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Evaluation of Vitamin Contents, Antioxidant and Antimicrobial Activities of Different Leaf Extracts of *Taraxacum officinale* (Dandelion)

I. E. Daniel[1*], K. N. Mathew[1] and P. L. John[1]

[1]Department of Chemistry, University of Uyo, PMB 1017, Uyo, Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author IED designed the study, wrote the protocol and the first draft of the manuscript. Author KNM managed the literature searches and the experimental process. Author PLJ identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Methanol and ethyl acetate leaf extracts of *Taraxacum officinale* (dandelion) were evaluated for phytochemical compounds, vitamins, antioxidant and antimicrobial activities. Phytochemical compounds and vitamins were determined using standard procedures while antioxidant activity was determined using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing/antioxidant power (FRAP) Assay. Antimicrobial activity against various pathogenic bacteria and fungi were screened using disc diffusion method. The results indicated that the bioactive compounds (total phenol, flavonoids, saponins, tannins and alkaloids) determined quantitatively were present in appreciable concentration in both extracts. The result also showed that both extracts contain a variety of vitamins (A, B complex, C and E), with vitamins C and A having the highest concentration while the B-vitamins (B1, B2 and B3) and vitamin E were present in moderate concentrations. Both extracts showed significant scavenging and reducing ability comparable to the reference antioxidant, ascorbic acid in a dose dependent manner, with methanol exhibiting the highest scavenging and reducing capacity. The antimicrobial activity of both extracts showed appreciable

**Corresponding author: E-mail: imaudoekwere@gmail.com;*

broad spectrum activity against the pathogenic bacteria and fungi strains tested at various concentrations. Methanol extract was found to be most effective compared to ethyl acetate extract. These results indicated that the leaf extracts of dandelion possess antioxidant and antibacterial activity against the tested bacteria possibly due to the presence of bioactive compounds and other nutrients.

Keywords: Taraxacum officinale; ascorbic acid; disc diffusion method; flavonoids; saponins.

1. INTRODUCTION

Recently, there has been extensive research of plants with antioxidant and antibacterial properties. Numerous studies have shown that aromatic and medicinal plants are sources of diverse primary and secondary metabolites such as Phenolic compounds, flavonoids, tannins, saponnins, terpenoids, steroids, carotenoids and alkaloids which display antioxidant and antimicrobial properties such as protecting the human body against both cellular oxidation reactions and pathogens [1,2,3]. Free radicals and oxygen species such as, hydroxyl radicals, superoxide and other singlet oxygen are generated in the human body under physiological conditions. Highly active free radicals and their uncontrolled production are responsible for numerous pathological processes such as cell tumour (prostate and colon cancers) and coronary heart diseases [4,5,6,7,8]. Oxidative stress occurs when the production of reactive oxygen metabolites exceeds the capacity of the antioxidant system of the cell, tissue or body. Exposure of human beings as well as plants to the negative effect of excessive free radicals have led to the development of defense mechanisms such as utilizing antioxidants naturally generated in situ in the body (endogenous antioxidants examples glutathione peroxidase, catalase and superoxide dismutase) or externally supplied through foods and supplements as dietary components (exogenous antioxidants) [9]. Furthermore, studies have shown that there is decline in viability and potency of the human's antioxidants as individual ages [10,11]. However, endogenous antioxidants may not be sufficient to maintain optimal cellular functions under increased oxidative stress as a result of these, dietary antioxidants may be necessary. The main sources of exogenous antioxidants include food and medicinal plants such as spices, fruits and vegetables [12]. This protective effect of dietary or exogenous antioxidant is often attributed to different antioxidant components, such as vitamin C, vitamin E, carotenoids, polyphenolic compounds and other phytochemicals [13,14,12]. Several studies indicate that medicinal plants contain compounds that are significant in therapeutic application against human and animal pathogen, including bacteria, fungi and viruses [15,16].

Multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in treatment of infectious diseases. This has led to the search for new antimicrobial substances from various sources like medicinal plants [17]. Considerable interest in natural antioxidants and antimicrobial agents obtained from plant sources is because of the side effects and toxicity often associated with the use of synthetic antioxidants and antibiotics [18,19,20,21].

Among the nutrients, vitamins are essential compounds found in plant foods that perform specific and vital functions of the body. They may act as a cofactor necessary to catalyze a biological reaction, required for proper vision, involved in cellular metabolism, cell growth, differentiation and development [22].

Taraxacum officinale is an herbaceous perennial plant of the family *Asteraceae*, commonly called dandelion. This plant grows in temperate regions of the world and is found mostly on the roadside, on scattered banks, in lawns, on shores of waterways and other areas with moist soils [23]. *Taraxacum officinale* produces stems that are typically 5-40 cm tall, though in some cases it may grow up to 70 cm high. The foliage may be upright-growing or horizontally spreading. The plant is characterized by hairy stems, milky latex, and basal leaves with one single flower head. The flower heads are ligulate and bisexual. The leaves are obovate in shape with strident or gentle teeth [24].

Young dandelion leaves can be eaten fresh, the roots are roasted and used as an additive in the production of coffee, and dandelion extract is used as a flavor in various food products (alcoholic beverages, soft drinks, frozen dairy desserts, sweets, baked goods, jellies, puddings, and cheese) [25].

The plant is used for treatment of jaundice and disorders of the liver, gallbladder and various gynaecological diseases such as breast and uterine cancers [26,27]. A decoction of the whole plant may be used in treating diabetes and also as a laxative, digestive stimulant and diuretic [28,29,30,31]. Dandelion are rich sources of flavonoids, triterpenes, phytosterols, coumarins β-carotene, iron (Fe), calcium (Ca) and recommended as a natural source of vitamin C (Ivanov et al. 2018). Some studies have revealed that extracts of *T. officinale* possess antioxidant, hepatoprotective, anti-inflammatory, and antitumor activities [25,32,33,34].

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

The leaves of *Taraxacum officinale* were collected from Use offot village in Uyo Local government Area of Akwa Ibom State, Nigeria. It was authenticated by a botanist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State and the sample with voucher number UUH4067 was kept in the herbarium. The leaves were thoroughly washed with distilled water and air dried to constant weight at room temperature for 10 days. The air-dried leaves were ground to fine powder and then stored in an air-tight container until further use.

2.2 Preparation of Extracts

The powdered leaf was divided into two parts and extracted separately with methanol and ethyl acetate using cold maceration method at room temperature. After 72 hours, the plant extracts were filtered and then concentrated using rotary evaporator at 40°C, and each extract was transferred into well labeled sterile glass vials and stored at 4°C before use.

2.3 Determination of Polyphenols

The total phenolic content in the extracts were determined by the modified Folin-Ciocalteu method as described by [35] and modified by [36]. Sample extract was dissolved in methanol (1 mg/ml). An aliquot of 0.5 ml of each plant extract (1 mg/ml) was mixed with 5 ml of Folin-Ciocalteu reagent which was previously diluted with distilled water (1:10 v/v). The mixture was shaken slightly and allowed to stand at 22°C for 5mins. After, 4 ml (75 g/l) of sodium carbonate (Na_2CO_3) was added, and the tubes containing the mixtures were allowed to stand for 30 min at 40°C to develop colour. Absorbance was then read at 765 nm using the spectrophotometer. Results were expressed as Gallic acid equivalent in (mg/g) of extracts. All samples were analyzed in triplicate.

2.4 Determination of Total Flavonoids

Total flavonoid contents were determined using the method of [37]. A volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content were calculated as quercetin equivalent (mg/g).

2.5 Determination of Total Alkaloids

Total alkaloids were determined according to standard method as described by [38]. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.6 Determination of Total Tannins

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min [39].

2.7 Determination of Total Saponins

The samples were ground and 20 g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture

was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated [40].

2.8 *In vitro* Antioxidant Activities

2.8.1 Antioxidant activity by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) method

The free radical scavenging activity of the different extracts was measured *in vitro* by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH method) as described by [41] DPPH solution was mixed with sample solutions at different concentrations (20 - 100. µg/ml). A control (Abs Control) containing methanol and DPPH solution was also realized. All solutions obtained were then incubated for 1 hour at room temperature. Absorbance was measured at 517 nm. Vitamin C was used as standard and the same concentrations of it were prepared as the test solutions. The percentage of inhibition of samples was calculated from obtained absorbance by the equation:

% inhibition = ((Abs control-Abs test)/Abs control) × 100. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC_{50} value for each of the test solutions.

2.8.2 Ferric reducing/antioxidant power (FRAP) assay

The reducing property of the extract was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution [42]. Briefly appropriate concentrations of the extracts were mixed with 2.5 ml of 200 mM of sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixture was incubated at 50ºC for 20 min after which 2.5 ml of 10% trichloroacetic acid was added. The mixture was then centrifuged at 650 rpm for 10 min. Supernatant (The upper layer) (5 ml) was mixed with equal volume of deionized water and 1 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power [42]. Ascorbic acid was used as positive reference. The experiment was done in triplicate.

2.9 Antimicrobial Activity

2.9.1 Collection of test organisms

Microorganisms used were obtained from the microbial stock collection unit of department of microbiology, university of Uyo, Akwa Ibom State. The test organisms used were 1 Gram – positive bacterium (*Staphylococcus aureus*), 4 Gram –negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa, Salmonella spp, Vibrio cholerea*) and two fungi (*Candida albicans, Aspergillus niger*). These organisms were sub cultured to obtain pure and fresh isolates. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Isolates were identified using standard microbiological procedures by carrying out gram's reaction and biochemical tests to confirm the species.

2.9.2 Preparation of test organisms before inoculation

McFarland standard was used as a reference to adjust the turbidity of bacterial suspensions. The bacterial suspensions were standardized following the CLSI guidelines for aerobic bacteria. All of the test microorganisms were grown in Mueller Hinton broth for 18–24 h, followed by the matching of bacterial suspension to the turbidity equivalent to 0.5 McFarland solutions (1-2×108 cfu/ ml).Different concentrations (10, 20, 40, 60 and 80 mg/ml) of the extracts were prepared and kept in corked test tubes.

2.9.3 Seeding of muller – hinton agar plates

0.1 ml of each diluted isolates was aseptically transferred into Muller – Hinton agar (Oxoid, UK) plates and aseptically spread evenly using sterile Hockey stick. The seeded plates were left for 30 mins for the isolates to diffuse into the medium. Sterile cork borer of 5mm was used to bore holes on the agar plates. 0.1 mL of each of the extracts was then dropped in the holes and labeled accordingly. The diameters of inhibition zones were measured and antibacterial activity was

considered for diameters of inhibition zone greater than 9 mm [43].

3. VITAMIN ANALYSIS

Vitamins such as Thiamine, Riboflavin, Niacin and Ascorbic acid were estimated by the method described by [44].

3.1 Determination of THIAMINE (Vitamin B1)

About 50 ml of ethanolic sodium hydroxide and 5 g of the sample were homogenized and filtered in a 100 ml flask. To the 10ml of the filtrate, 10 ml of potassium dichromate was added. A blank was prepared simultaneously and the colour developed was read at 360 nm.

3.2 Determination of Riboflavin (Vitamin B2)

5 g of the sample was extracted with 100 ml of 50% ethanol solution and shaken for 1 h. This was filtered into a 100 ml flask; 10 ml of the extract was pipette into 50 ml volumetric flask. 10 ml of 5% potassium permanganate and 10 ml of 30% H_2O_2 were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance measured at 510 nm in a spectrophotometer.

3.3 Determination of Niacin (Vitamin B3)

5 g of the sample was treated with 50 ml of 1 N sulphuric acid and shaken for 30 min. 3 drops of ammonia solution were added to the sample and filtered. 10 ml of the filtrate was pipette into a 50 ml volumetric flask and 5 ml potassium cyanide was added. This was acidified with 5 ml of 0.02 N H_2SO_4 and absorbance measured in the spectrophotometer at 470 nm wavelengths

3.4 Estimation of Ascorbic acid (Vitamin C)

Vitamin C was determined by dichlorophenol Indophenol dye reduction method with slight modifications [45]. About 0.5 g of each extract was weighed and macerated with 12 ml of 0.4% oxalic acid in a test tube for 30 minutes. Afterward it was centrifuged for 5 minutes and the solution was filtered using Whatman filter paper. One ml of the filtrate was transferred into a dry test tube and 9 ml of 2,6- dichlorophenol indophenol solution was added to it. The absorbance was taken at 15 and 30 seconds interval at 520 nm.

3.5 Estimation of Vitamin A (Retinol)

To 0.5ml of the sample, 0.5 ml of chloroform and 2 ml of Trifluoroacetate reagent (TFA) were added in a test tube. The absorbance was recorded at 600 nm.

3.6 Determination of Vitamin E (Tocopherol)

This was determined spectrophotometrically using a modified standard method of [46]. 0.5 g of each of the plant extracts was extracted with 0.5 ml of ethanol and shaken for 1 minute. 3 ml of xylene was then added and centrifuged to separate extract. 1 ml of the extract was added to 1 ml of 2, 2'- dipyridyl reagent with 5 mL $FeCl_3$ and 5 mL of H_3PO_4 and an orange colouration was observed. The blank and standard (1 tablet of vitamin E) was prepared in the same way excluding the extract. Absorbance of test sample and standard sample was read at 539 nm.

4. RESULTS AND DISCUSSION

Polyphenolic compounds are very important plant bioactive component reported to possess strong antioxidant, antibacterial and numerous biological activities due to their molecules structures reported to contain a hydroxyl group or phenolics ring [47,48]. Several studies have shown that these compounds are very effective in scavenging free radicals due to their redox properties, thereby having the capacity to link with proteins and bacterial membranes to form complexes [49].

The total phenolic content of the methanol and ethyl acetate extracts from leaves of *Taraxacum officinale* was determined by Folin-Ciocalteu method and the results were expressed as mgGAE/g (Table 1). The results obtained in this study showed a considerable level of phenolic content in both leaf extracts. The highest concentration was recorded for methanol extract (15.3±0.11mg GAE/g), nevertheless, there was significant difference in the concentration of polyphenols in both extracts. This may be attributed but not limited to the polarity of the solvents used as well as the chemical nature of the endogenous extractable compounds. The concentration of polyphenol in this study is within the range reported by [50]. The presence of

polyphenols in both extracts enhances the medicinal uses of *Taraxacum officinale.*

The results of the concentration of flavonoids presented in Table 1 show varying concentrations of flavonoids in methanol and ethyl acetate extracts of *Taraxacum officinale.* The results also showed that *Taraxacum officinale* contained significantly higher concentration of flavonoids, with methanol extract having the highest flavonoid content (16.76±0.21mg/g).

The antioxidant activity of flavonoids (such as flavones, flavanols and condensed tannins) is due to their free OH groups, especially 3-OH. They suppress the formation of reactive oxygen species, inhibit metabolic processes, nucleic acid biosynthesis, platelet aggregation, and mast cell histamine release (anti-inflammatory effect), and they exhibit antimicrobial, anti-inflamatory, antiallergic, antineoplastic, antiviral, antithrombotic and vasodilatory activities [51,52].

Alkaloids are nitrogen – containing naturally occurring compounds. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bactericidal properties. Their antimicrobial properties are probably due to their ability to intercalate with DNA of the microorganisms [53,54]. The extracts of dandelion contain moderate concentration of alkaloids and this justifies its use a broad spectrum antimicrobial agent.

The presence of tannins in moderate concentrations in the different leaf extracts of dandelion (2.6±0.13 and 0.2±0.01mg/g for methanol and ethyl acetate extracts respectively) indicated that *Taraxacum officinale* may be used as diuretics against stomach and duodenal tumours and they play a major role in the treatment of inflamed tissues [55,56,57]. Tannins are well known for their anti-oxidant and anti-microbial properties, as well as for soothing relief, skin regeneration and anti-inflammatory activities [58].

The concentration of saponins in the extracts of dandelion was moderate, with methanol extracts containing significantly higher concentration.

Saponins from fruits and vegetables are important dietary supplements and are known to exhibit antimicrobial activities and protect plants from microbial pathogens [59]. They could be beneficial in modulating blood lipids, lower cancer risks and improve blood glucose response as well as possess antioxidant activity [60,61]. The presence of these phytochemicals in the different leaf extracts of *Taraxacum officinale* studied reveals that the plant possesses various pharmacological activities, hence justifying its medicinal importance.

Vitamins refer to a class of micro nutrients that play essential roles to human health and are classified into water soluble (ascorbic acid, niacin, riboflavin, and thiamine) and fat soluble vitamins (retinol and tocopherol) [62].

The vitamin content of the various extracts of *Taraxacum officinale* determined using standard procedures are presented in Table 2. From the results obtained, the methanol extract contained the highest concentration of water soluble vitamins (ascorbic acid, niacin, riboflavin and thiamine) while ethyl acetate contained the highest concentration of fat soluble vitamins (vitamins A and E).

Vitamin A is a natural antioxidant that inhibits free radicals and is essential for normal vision, gene expression, growth and immune function by its maintenance of epithelial cell functions [63]. The highest value of vitamin A was observed in ethyl acetate extract. On comparing the concentration of vitamin A obtained in this study with recommended daily allowance (RDA) which is 750 - 1200µg/day, it was observed that the levels of vitamin A in both extracts were lower than the recommended daily allowance limits.

Table 1. Phytochemical composition of the leaf extracts of *Taraxacum officinale* and expressed as mg/ g dry weight

Phytochemicals	Methanol extract	Ethyl acetate extract
Alkaloids	0.49 ±0.01	0.38 ±0.03
Flavonoids	16.76±0.21	13.09±0.04
Saponins	2.8 ±0.021	0.17±0.01
Total phenol	15.3±0.11	5.3±0.02
Tannins	2.6±0.13	0.2±0.01

Table 2. Vitamin composition of leaf extracts of *Taraxacum officinale* expressed in mg/100 g dry weight

Vitamins	Methanol extract	Ethyl acetate extract
Vitamin A	8.03±1.2	10.41±1.4
Vitamin C	32.07±1.51	30.03±1.3
Vitamin E	2.8±0.4	3.0±0.2
Vitamin B1	0.16±0.01	0.12±0.01
Vitamin B2	0.24±0.3	0.19±0.12
Vitamin B3	0.12±0.01	0.08±0.01

Ascorbic acid (Vitamin C) is a potent antioxidant that inhibits, minimizes and terminates the propagation of the free radicals by donating hydrogen and electron thus changing its structure from ascorbic acid to dehydroascorbic acid [64]. Ascorbic acid facilitates the transport and uptake of non-heme iron and utilization [65]. They also act synergistically with tocopherol to regenerate the tocopheryl radicals. Vitamin C is required for maintenance of normal connective tissues and wound healing and also helps in the development of bones, teeth and prevention of scurvy [66,67]. The recommended daily allowance of ascorbic acid is 60 mg (for adults) and 20 mg (for children) [68]. From the result obtained, the vitamin C concentration in both extracts was lower than the recommended daily allowance for adult but above permissible limit for children.

Vitamin E is a major lipid soluble antioxidant and is most effective chain breaking antioxidant within the cell membrane. It protects the cell membranes from lipid peroxidation and oxidative stress/damage caused by free radicals [69,70]. Vitamin E exerts it antioxidant activity by donating the hydrogen or the hydroxyl group of its chroman ring to neutralize the free radical [71]. It is vital to the formation and normal function of red blood cell and muscles [63]. The result obtained for vitamin E in this work was 2.8±0.4mg/100g for methanol while ethyl acetate extract contained 3.0±0.2mg/100g vitamin E. On comparing the result in this study with the recommended dietary allowance of 15mg/day as stipulated by WHO, it was observed that level of vitamin E in both extracts were below the recommended dietary allowance.

The water soluble B vitamins (B1 (thiamin), B2 (riboflavin) and B3 (niacin)) were determined in methanol and ethyl acetate extracts of dandelion and results presented in Table 2. The results revealed that both extracts contained moderate levels of these vitamins. However, the concentration of all the B vitamins were below the recommended daily allowance (RDA) for vitamins (thiamin = 1.4 mg, riboflavin =1.7mg and niacin = 18mg).

Thiamine (Vitamin B1) is essential for nervous system function, energy production, stimulating appetite and as a coenzyme in metabolism of carbohydrates.

Riboflavin (Vitamin B2) catalyses the formation of niacin, produces energy from proteins, carbohydrates and fat and serves as a cofactor in oxidative phosphorylation and reduction reactions [72]. Niacin (VitaminB3) has the ability to lower blood lipids and is sometimes used in treating hyperlipidaemia. It also plays an important role in DNA repair and metabolism as well as coenzyme formation [73,74].

It should be noted that though, the vitamin content of the leaves are low, consumption of this plant material will contribute in meeting the daily vitamin requirement as stipulated for healthy adults. Worthy to also note is that medicinal activity of plant extracts is not limited to only phenolics, but may come from the presence of other secondary metabolites such as volatile oils, carotenoids and vitamins [75].

4.1 Antimicrobial Activity

The results of antimicrobial activity of the different leaf extracts of *Taraxacum officinale* against seven standard microorganisms; 1 Gram –positive bacterium (*Staphylococcus aureus*), 4 Gram –negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa Salmonella spp, Vibrio cholerea*) and two fungi (*Candida albicans, Aspergillus niger*) were presented in Table 3. The antibacterial efficacy of methanol and ethyl acetate extracts of dandelion against these human pathogenic bacteria showed different selectivity for each microorganism. The antimicrobial activity of the different extracts was directly proportional to the concentration of the extracts, with the highest concentration recording

the highest activity. Methanol extract showed maximum inhibition at 80 mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa* at 32±0.01 and 28±0.1 mm respectively while *Vibrio cholerea* showed the least sensitivity (14±1.11 mm) at 80 ml/ml. Test fungi (*Candida albicans and Aspergillus niger*) showed varied susceptibility to extracts. The susceptibility of *Candida albicans* to methanol extract ranged from 7.0 ±0.03 - 12±0.3 mm while no activity was observed against *Aspergillus niger.* The zone of inhibition of *Staphylococcus aureus* ranged from 8.0±0.03 - 16±02 mm while that of *Salmonella spp* varied from 10±0.02 to 17±0.25 mm in methanol extract.

Similarly, *Escherichia coli* and *Pseudomonas aeruginosa* showed higher sensitivity against ethyl acetate extract at 80 mg/ml (20±0.21 and 20±0.03mm respectively). The least activity at 80 mg/ml was recorded for *Salmonella spp* (10±0. 22 mm). *Staphylococcus aureus* was resistant to the effect of ethyl acetate extract as no activity was recorded at different concentrations.

The antifungal activity of the various concentrations of ethyl acetate extracts of *Taraxacum officinale* against the various strains of fungi such as *Aspergillus. niger* and *Candida albicans* revealed that the extract inhibited the growth of the fungi at different concentrations. The zones of inhibition for *Aspergillus. niger* and *Candida albicans* at 80 mg/ml was 17±0.15 and 15±0.02 mm respectively.

Generally, the antimicrobial activity of the methanol and ethyl acetate extracts of *Taraxacum officinale* revealed that gram positive and gram negative bacteria as well as fungi were susceptible to these extracts, indicating that dandelion may be used as a broad spectrum antimicrobial agent. This further corroborates its use in the treatment of various ailments. It was also observed that methanol showed the highest antimicrobial activity compared to ethyl acetate extract.

4.1.1 Antioxidant activity by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) method

The result of DPPH scavenging activity assay of the different extracts of *Taraxacum officinale* in this study are presented in Fig. 1. The results indicated that these extracts scavenged free radicals in a concentration dependent manner. The DPPH scavenging activity of methanol and ethyl acetate extracts at 100 µg/ml which was the highest concentration was 85.3% and 60.1% respectively, compared to the standard vitamin C (88.4%). All of the assessed extracts of dandelion were able to reduce the stable, purple-colored radical DPPH to the yellow colored DPPH-H form. However, methanol extract have prominent antioxidant activity which may be attributed to the presence of bioactive compounds. Usually, higher total phenol and flavonoids contents lead to better DPPH scavenging activity [76,77]. As known, polyphenols have a metal chelating potential and their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides and can be justified by their chemical structure [78,79,80]. The IC_{50} value was calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the IC_{50} value, the higher the antioxidant activity of samples [81]. The observed IC_{50} value showed that methanol extract exhibited the highest antioxidant activity (2.88 µg/ml), which was comparable to vitamin C (2.18 µg/ml) used as standard.

4.1.2 Ferric reducing/antioxidant power (FRAP) Assay

Ferric Reducing Antioxidant Power (FRAP) assay is a quantitative assay for measuring the antioxidant potential within a sample. Ferric iron ($Fe^{3+)}$ is reduced, by electron-donating antioxidants present in the extracts, to its ferrous form (Fe^{2+}). This assay is used to evaluate the capacity of natural antioxidant to donate an electron or hydrogen [82].

4.2 Antioxidant Activity

The ferric reducing activity of the leaf extracts of dandelion were presented in Fig. 2. The reducing capacity of the extract, another significant indicator of antioxidant activity was also found to be appreciable comparable with ascorbic acid. The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain and by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation [83,84,85]. Like the radical scavenging activity, extracts exhibited a concentration – dependent reducing power. The LC_{50} value of the reducing power of the extracts revealed that methanol extract had a higher reducing ability than ethyl acetate extract.

On comparing the LC_{50} of both extracts with vitamin C, it was observed that the standard had a better reducing power than the extracts. Results showed that the antioxidants present in the extracts are capable of reducing ferric complex Fe^{3+} into ferrous Fe^{2+} form.

Table 3. Antimicrobial Activity of methanol and ethyl acetate leaf extracts of *Taraxacum officinale* against the human pathogenic bacteria by disc diffusion method

Crude extracts	Isolates	Zone of inhibition (mm)				
		10 mg/ml	20 mg/ml	40mg/ml	60 mg/ml	80 mg/ml
Methanol	*Staph. aureus*	8.00±0.03	10.00±0.32	11.0±1.21	13±0.1	16±0.2
	Asper. niger	NA	NA	NA	NA	NA
	Candida albican	7.0±0.03	8.0±0.01	10.0±1.1	10.0±0.01	12±0.3
	Vibrio cholera	10.0±0.01	10±0.025	11±0.03	12±0.01	14.0±1.11
	Escherichia coli	17.2±0.2	19±0.01	24.0±0.3	28±0.04	32±0.01
	Salmonella spp	10±0.02	10±0.12	12±1.5	14±1.25	17±0.25
	Pseudo. aeruginosa	14±0.01	17±1.33	21±0.23	25±0.01	28±0.1
Ethyl acetate	*Staph. aureus*	NA	NA	NA	NA	NA
	Asper. niger	8±0.02	9±0.01	11±0.6	14±0.11	17±0.15
	Candida albican	10±0.01	10±0.13	12±0.01	14±0.12	15±0.02
	Vibrio cholera	NA	7±0.01	9±0.02	14±0.02	16±0.16
	Escherichia coli	10±0.01	11±0.02	13±0.01	17±0.11	20±0.21
	Salmonella spp	NA	7±0.02	8±0.03	9±0.05	10±0.22
	Pseudo. aeruginosa	11±0.3	13±0.01	15±1.2	17±0.34	20±0.03

KEY: NA = No activity, Staph. Aureus = Staphylococcus aureus, Asper. Niger = Aspergillus niger, Pseudo. Aeruginosa= Pseudomonas aeruginosa

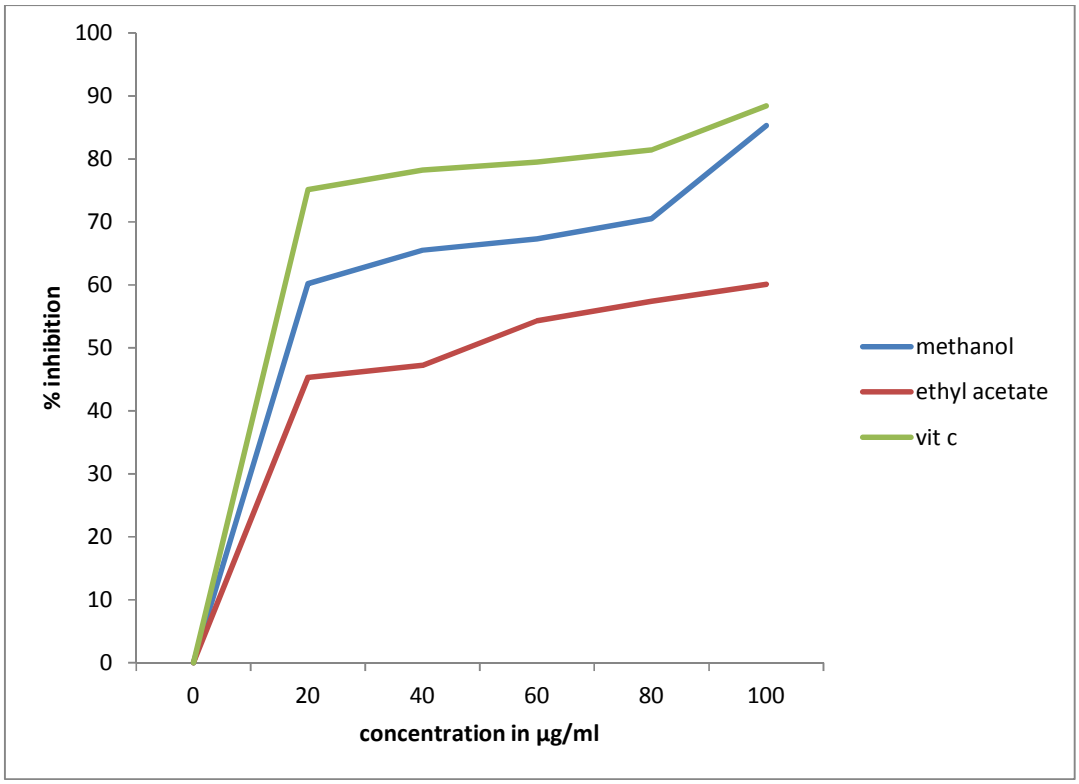

Fig. 1. DPPH scavenging activity of leaf extract of *Taraxacum officinale*

Table 4. LC_{50} in µg/ml for antioxidant activity of extracts of *Taraxacum officinale*

Antioxidant activity	Methanol extract	Ethyl acetate	Vitamin C
DPPH	2.88	4.10	2.18
FRAP	329.3	467.75	172.16

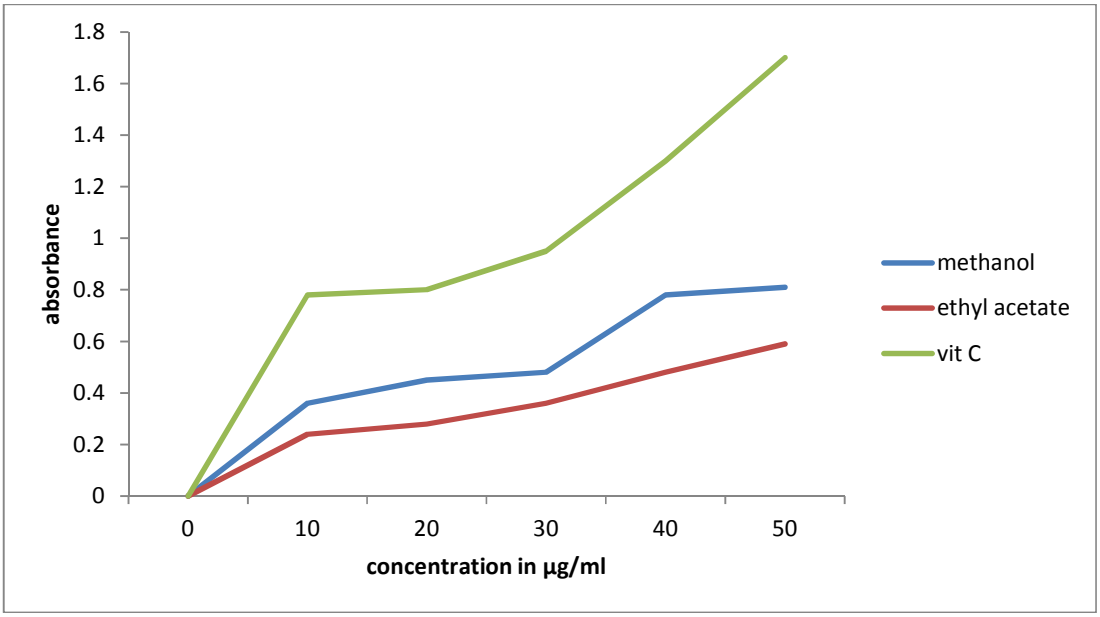

Fig. 2. Ferric reducing power activity of leaf extract of *Taraxacum officinale*

5. CONCLUSION

Methanol and ethyl acetate extracts of dandelion obtained in this study were evaluated for its bioactive compounds, vitamin content, antioxidant and antimicrobial activities. The results revealed that these extracts contained moderate to significant concentration of all the phytochemical compounds tested for. These extracts equally contained appreciable concentration of vitamins such as vitamins A, B complexes, C and E.

The extracts of dandelion exhibited strong reducing and free radical scavenging properties, which may be due to the presence of antioxidant vitamins (vitamins A, C and E), flavanoids, tannin, alkaloid and polyphenols compounds. The antibacterial activity of the extracts against tested bacteria and fungi strains shows that it has the potential to be used as broad spectrum antibiotics for the treatment of various ailments.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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