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Original article

PHYTOCHEMICAL, ELEMENTAL ANALYSIS AND ANTIFUNGAL ACTIVITY OF ERIGERON FLORIBUNDUS (BILBAO FLEABANE) LEAF EXTRACT AGAINST MALASSEZIA FURFUR

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Abstract

Background: Elemental composition and phytochemical content of plants viz; saponins, flavonoids, alkaloids, tannins, polyphenols, terpenoids and their concentrations are potentials for their antifungal or antimicrobial activity.
Aim: This study is aimed at evaluating the phytochemical, elemental composition and antifungal activity of the essential oil and dichloromethane extract of *Erigeron floribundus* (Kunth) Sch.Bip., against *Malassezia furfur* - the fungi that causes *Pityriasis versicolor* (eczema).
Method: Assays for the antifungal activity of were performed using the agar dilution method at serial concentrations ranging from 2 to 0.06 mg/ml. Phytochemicals were determined using suitable Official Analytical (AOAC) methods.
Results: The essential oil of *E. floribundus* was the most potent, inhibiting the fungi at 0.25 mg/ml (IZD = 27 mm), while the dichloromethane extract inhibited the organism at 0.5 mg/ml (IZD = 25 mm), showing a better activity than the control antifungal, clotrimazole which showed an MIC of 1 mg/ml and an inhibition zone diameter (IZD) of 18 mm. Saponins showed the highest concentration of 900 mg per 5 g in the phytochemicals analyzed; with tannins showing the lowest of 56.6 mg per 5 g. The concentrations of carbon, nitrogen, magnesium, sodium and Sulphur were 68.7 mg per 100 g, 49.4 mg per 100 g, 324.5 ppm, 78.50 ppm and nil respectively.
Conclusion: The essential oil and dichloromethane extract of *E. floribundus* revealed a good potential as an antifungal agent against *M. furfur* based on their minimum inhibition concentration, comparing favorably with the conventional antifungal agent used as control in this study. Other phytochemicals in the plant may also contribute to its use as traditional treatment of eczema/skin disorders.

INTRODUCTION

Malassezia furfur (*Pityrosporum ovale*) is a lipophilic yeast-like fungus that causes *Tinea versicolor* (*Pityriasis versicolor* i.e eczema) a common superficial fungal infection [1]. It is a common skin commensal that can exist as both a yeast and a mold (a dimorphic fungus). The skin of the human chest, back, and scalp are rich in fatty acids providing *Malassezia furfur* the required source of exogenous lipids for its growth [2]. It is normally a harmless component of normal skin flora but that in some people, causes *Tinea versicolor*. Most affected people are healthy. Factors that may predispose to *Tinea versicolor* include heat and humidity and immunosuppression due to corticosteroids, pregnancy, under nutrition, diabetes or other forms of disorders. Hypopigmentation in *Tinea versicolor* is due to the inhibition of tyrosinase caused by *Malassezia furfur* production of azelaic acid [3].

Tinea versicolor (Pityriasis versicolor i.e eczema) is a skin infection with *Malassezia* that manifests as multiple asymptomatic scaly patches varying in colour from white to tan, and brown to pink [3]. Diagnosis is based on clinical appearance and potassium hydroxide wet mount of skin scrapings. Treatment is with topical or sometimes oral antifungals. The occurrence of fungal infections is increasing at alarming rates, especially among immune-compromised subjects, such as AIDS patients, transplanted patients and neonates [4]. Among the pathogens, species of *M. furfur* are generally associated with these infections, whose incidence is attributed to a variety of factors, either exogenous or endogenous.

Actually, conventional treatments for fungal infections are not fully effective, since the available drugs lead to secondary effects or to development of resistance [4]. Therefore, the search for new drugs and alternative therapies (including natural products) for the treatment of *M. furfur* infections has become critical. In this aspect, plants and their derivatives have been contributing to pharmacological research due to their potential as source for a variety of biologically active ingredients used in drugs has been identified in some species, however, it should be taken into consideration that besides its beneficial effects, the use of plants may interface with conventional treatments by interaction with drugs, thus potentiating or minimizing clinical efficacy [4]. These biologically active ingredients in plants used in drugs formulation contains phytochemicals; organic compounds manufactured by plants through primary/secondary metabolism. These include alkaloids, phenols, saponins, flavonoids, tannins, phytates, etc. [5]. Antifungal activity exhibited by *Erigeron floribundus* may be attributed to the presence of these secondary metabolites [6].

This study seeks to establish that essential oil and dichloromethane extracts from *Erigeron floribundus*, an herbaceous plant growing in tropical areas and can reach a height of 2m. The species belongs to the family of *Asteraceae*. The leaf is simple, linear, pubescent and dentate. The inflorescences are yellow panicles with dry indehiscent fruits, can inhibit growth of *Malassezia furfur* and provide alternative cure for *Pityriasis versicolor* (eczema).

Terpenoids are very important in attracting useful mites and consume the herbivorous insects [7]. Plants having alkaloids are used in medicines for reducing headache and fever. These are attributed for antibacterial and analgesic properties [8].

The beneficial and side effects of the use of this plant in treating skin disorders prompts the analysis of phytochemicals such as flavonoids, tannins, alkaloids, phenols, saponins and terpenoids present. Among these groups of compounds, the essential oils and flavonoids can be incriminated in the antifungal activity of *Erigeron floribundus (Kunth) Sch.Bip.* [9].

Several studies have reported elemental contents in plant extracts, which are consumed by man either as an herbal health drink or medicine [10]. These elements are present at varying concentrations in different part of the plants, especially in roots, seeds and leaves which are used as dietary items as well as ingredient in traditional medicinal preparations.

Considering the importance of trace elements in various human metabolic processes and also considering their curative properties, in the present study, we applied one of the sensitive analytical techniques like Atomic Absorption Spectroscopy (AAS) to study the elemental contents in *Erigeron floribundus*. The overall impact of these essential elements on human health especially as it relates to skin infections is also discussed. Thus, elemental analysis of this plant is carried out to determine the concentrations of Sulphur, magnesium, sodium, carbon and nitrogen present in the plant.

In literature, it has been abundantly established that the plant is effective in the treatment of skin disorders by the rural populace as well as those from the urban areas [9]. The essential oils, dichloromethane and aqueous extract from *E. floribundus* have been investigated for their antimicrobial properties against a variety of fungal and bacterial infections. Studies have shown the use of the essential oils from *E. floribundus* to inhibit the growth of a wide range of dermatophytes including *Epidermophyton floccosum, Microsporum caris, M. gypseum, M. langeronii, Trichophyton mentagrophytes, T. rubrum, I. soudanense* and a filamentous fungus, *scopuariopsisbrevicaulis*. In another study, the *E. floribundus* oil showed a good activity against *Staphylococcus aureus*, HCT 116 colon carcinoma cells and *Trypanosomia brucei* including *Candida albicans*.

The use of essential oil dichloromethane, methanol and aqueous extract of *E. floribundus* has been employed to inhibit growth of bacteria and fungi of human origin. Petrelli [11] showed in a study that the essential oil of *Erigeron floribundus* showed a good activity against *Staphylococcus aureus* with inhibition zone diameter, IZD of 14mm, Minimum Inhibitory Concentration (MIC) of 512µg/ml. The study also investigated the inhibitory effect of *E. floribundus* essential oil on nicotinate mononucleotide adenylyl transferase (NadD), a promising new target for developing novel antibiotics and *Trypanosoma brucei*, the protozoan parasite responsible for Human African Trypanosomiasis. The essential oils also exhibited strong cytotoxicity on HCT 116 Colon Carcinoma cells. The IC_{50} and 14.89µg/ml, 33.5µg/ml and 14.89µg/ml for NadD, *T. brucei* and HCT 116 colon carcinoma cells respectively.

The leaves of *E. floribundus* (Kunth) Sch.Bip. (Asteraceae) are used by some traditional healers in West Africa in HIV/AIDS therapy. Yapo [12] in this study established that the plant extract dose tested, induced a significant increase in neutrophils (< 0.001) total lymphocytes and TCD4 + (P<0.0001) in rabbit blood and thus provides some justification for its use in the traditional treatment of AIDS.

E. floribundus is a reputed medicinal plant used in Cote d'Voire, West Africa for the treatment of skin disorders [9]. In the study, antifungal activity of *E. floribundus* showed that only the dichloromethane extract exhibited an activity against *Microsporuncanis* and a broad spectrum of good antifungal activity against all the remaining fungi tested; including *Epidermophyton floccosum, M. gypseum, M. lanheronii, Trichiphyton mentagrophytes, T. rubrum, T. soudanense* and one strain of the filamnentous fungus, *Scapulariopsisbrevicaulis.* The assays were performed using the agar dilution methods at serial concentrations ranging from 2 to 0.06mg/ml.

The role of sulphur, magnesium, nitrogen and sodium in medicine is well known for their effect in most drugs which have antifungal, anti-inflammatory, anti-carcinogenic and anti-oxidant properties. There is however, no literature on the mineral content of the plant, an aspect that this research seeks to provide information on.

The role of some inorganic elements like vanadium, zinc, sodium, potassium, magnesium, aluminum, iron, nickel, cadmium, copper, cobalt and manganese in the improvement of impaired glucose tolerance and their indirect role in the management of diabetes mellitus, hypoglycemic, wound healing and anti-inflammatory effects are being used, which contain both organic and inorganic constituents. In the study, carried out by Narayanan [13] an attempt was made to analyze the inorganic elements present in *Aloe vera* leaf gel. The concentration of various element; potassium, magnesium, sodium and zinc in the sample was more than 200 mg. The concentration of the other elements analyzed in the sample decreased in the order Fe>Al>V>Cu>Mn>Pb>Ni>Co>Cd. Sodium and potassium were estimated by a flame photometer. The rest of the elements were analyzed using atomic absorption spectroscopy (AAS- varion200AA).

Lokhande [14] in their study on mineral content of some ayurvedic Indian medicinal plants by instrumental neutron activation analysis and AAS techniques, reported that many ayurvedic preparations appear to demonstrate significant success in treatment and cure of complex diseases. The elements Cr, Ca, Cd, Ni, Pb and Hg were analyzed by AAS technique by measuring the absorbance of the species at its resonance wavelength.

The aim of this study is to determine and establish the Minimum Inhibitory Concentration (MIC) and The Minimum Fungicidal Concentration (MFC) values of the essential oil, dichloromethane and aqueous extract of the leaves of *Erigeron floribundus* against *Malassezia furfur* (the fungi that causes eczema) and to determine the concentration of some phytochemicals (flavonoids, Saponins, phenols, alkaloids and terpenoids) and elements (Sulphur, carbon, nitrogen, magnesium and sodium) present in the plants.

MATERIALS AND METHODS

Plant Materials

The leaves of *E.floribundus* (Kunth) Sch.Bip. were harvested by one of the authors (Mr. Alfred Enang) in Iyamoyong, Obubra Local Government Area of Cross River State, Nigeria in June 2019. The plant was identified at the herbarium of the Department of Botany, University of Calabar by Dr. Effa Anobeja Effa. The plant was dried at 30-40^{0}C using a mamart thermostatic oven model (F-Nr; C508.0270, type: UNB500). It was then allowed to cool and crushed into powder, using a manual blender model (F-NO1Quaker City Mill Philadelphia PA. USA) and was packaged, ready for extraction, phytochemical and elemental analysis. These analyses were carried out both in Nigeria and India where necessary, some in Jawaharlal Nehru Centre for Advance Scientific Research, Jakkur, Bangalore, India.

Fig. 1: *Erigeron floribundus* (Bilbao fleabane) plant.

Preparation of Plant Extract (Solvent Extraction)

The powdered dried sample (100g) was extracted successively with five times their weight of water and dichloromethane at room temperature for 15 hours. The filtrate was evaporated on a rotary evaporator (40^{0}C) to yield the crude extracts. For the aqueous extracts, 50g of the powder in 250ml distilled water were boiled for 15 minutes. Each of the extracts was lyophilized and weighed.

Steam Distillation (Essential Oil Extraction)

Powdered portion of plant material (500g) were poured into the steam with a heating mantle. Pressurized steam was injected from the mantle through the plant material, releasing the plants aromatic molecules and turning them into vapour. The vapourized plant compounds travel to the condenser where two separate pipes make it possible for hot water to exit and the cold water enter the condenser. This makes the vapour

cool back into liquid form. The aromatic liquid by-product drops from the condenser and collects inside a receptacle underneath it. The essential oil being lighter than water, then float to the top and is siphoned off. Once obtained, the oil was transferred into a glass flask and kept at -20^{0}C for biological experiments.

Phytochemical Analysis

Alkaloid Determination

Alkaloid content of *E. floribundus* was obtained by weighing 5 g of the powered sample into a beaker. 100 cm^3 of 100% acetic acid in ethanol (1:1 ratio) was measured into the sample container and covered to stand for 4 hours. The extract was filtered after four hours. It was then concentrated using water bath to a quantity of the original volume. Ammonia solution was added to the concentrated sample extract drop wise until the precipitate was complete. The precipitate was allowed to settle, then filtered and washed with dilute ammonium hydroxide. The residue left was taken as the crude alkaloid. It was then dried in an oven and weighed. The difference between the weight of the empty beaker and that of the beaker plus residue was recorded [15].

Flavonoid Determination

In determining the crude flavonoid of *E. floribundus*, the method of Bohm and Kocipai-Abyazan [16] was followed thus: Exactly 50 cm^3 of 80% aqueous methanol was added to 5.0 g of sample in a 250 cm^3 beaker, covered and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of each plant sample. Each plant sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed to a constant weight.

Saponins Determination

In determining the content of saponins in *E. floribundus*, the method reported by Obadoni and Ochuko [17] was followed thus: 5 g of the sample powder was dispersed in 50cm^3 20% ethanol in a beaker. The suspension was heated over a hot water bath for 4 hours with a continuous stirring at about 60^{0}C. The mixture was filtered after 4 hours and the residue was re-extracted with another 25 cm^3 of 20% ethanol. The combined extract was concentrated to reduce to 40 cm^3 over the water bath at 90^{0}C. The mixture was transferred into a separatory funnel and 20cm^3 of diethyl ether was added and shaken thoroughly. The aqueous layer of the extract was recovered while the ether layer was discarded. The purification process was repeated and 60 cm^3 of n-butanol was added and the extract was washed twice with 10cm^3 of 5% aqueous sodium chloride. The remaining extract was evaporated in a water bath and dried in an oven to a constant weight.

Extraction of Crude Terpenoids

The crude terpenoids of *E. floribundus* was obtained by soaking 5 g of the powered material in 50 ml of 95% ethanol for 24 hours. The extract was filtered and the filtrate extracted with petroleum ether (boiling point 60^{0}C to 80^{0}C) and concentrated to dryness. The dried ether extract was treated as crude terpenoids [18].

Determination of Total Polyphenols

Total polyphenol content was estimated using Folin-ciocalteu (Fc) assay which is widely used in routine analysis. A known amount of aqueous extract (10 mg/ml) was mixed with 1.0ml of Fc reagent and 0.8 ml of 2% $Na_2CO_3$was made up of 10ml using water-methanol (4:6) as diluting fluid. Absorbance was read at 740 nm after 30 minutes using spectrophotometer. Tannin acid (0-800mg/L) was used to produce standard calibration curve. The total phenolic content was expressed in mg of tannic acid equivalent (TAE)/100 g sample [19].

Tannins Determination

Tannins content of *E. floribunndus* was determined by weighing 5 g of the sample into a plastic bottle and 50 cm^3 of water was added and shaken for 1 hour in a shaker. It was then filtered and 3cm^3 of the extract was measured into a test tube and mixed with 3 cm^3 of 0.1M $NH_4Cl(aq)$ and 3 drops of ferrocyanide. It was allowed to stand for 10 minutes, then measured in the UV-Vis spectrometer at 605 nm [15].

Elemental Analysis

Magnesium analysis

The concentration of magnesium present in the plant was determined by digesting 2.0 g of the sample in powdered form with mixture of nitric acid, hydrochloric acid and perchloric acid in the ratio 9:4:2 respectively. The digested sample was diluted suitably with 100 ml deionized water and filtered. The digested and filtered solution was then used for analysis of the elements by Atomic Absorption Spectrophotometer (AAS-Perkin Elmer 3100 model) using suitable hollow cathode lamps. The procedure above was according to Sobukola [20] and Akpe and Ubua [21] with modification.

Sulphur analysis

Sulphur was determined by turbidimetric determination of sulphate in plant digest using Bausch and Lomb spectronic 70, following the method of Chaudhum [22] with some modification thus: Ten millilitres (10 ml) of the sample aliquot was pipetted into a 25 ml volumetric flask. Distilled water was added to make the volume approximately 20 ml. 1 ml of gelatin-$BaCl_2$ reagent was added and made to mark with distilled water. The content was then mixed thoroughly and allowed to stand for 30 minutes. The Percentage Transmittance (%T) and Optical Density (O.D.) were determined at 420 nm within 30 minutes on a B & L Spectronic 70-electrocolorimeter. The content was shaken in the flask before pouring into the photo-test tube.

3.5.3 Carbon analysis

An ASTM (e777-08) standard test method was used for the analysis of carbon in the plant sample as reported by [Jenkins [23]. The determination is made by burning the sample to convert all of the carbon to carbon dioxide. 0.2 g of the plant sample was weighed to the nearest 0.1 mg into a combustion boat. The combustion (850 to 900 ^0C) is carried out by high purity oxygen. The carbon dioxide is then recovered in an absorption train. The absorbers were then removed to the vicinity of the balance, allowed to cool to room temperature for 15 to 20 minutes, vented momentarily to the atmosphere, wiped with a lint-free cloth in the areas handled and finally weighed to the nearest 0.1 mg. The combustion tube packing is used to remove any interfering substances. This test method gives the total percentage (weight percent) of carbon in the plant sample analyzed.

Nitrogen Analysis

The ASTM (e778) standard test method was used for the analysis of nitrogen in the plant sample as reported by [Jenkins [23]. In the acid test method adopted for this analysis, the N_2 in the sample is converted into ammonium salts by destructive digestion of the sample with a hot, catalyzed mixture of concentrated H_2SO_4 and K_2SO_4. The salts were subsequently decomposed in hot alkaline solution from which the ammonia is recovered by distillation and finally determined by acidimetric titration. 1 g of the finely grinded plant sample was weighed into a weighing scoop and the sample was carefully transferred into a 500 ml Kjeldahl flask containing 7 to 10 g of K_2SO_4 and 0.8 g of mercury. iii. 30 ml of H_2SO_4 (Sp. Gr. 1.84) was added to the mixture rotating by the flask to wash any sample adhering to the walls into the mixture. v. The content was heated to boiling, with the heat input controlled in a manner that the H_2SO_4 vapours condenses no more halfway up the neck of the flask. The digestion was continued until all sample particles are oxidized, evidences by a nearly colourless solution. A few drops of $KmnO_4$ were added to ensure complete oxidation. The solution was heated further to destroy the excess permanganate, leaving a decolorized solution. The cooled digestion mixture was diluted to about 300 ml with water and the heat of dilution removed by cooling the flask. 20 ml of H_3BO_3 solution was added into a 250 ml Erlenmeyer flask and 6 drops of mixed indicator solution was added. With the distillation apparatus set up, the ammonia collected in the Erlenmeyer flask containing the H_3BO_3 was titrated to the mixed indicator end point using 0.2 N H_2SO_4 as the titrant. A blank

determination was run in the same manner as above, using 1 g (weighed to the nearest 1 mg) of sucrose as the sample material.

The nitrogen in the sample was calculated as follow:

$$\text{Nitrogen \%} = \frac{(A - B)\ N\ X\ 0.014}{C} X\ 100$$

Where:

A = ml of H_2SO_4 required for titration of the sample
B = ml of H_2SO_4required for titration of the blank
N = Normality of H_2SO_4
C = grams of sample used
0.014 = milliequivalent weight of nitrogen

Sodium analysis:

Sodium was analyzed by flame photometry following the method reported by Banerjee and Prasad [24] thus: The FP8800 Flame Photometer (A. KRUSS Optronic Model) was calibrated using a standard solution of sodium (in ppm range). Fifty millilitres (50 ml) of the sample extract was poured into the sample holder of the photometer for analysis. The photometer gave a yellow colour flame at 589 nm. The concentration of sodium in the sample was determined form the instrument's calibration curve corresponding to the emission intensity of sodium in the sample.

Antifungal Analysis

The organism was obtained as clinical isolates from the skin scrapings of the affected area of patients from Primary Health Centre, Iyamoyong, Obubra Local Government Area of Cross River State, using a surgical blade, into a sterile bottle containing a mycological saline (peptone water) to keep it alive prior to culturing.

Sabouraud dextrose agar, used as media was prepared according to manufacturer's directions. All glass wares were washed with detergents and properly rinsed with water and then sterilized by autoclaving before use.

The sample was serially diluted using peptone water as diluent. 9 ml of peptone water was introduced into test-tubes aseptically and sterilized. After cooling of the peptone water, one gram of the sample was therefore weighed into the first test-tube labelled 10^{-1} and a four-fold serial dilution was carried out accordingly. One ml of the diluted sample was pour plated in triplicate on Sabouraud dextrose agar supplemented with olive oil. Plates were incubated at 37^0C for 72 hours in an incubator. After 72 hours, discrete colonies were counted and further sub-cultured into fresh prepared media of Sabouraud dextrose agar to obtain a pure culture of the test organism. Morphological cultural characteristic of the organism noted, confirmed it to be *Malassezia furfur*.

From the stock cultures, which was maintained on SDA slants, colonies were transferred into Sabouraud dextrose broth using sterile inoculating wire loop and incubated at 37^0C for 24 hours, for each test organism.

The overnight broth culture was diluted appropriately by gradually adding normal saline to it and the density of the inoculums, standardized by comparing with 0.5 McFarland standard of barium sulphate solution.

The antifungal sensitivity testing of the plant extract was performed using agar well diffusion method on Muller Hinton agar. Clotrimazole 1% was used as the control antifungal. The medium was prepared, sterilized, poured into petri dishes and allowed to solidify. 1.00ml cultures of each test organism from the standardized inoculants was then inoculated onto the Muller Hinton agar plates aseptically and ensured that it was evenly distributed. Then using a sterile cup borer, agar wells were created and different concentrations of the plant extracts and the control, serially diluted in dimethyl sulfoxide (DMSO) were introduced into the perforated agar and control plates respectively. The plates were allowed to stand for 30 minutes at room temperature before incubation at 37^0C for 48 hours. Zones of inhibition (diameter) formed on the medium were measured with a transparent metre rule and expressed in millimetres for both plant extracts and the control.

The minimum inhibitory concentration (MIC) is defined as the lowest concentration that produced no visible fungal growth after the incubation time. The MIC of the plant extracts and essential oils on test fungi was carried out using tube dilution technique. 1.00 ml standardized suspension of the test fungi was inoculated

into a series of tubes of Sabouraud dextrose broth. The plant extracts and essential oils were dissolved in dimethyl sulfoxide (DMSO) and diluted to give two-fold serial dilutions that were incorporated into growth medium (tubes). The resulting concentrations ranged from 2 to 0.06 mg/ml. The tubes were incubated in duplicate at 37^0C for 24 – 48 hours. MIC was then read after incubation as the least concentration of the plant extracts and essential oils that inhibited the growth of the test fungi using turbidity as an index.

The minimum fungicidal concentration is defined as the lowest concentration where no fungi growth is observed on the fleshly prepared plates. This was determined by first selecting tubes that showed no growth during MIC determination. A loop-full from each tube was then sub-cultured onto agar plates and incubated at 37^0C for 24-48 hours. Therefore, the least concentration of the MIC test at which no growth in the subcultures plates was observed was noted as the minimum fungicidal concentration (MFC).

RESULTS AND DISCUSSION

Phytochemical Composition

The results of the analysis of the phytochemical composition of *E*. *floribundus* are presented in table 1 below.

Table 1 Phytochemical concentration in *Erigeron Floribundus*

Phytochemical	Concentration(mg/5 g)
Saponins	900±5.0
Flavonoids	400±3.0
Tannins	56.6±1.4
Polyphenols	107.8±2.2
Alkaloid	100±2.0
Terpenoids	200±3.0

Values reported in Mean ±S.D. Format with N=3.

The study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoids, flavonoids, alkaloids, tannins and essential oils were present in the plant sample, corroborating the report by Trabi [25] on the presence of secondary metabolites in *E. floribundus*. This research is quite innovative, as it gives information on the concentrations of these phytochemicals in the plant. The result of the phytochemical analysis shows that the leaves were rich in flavonoids, saponins, alkaloids, terpenoids, polyphenols and tannins with saponins being the most abundant of them all.

The study showed that saponins have the highest concentration of 900 mg per 5 g of the sample representing 18% of the phytochemical content of the plant. Saponins have been reported for their ability to kill disease-causing bacteria, scavenge oxidative stress and inhibit tumor growth. That is why they are used in some medications [39]. Their antibacterial and foaming properties informs their addition to shampoos, soaps, household cleaners and make-up products [26].

Flavonoids concentration in the plant under study is 400 mg per 5 g, representing 8% of the phytochemical content of the plant. Flavonoids have been shown to have direct antibacterial activity, synergistic activity with bacteria virulence factors in numerous in vitro and a limited number of in-vivo studies [27]. These compounds in plants are reported to afford protection against ultraviolet radiation, pathogens and herbivore [28].

The study showed that tannins have the lowest concentration in the plant. Tannin contents in the plant was 56.6 mg per 5 g representing 0.9% of the phytochemicals analyzed. Tannins have been reported for their wound healing properties, anti-inflammatory, analgesic and antioxidant properties. Tannic acid and propyl gallete have been found to inhibit food borne bacteria, aquatic bacteria and off-flavour-producing micro-organisms. Their antimicrobial properties seemed to be associated with the hydrolysis of ester linkage

between gallic acid and polyols, hydrolyzed after ripening of many edible fruits. Tannins in these fruits serve as natural defense mechanism against microbial infection [29].

The amount of polyphenols in the plant studies was found to be 107.8 mg per 100 g representing about 2% of the phytochemical content of the plant. Polyphenols have been reported to be one of the most numerous and diverse group of secondary metabolites, their anti-antioxidants properties provide the basis for antimicrobial effects.

Alkaloids concentration in the plant under study was found to be 100 mg per 5 g. Plants having alkaloids are used in medicines for reducing headaches and fever. These are attributed for antibacterial and analgesic properties [30]. Alkaloids are the active components of numerous medicinal plants or plant-derived drugs and poisons and their structural diversity and different physiological activities are unmatched by any other group of natural products [31].

The concentration of terpenoids in the plant studied is 200 mg per 5 g of the plant sample. Terpenoids are reported to have anti-inflammatory, anti-viral, anti-malarial, anti-bacterial properties. They are also found to inhibit cholesterol synthesis [32].

Mineral Composition

The results of the analysis of the mineral content of *E. floribundus* are presented in Table 2.

Table 2 Mineral contents of *Erigeron floribundus*

Elements	Concentration
Carbon	68.7±1.5 mg/100g
Nitrogen	49.4±1.2 mg/100g
Magnesium	324.5±2.0 ppm
Sodium	78.50±1.5 ppm
Sulphur	ND

ND = Not Detected, Values reported in Mean ±S.D. Format with N=3.

In the present study, five elements were analyzed viz; Sulphur, magnesium, nitrogen, sodium and carbon (Table 3). Carbon and nitrogen were found to have the highest concentration respectively. The concentration of magnesium was observed to be higher than that of sodium, while, and quite curiously, Sulphur was not detected in the plant under study. The concentration of carbon was found to be 68.7 mg per 100 g representing 6.87% followed by nitrogen with a concentration of 49.4 mg per 100 g representing 4.94% of the mineral content of the plant.

The content of magnesium was found to be 324 ppm while sodium had the lowest concentration with 78.50 ppm. Each element is important in the structural and functional integrity of the living cells. Presumably, *Erigeron floribundus* contains trace elements in a bioavailable form and their impact on the overall pharmacological action cannot be ruled out.

Carbon plays an important role in the physiology of the human body. It is the basis of protein, fats and nucleic acids in the human body [12]. The pharmaceutical industry uses nitrogen gas mixture for lung function test. The medicinal gas is also used for cryosurgery removal of some cancers and skin lesions and also for the storage of tissue, cells and blood in cryogenic temperatures to avoid oxidation of the sample.

Magnesium is one of the major minerals which is related to the carbohydrate and fat metabolism. It is a mineral that is important for normal bone structure in the body. It is also required for the proper function of nerve muscles and other parts of the body.

Sodium is essential for regulation of osmotic pressure of the body and helps to maintain acid-base and water balance of the body. Its deficiency causes loss of body weight and nerves disorder. In foods, sodium chloride is used to add taste and preserve food [34]. Although sulphur was not detected in the plant under study, it has been established that sulphur have antibacterial effects against bacteria that causes acne. It is applied to the skin for acne, hay fever, skin redness, dandruff, scaly and red skin patches, an itchy skin

infection caused by mites (scabies), lice, cold sores, warts and poison oak, ivy and sumac infections. It also helps to promote the loosening and shedding of skin [35].

Even though the direct link between the essential elemental content of medicinal plant and their curative capacity is not yet established, the experimental data of the present work will be of immense importance in the synthesis of new drug formulations. Further, the data obtained on individual element concentration in *Erigeron floribundus* will be useful in deciding the dosage of herbal drugs prepared from this variety, for the management of skin infections and other related disorders.

Antifungal Activity

The results of the antifungal analysis of the essential oil, dichloromethane and aqueous extracts of *E. floribundus* against *M. furfur* are presented in Table 3 below.

Table 3. Antifungal activity of *Erigeron floribundus* against *Malassezia furfur*

Extract	MIC(mg/ml)	MFC(mg/ml)	IZD (mm)
Essential oil	0.25±0.01	0.5±0.05	27±0.1
Dichloromethane	0.50±0.01	0.5±0.05	25±0.1
Aqueous	ND	ND	ND
Clotrimazole	1.0±0.1	2.0±0.1	18±0.1

ND = Not Detected, Values reported in Mean ±S.D. Format with N=3.

The antifungal activity investigation revealed that *M. furfur* was more susceptible to the essential oil than it was to the dichloromethane extract obtained from *Erigeron floribundus*. *Erigeron floribundus*is a medicinal plant commonly used in traditional medicine in Cote d' Ivoire and in other African countries to treat skin infections.

In this study, the dichloromethane extract was effective on clinical isolates of *M. furfur* with MIC value of 0.5 mg/ml while the essential oil was inhibiting the yeast growth at 0.25 mg/ml. Both the essential oil and dichloromethane extract showed a good antifungal activity against *M. furfur* than the reference anti-fungal clotrimazole, which inhibited the yeast at 1 mg/ml. No antifungal activity was observed for the aqueous extract. To the best of our knowledge, this is the first study reporting on the antifungal activity of the essential oil and dichloromethane extract of *E. floribundus* against *Malassezia fufur* - the causative organism of *Pityriasis versicolor* (eczema).

In previous studies however, Trabi [25] obtained MIC values of 0.25 mg/ml against the strains of *Epidermophyton floccosum*, *microsporum gypseum, M. largeronii, T. rubrum, T. sudanense* and *S. brevicaulis.* while Petrelli [11] obtained an MIC of 0.512 mg/ml against *C. albicans* from essentials oil of *E. floribundus*.

Phytochemical determination of *E. floribundus* yielded considerable amount of saponins, flavonoids, tannins, polyphenols, alkaloids, and terpenoids. The antifungal activity exhibited by this plant may be attributed to the presence of these secondary metabolites. The antifungal activity observed for the dichloromethane extract suggest that the active metabolites of *E. floribundus* are mostly lipophilic. The lipophilic nature of these compounds may be due to the presence of a phenyl chain. Therefore, these compounds can be extracted by non-polar solvents such as dichloromethane. The prenylated groups render the active molecules more lipophilic, inducing antimicrobial activity within interactions with cell membranes. This may probably explain the lack of activity observed for the aqueous extract which is polar [25]. The Inhibition Zone Diameter (IZD) of 27 mm, 25 mm was indicative of a good activity for the essential oil and dichloromethane extract respectively, while minimum fungicidal concentration (MFC) was 0.5 mg/ml for both the essential oil and dichloromethane extracts as against the control antifungal with an MFC of 2 mg/ml and IZD of 18 mm.

In summary, the essential oil and dichloromethane extract of *E. flobribundus* was found to show a good activity against the organism and had lower MIC and MFC values than the control antifungal-clotrimazole. The essential oil showed a Minimum Inhibitory Concentration (MIC) value of 0.25 mg/ml, a Minimum

Fungicidal Concentration of 0.5 mg/ml and an Inhibition Zone Diameter (IZD) of 27 mm. Moreso, the dichloromethane extract showed an MIC of 0.5 mg/ml and an MFC of 0.5 mg/ml against *M. furfur*, with an IZD of 25 mm indicative of a good activity; while the control-clotrimazole, was inhibiting the organism at 1.0 mg/ml, 2.0 mg/ml and 18 mm of MIC, MFC and IZD respectively

CONCLUSION

Erigeron floribundus in this study, proved to be rich in saponins, alkaloids, tannins, terpenoids, flavonoids and polyphenols. The anti-bacterial, anti-inflammatory, anti-viral and anti-fungal activities of this medicinal plant may be due to the presence of the above-mentioned secondary metabolites. The study also offers a scientific basis for the use of *Erigeron floribundus* in traditional medicine for treatment of skin infections and other related disorders. The essential oil of this plant and its dichloromethane extract have revealed good potentials as an antifungal agent against *M. furfur* and can thus, providing evidence for the treatment of eczema and other related skin infections.

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Conflict of Interest: The authors declare that there is no conflict of interest with the manuscript.

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