzymes that aid in the digestion of nutrient by the fungal spores. Beatriz *et al*. (2018) reports that phenolic compound at a very little concentration inhibits the growth and sporulation of several fungi species leading to fungistatic or fungicidal activities. Similar potential of phenolic was also observed from the juice extract of *Gmelina arborea* on *Candida* species (Adamu *et al*., 2019). Flavonoids are structurally diverse secondary metabolites in plants that are reported to inhibit fungal growth by disrupting plasma membranes, inducing mitochondrial malfunction, and reducing cell wall construction, cell division, RNA (Ribonucleic acid), and protein synthesis, as well as the efflux mediated pumping system (Al Aboody and Mickymaray, 2020). Carbohydrates were present in the extract. Several studies has detected same in the leaves of other plants especially as the site of photosynthetic activities, though some novel mono-substituted carbohydrate fatty acid (CFA) esters are known to be fortified with side chains of bioactive compounds (Josephs and Dowe, 2016). Tannins are unabsorbable, complex structure having binding properties that may produce effects on the gastrointestinal tract including antioxidant, free radical scavenging, antimicrobial, antiviral, anti-mutagenic, anti- carcinogenesis, anthelmintic, hepatoprotective effects harmful pro-oxidative enzyme inhibitor, and anti-nutrient effects inhibitor (Takechi *et al*., 2023). Tannins appear to exert these effects in varieties of methods, including inhibition of extracellular microbial enzymes, deprivation of microbial growth substrates, or direct action on microbial metabolism via suppression of oxidative phosphorylation (Díaz *et al*., 2014). Terpenoids are for dissolution of the cell wall of microorganisms by weakening the membranous tissue (Sebei *et al*., 2015). Terpenoid also have strong antmicrobial effects (Sebei *et al*., 2015; Park *et al*., 2016). The monoterpenoids in terpenoids are mainly found in the genus Mentha, and previous studies have found that most of the compounds extracted from plants of the genus mentha have strong antimicrobial activity (Park *et al*., 2016).

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Glycosides are classified on the basis of type of sugar component, chemical nature of aglycone or pharmacological action (Alli *et al*., 2011). Glycosides are purely bitter principles that are commonly found in plants of the Genitiaceae family and though they are chemically unrelated but possess the common property of an intensely bitter taste. The bitters act on gustatory nerves, which results in increased flow of saliva and gastric juices (Altemimi *et al.,* 2017). Chemically, the bitter principles contain the lactone group that may be diterpene lactones (e.g. Andrographolide) or triterpenoids (e.g. Amarogentin). Some of the bitter principles are either used as astringents due to the presence of tannic acid, as antiprotozoan, antifungal or to reduce thyroxine and metabolism (Anokwuru *et al*., 2011).

The quantified phytochemicals revealed variation with respect to the distinct plant extract, where it was observed that *Ocimum canum* had the highest quantities of alkaloid, flavonoids, saponins, phenols, tannins as well as steroids. This could be as a result of environmental factors or post-harvest storage and processing methods of the plant.

In this study, the radial mycelial growth of all the isolated fungi was found to be significantly inhibited by the plant extracts although, the efficacy of the extracts was found to vary with concentration. This indicated that *Ambrosia maritiama, Ocimum basilicum, Ocimum canum* and *Moringa oleifera* had fungicidal potential recording 100% mycelia growth inhibition whereas *Adansonia digitata, Ocimum gratissimum* and *Vernonia cinerea* had fungistatic effect within the period of observation. It was further revealed in the study that *Ocimum canum* performs better than the positive control even at lower concentration which could be as a result of higher quantities of the major phytochemical compound it possessed. It was further revealed in this study that the higher the quantities of phytochemicals, the higher the inhibitory effect and vice versa.

These findings agree with the findings of Ana *et al*. (2023); Tripathi *et al*. (2023). Antifungal efficacy of *Adansonia digitata* extract from this study is in conformity with the findings of several researchers. Lagnika *et al*. (2012) reported the antifungal efficacy of *Adansonia digitata* against *A. flavus, Aspergillus niger, Aspergillus nudulans, Aspergillus parasiticus, and Aspergillus terreus*.

Amrish and Vinod (2015) also reported the antifungal potential of *Adansonia digitata* extract against *Aspergillus niger*, *Aspergillus clavatus*, *Candida albicans.* Similarly, Omkar *et al*. (2023) also reported the antifungal properties of *Adansonia digitata* leaf extract against *Ampelomyces quisqualis, Aspergillus flavus, Aspergillus niger, Drechslera avenaceum, Fusarium* oxysporum, *Fusarium solani* and *Trichoderma viride*. Abdelgaleil *et al*. (2011) reported the antifungal action of *Ambrosia maritiama* against *Botrytis cinerea* and *Fusarium oxysporum*. Ahmadu *et al.* (2021) reported the antifungal efficacy of *Moringa oleifera* leaves extract on *Botrytis cinerea*. Antifungal activity of *Moringa oleifera* leaf extract was reported against *Saccharomyces cerevisiae*, *Candida tropicalis*, *Apergillus niger* and *Aspergillus flavus* (Pinal *et al*., 2014).

The efficacy of methanol leaf extract of *Moringa oleifera* was also reported by Sonali *et al*. (2023) against *Fusarium oxysporum*. Margaret *et al*. (2021) also reported the *in vitro* efficacy of *Moringa oleifera* methanol extract against *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* sp., *Rhizopus* sp., and *Trichoderma* sp. Juliana *et al*. (2022) reported the antifungal efficacy of *Ocimum gratissimum* extract against *Aspergillus flavus*, *Rhizopus* sp, *Fusarium solani* and *Aspergillus niger*. Chimeka *et al*. (2020) also reported the antifungal potentials of *Ocimum gratissimum* extract against *Aspergillus niger, Aspergillus flavus, Galactomyces candidum, Trichoderma viridae, Rhizopus delemar, Fusarium solani* and *Penicillium chrysogenum*.

Antifungal activity of *Ocimum canum* was also in conformity with the findings of many researchers. The antifungal efficacy of *Ocimum canum* extract is in conformity with the findings of Manoranjan *et al*. (2018) who reported its efficacy against *Trichoderma* sp., *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp and *Penicillium* sp. Similarly, Kumar *et al*. (2023) also reported the efficacy of *Ocimum canum* extract against storage moulds viz. *Alternaria alternata*, *Aspergillus candidus*, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, Aspergillus paradoxus, Aspergillus terreus, Aspergillus versicolor, Cladosporium cladosporioides, Curvularia lunata, Fusarium nivale, F. oxysporum, Penicillium chrysogenum* and *Trichoderma viride*. The antifungal efficacy of *Ocimum basilicum* was in agreement with the findings of some researchers.

Kafeel *et al*. (2016) reported the efficacy of *O. basilicum* methanolic extract against *Aspergillus* sp. and *Curvularia* sp. Akpo *et al.* (2023) also reported the antifungal potentials of *O. basilicum* against *A. niger*, *A. flavus*, *B. cinerea*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Fusarium solani*. Antifungal activities of *Vernonia cinerea* conforms with the findings of Liendo *et al*. (2021) who reported the efficacy of *Vernonia cinerea* extract against *Aspergillus niger* and *Fusarium oxysporum*.

Biological control is generally favoured as a method of plant diseases management because it does not have demerits of chemicals (Ana *et al*., 2023; Tripathi *et al.,* 2023). Kuhn and Hargreavez (1997) observed that substances found fungicidal *in vitro* in almost all cases kill the fungi *in vivo*. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Tripathi *et al*., 2023). They still remain the principal source of pharmaceutical agents used in orthodox medicine (Meena *et al*., 2018**).**

In conclusion, this study revealed nine (9) phytochemicals associated with the selected plant leaf methanol extracts with *O. canum* having the highest concentrations of Alkaloids, Flavonoids, Saponins, Phenols, Tannins and Steroids; while, *A. maritiama*, *O. basilicum* and *M. olifera* had the highest concentration of Carbohydrates, Tritipenes and C/glycosides respectively. There is significant variation (p<005) in the effect of leaf methanol extract of the selected plants and Mancozeb (20 mg/ml) on the mycelial growth inhibition of isolated fungi species, with *O. canum* having high efficacy against four fungi species studied recording 100% mycelial growth inhibition. Investigations into the mechanisms by which *O. canum* extract inhibits the growth of *A. flavus*, *A. niger*, *F. oxysporum*, and *F. solani* can aid in developing new antifungal agents. Further isolation and characterization of bioactive compounds of *O. canum* is highly recommended since it is the most active in terms of the antifungal activity. Plant selection plays a crucial role in natural product development, considering the variations in phytochemical compounds across different plant species, studying *A. maritiama*, *O. basilicum*, and *M. olifera* can uncover the potential uses of their abundant carbohydrates, triterpenes, and C/glycosides, respectively, in medicine, cosmetics, or functional foods.

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