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Abstract

Lactuca taraxacifolia is an important medicinal plant used locally in the treatment or prevention of many human diseases and illnesses. The aim of the study was to investigate the chemical composition, pH, TPC, TFC, TAA, carotenoid, antioxidant, anti-arthritic, anti-inflammatory and bactericidal activities. These were measured using GC-MS, pH meter, Folin-Ciocalteu?s, AlCl3, 2,4-DNPH, acetone-hexane, DPPH, PTAC, BSA and agar-well diffusion methods respectively. The pH of the aqueous solution was 6.06. The GC and GC-MS analyses revealed the presence of 47 organic compounds making up 81.45

Index terms— lactuca taraxacifolia, phytochemical, pharmacological activities, antioxidant, anti-arthritic, antiinflammatory, antimicrobial activities.

1 I. Introduction

lants have limitless abilities to synthesize phytochemicals that have enormous therapeutic potentials (Suresh et al., 2012; Jain et al., 2015; **??**hittu and Akor, 2015). Secondary metabolites from plants are important component of alternative and complementary medicines as drugs derived from plants are still the main source of health care for the majority of rural dwellers (Shakya, 2016; Amira and Oloyede, 2017; Elamin, 2017). They are effective in the treatment of infectious diseases and simultaneously they also mitigate many of the side effects that are often associated with synthetic drugs (Rios and Recio, 2005; Jain et al., 2015).

Lactuca taraxacifolia (Willd) Schum. (Asteraceae) has been domesticated as a leafy vegetable in West Africa. L. taraxacifolia is used as a remedy for prevention and treatment of diseases such as measles, yaws, conjunctivitis, hyperthesion, cancer etc. It is reported to possess hypolipidaemic, antihypertensive effects **??**Adebisi, 2004; **??**bi et al., 2006; **??**akpere and Aremu, 2008; **??**airo et al., 2015). The leaves of L. taraxacifolia are used in stimulate lactation and also to induce multiple births in animals (Adinortey et al., 2012). The leaves are rubbed on limbs to aid walking in children. The milky latex of the plant is used to treat conjunctivitis (Sakpere and Aremu, 2008). This plant had been known for their nutritional quality for long; the plant is used as vegetable and eaten as salad or cooked as soups (Adinortey et al., 2012; **??**detutu et al., 2013; **??**uffina et al., 2016). It has been observed to be a good source of essential mineral elements (Soetan et al., 2010; Gbadamosi et al., 2012).

To the best of our knowledge, there is paucity of information on the chemical composition and pharmacological properties of L. teraxacifolia so far. Therefore, the present research was undertaken for with the aim at looking into the phytochemical, pH, ascorbic acid, total phenolic content, total flavonoid content, ?carotene, lycopene, antioxidant, anti-arthritic, antiinflammatory and bactericidal potentials of the leaf extract of L. taraxacifolia from Nigeria.

2 II. Materials and Methods

3 b) Measurement of pH

Pulverised leaves of L. taraxacifolia were soaked in distilled water for ?2.5 hr and then filtered. The pH values were measured in the fresh filtered solution using digital portable pH meter (Naka et al., 2016).

4 c) Gas Chromatography-Mass Spectroscopy Analysis

The leaf methanolic extract of L. taraxacifolia was analysed using Shimadzu GC-MS-QP2010 Plus (Japan). The separations were carried out using a Restek Rtx-5MS fused silica capillary column (5%diphenyl-95%-

dimethylpolysiloxane) of 30 m× 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The conditions for analysis were set as follows; column oven temperature was programmed from 60-280 °C (temperature at 60 °C was held for 1.0 min, raised to 180 °C for 3 min and then finally to 280 °C held for 2 min); injection mode, Split ratio 41.6; injection temperature, 250 °C; flow control mode, linear velocity (36.2 cm/sec); purge flow 3.0 ml/min; pressure, 56.2 kPa; helium was the carrier gas with total flow rate 45.0 ml/min; column flow rate, 0.99 ml/min; ion source temperature, 200 °C; interface temperature, 250 °C; solvent cut time, 3.0 min; start time 3.5 min; end time, 24.0 min; start m/z, 50 and end m/z, 700. Detector was operated in EI ionization mode of 70 eV. Components were identified by matching their mass spectra with those of the spectrometer data base using the NIST computer data bank, as well as by comparison of the fragmentation pattern with those reported in the literature (Oyebanji and Ololade, 2017).

5 d) Determination of Total Phenolic Content (TPC)

The TPC of the leaf extract of L. taraxacifolia was determined using Folin-Ciocalteau method. 1000 µgml -1 of the extract was mixed with 1.0 ml of 10% Folin-Ciocalteu reagent in distilled water and then neutralized with 4 ml of 7.5% sodium carbonate solution. The sample was maintained at room temperature for 3 hrs with periodical mixing, the absorbance at 760 nm was measured using UV-visspectrophotometer. The index of TPC in the juice was determined as µgmg -1 of gallic acid equivalent (GAE) using an equation obtained from the calibration curve of gallic acid graph (Amira and Oloyede, 2017).

6 e) Total Flavonoid Concentration (TFC)

The TFC of the extract of L. taraxacifolia was determined by spectrophotometry, using aluminium chloride method and quercetin as standard. Briefly, 1.0 ml of the extract, 0.10 ml of 10% aluminium chloride (AlCl 3 .6H 2 O), 0.10 ml of sodium acetate (NaC 2 H 3 O 2 . 3H 2 O) (1 M) and 2.80 ml of distilled water. After incubation for 40 min, absorbance was measured at 415 nm using a UV-Vis-spectrophotometer. To calculate the concentration of flavonoids, we prepared a calibration curve using quercetin as standard. The index of TFC concentration is expressed as quercetin equivalents (QE) in µg per mg of juice. All assays were carried out in triplicate (Formagio et al., 2014).

7 g) Determination of Carotenoid: Lycopene and ?-

Carotene Contents 200 mg of the leaves of L. taraxacifolia were homogenized with 10 ml of acetone-hexane mixture (ratio 4:6) to determine the lycopene and ?-carotene contents. The homogenate was centrifuged at 5000 x g for 10 min at 4°C. Automatically, two phases separated and an aliquot was taken from the upper solution (supernatant) for measurement of optical density at 663, 645, 505, and 453 nm in a UV-Vis-spectrophotometer. The assays were carried out in triplicates, the results were mean ± SD with acetone:hexane as blank. Lycopene and ?-carotene contents were calculated according to the equations: Lycopene = -0.0458A 663 + 0.204A 645 + 0.372A 505 -0.0806A 453 ; ?-Carotene = 0.216A 663 -1.22A 645 -0.304A 505 + 0.452A 453 . Lycopene and ?-carotene were finally expressed as mgg -1 fw. Where A= absorbance recorded at specific wavelengths **??**Wei et al., 2013).

8 h) Determination of Free Radical Scavenging and Antioxidant Activities i. In vitro DPPH Assay

The antioxidant and free radical scavenging of the extract of L. taraxacifolia were measured by using 2,2?-diphenyl-1-picryl-hydrazyl. Briefly, the reaction mixture (2.0 ml) consists of 1.0 ml of DPPH in methanol (0.004%) and 1.0 ml of various concentrations of the extract. It was incubated for 30 min. in dark, and then the absorbance was measured at 517 nm. The control was prepared by DPPH and methanol in place of sample. In this assay, the positive control was ascorbic acid. The percentage of inhibition can be calculated using the formula:

9 I% = [(A blank -A ext)/A blank] x 100

Where: A blank is the absorbance of blank solution and A ext is the absorbance of the extract. The dose response curve was plotted and IC 50 value for the extract and the standard were calculated **??**Ololade et al., 2016).

Antioxidant Activity Index: The antioxidant activity index (AAI) was calculated as:AAI = [DPPH initial concentration]/[IC 50]

f) Determination of Total Ascorbic Acid (TAA) 0.1 ml (1000 µgm -1) of the extract was added to 1.0 ml 2,4-dinitrophenylhydrazine (2,4-DNPH). It was allowed to stand for 30 min. and the absorbance was read in triplicate at 515 nm, using distilled water as blank. Ascorbic acid was used as a reference and for the calibration curve; result was expressed in microgram per milligram of ascorbic acid equivalent (Benites et al., 2015). AAI was classified as weak, when AAI < 0.5, moderate, when AAI ranged between 0.5-1.0, strong, when AAI ranged between 1.0-2.0, and very strong, when AAI > 2.0 (Ololade and Olawore, 2017).

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11 . Phosphomolybdate Total Antioxidant Capacity (PTAC) Assay

The PTAC of the extract of L. taraxacifolia was determined with phosphomolybdenum using ascorbic acid as the standard. An aliquot of 1.0 ml of the extract solution was combined with 1.0 ml of reagent (0.6 M sulphuric acid, 28 µM sodium phosphate and 4 µM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 o C for 90 min. after the samples had cooled to room temperature, the absorbance of the aqueous solution of each were measured at 695 nm in UV spectrophotometer. The blank solution contained 1.0 ml of reagent solution and the appropriate volume of the same solvent was used for the sample and it was incubated under the same conditions as the rest of the samples. The total antioxidant capacity was expressed as equivalents of ascorbic acid (Borokini et al., 2017).

12 I% = [(A ts -A pc)/A tcs] x 100

A ts is the absorbance of test solution; A pc is the absorbance of the product control and A tcs is the absorbance of test solution. The dose-response curve was plotted and IC 50 value for the extract was calculated (Alamgeer et al. 2017).

13 j) In vitro Bactericidal Potential

The antibacterial potentials of the extract were carried out using Agar-well diffusion method against Gram-positive bacteria:

(Enterococcus faecalis, Micrococcus varians, Streptococcus agalactiae and Staphylococcus aureus), Gram-negative bacteria: (Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens and Salmonella typhimurium). Bacteria were incubated and grown overnight at 37°C in Nutrient agar. The cultured bacteria were adjusted to 0.5 McFarland standards, 20 ml of sterilized Nutrient agar medium was homogenized and aseptically poured into sterile Petri dishes and plates were swabbed with inocula of the test organisms, and kept for 30 min. for adsorption. A sterile cork borer of 6mm in diameter was used to make uniform wells into which were added different concentrations (1000, 500 and 250 µgml -1) of the extract. The plates were allowed to stay in a refrigerator for 1 hour to allow proper diffusion of the juice solution into the medium. Synthetic antibiotic gentamicin (10?g/disc) was used as positive control. The plates were then incubated at 37 °C for 24 hr before visual assessment of the inhibition zones. The zone of inhibition was measured to the nearest size in millimetre (mm) using standard rule. The assay was carried out in aseptic conditions in order to achieve consistency .

14 III. Results and Discussion

15 a) pH of the Leaves of L. taraxacifolia

The pH of the distilled water leaf extract of L. taraxacifolia was 6.06 and within the standard limit (pH 3.40-6.10) that insures freshness for consumption (El-Sohaimy et al., 2015), this showed that the leaf of the plant had weak acidic property.

16 b) Chemical Constituent of the Leaf Extract of L.

taraxacifolia A total of 47 compounds were identified in the leaf methanolic extract of L. taraxacifolia, accounting for 81.45% of the total extract (Table 1), and the main constituents identified were palmitic acid (8.5%), methyl-11-octadecenoate (7.7%), erythritol (7.5%), glycerol (6.5%), linolelaidic acid, methyl ester (6.2%) and phytol (5.5%). The chemical composition of leaf extract of L. taraxacifolia investigated in this study was entirely different from what was obtained from other species of Lactuca. Previous studies on the chemical composition of fresh and dry leaves essential oils of Lactuca sativa from Sultanate of Oman showed that the composition was dominated by durenol (52.00% and 49.79%), thymol (11.55% and 10.73%) and ?-pinene (5.11% and 4.05%) (Al-Nomaani et al., 2013). Likewise, E-Ethyl-(Z)-3-(4acetylphenylthio) cinnamate (33.01%), acetate, (3?)-lup-20 (29)-en-3-ol (15.11%), 5,12-dihydroxy-, (5a,12?)ergost-25-ene-3,6-dione (10.46%) and 3-ethoxy-1-(3H)isobenzofuranone, (7.79%) were the most abundant component in GC-MS analysis of the methanolic entire extract of Lactuca runcinata (Kanthal et al., 2014).

17 Where: i) In-vitro Anti-Arthritic and Anti-Inflammatory Activities of the Extract on Inhibition of Protein Denaturation (Bovine Serum Albumin Assay)

In vitro anti-arthritic/anti-inflammatory activity of the extract was evaluated against protein denaturation method using BSA. Test solution (0.5 ml) composed of 0.05ml of the extract at different concentrations (1000-100 µgml -1) and 0.45 ml of BSA (5% aqueous solution). Test control solution (0.5 ml) consisted of 0.05 ml of distilled water and 0.45mL of BSA (5% aqueous solution). Product control solution consisted of 0.05ml of the extract at different concentrations (1000-100 µgml -1) and 0.45 ml of distilled water. Standard solution (0.5 ml) consisted of 0.05ml aspirin (3000 µgml -1) plus 0.45ml of BSA (5% aqueous solution). Solutions were incubated at specific temperature (37 o C) for 20 min. Solutions were cooled and 2.5 ml of phosphate buffer (pH 6.4) was added to all the solutions and temperature was increased progressively up to 70 o C for 5 min. Absorbance of the resultant solution was measured using UV visible spectrophotometer at 660 nm. The percentage inhibition of protein denaturation was determined using the following formula:

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18 c) Total Phenolic Content (TPC)

The TPC of the extract was 3,041.50 µgmg -1 GAE (Table 2). This might be due to the presence of low molecular mass phenolic compound such as 2,2'methylenebis[6-(1,1-dimethylethyl)-4-methylphenol in the leaf extract. TPC determined in this study for L. taraxacifolia was higher than those reported in other . var. longifolia had the total phenolic contents of 235.31 mg CE/g extract (Edziri et al., 2011). The phenolic compound loses an H + ion to produce a phenolate ion, which reduces Folin-Ciocalteu reagent **??**Ahmed et al., 2015). Phenolic compounds are known as free radical terminators and strong chain breaking antioxidants, so this may contribute directly to antioxidative action of the plant (Flora, 2009). Studies had shown that consumption of phenolic antioxidant prevents chronic disease such as cancer, cardiovascular diseases (CVD), diabetes, cirrhosis, malignancy, stroke and arthritis **??**Zhang et al., 2015;Dzia?o et al., 2016). The outstanding pharmacological potential of phenolic compounds is due to their ability to block specific enzymes that cause inflammation. They also modify the prostaglandin pathways and thereby protect platelets from clumping Ezenagu, 2008, Okwu andNnamdi, 2008;Osuntokun and Olajubu, 2014).

19 d) Total Flavonoid Content (TFC)

The TFC of the extract was 59.05 µgmg -1 QE (Table 2). Flavonoids limit the risk of degenerative diseases associated with oxidative damage. Flavonoids are very important plant secondary metabolites because their hydroxyl groups confer scavenging ability on them (Ghasemzadeh and Ghasemzadeh, 2011). The broad medicinal properties of flavonoids are attributed mainly to their antioxidant properties (Dai and Mumper, 2010; **??**angeetha et al., 2016;Ganesan and Xu, 2017). Flavonoids slow down the oxidative degradation of lipids, improve the quality and nutritional value of food and biological response modifiers (Kumar, 2014;Mojzer et al., 2016). They have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activities **??**Panche et al., 2016). Flavonoid exerts protection against chronic disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages **??**Shukla et al., 2014;Karau et al., 2015;Fernandes et al., 2017).

20 e) Total Ascorbic Acid (TAA)

The TAA analysis of the investigated extract of L. taraxacifolia showed the presence of high amount ascorbic acid and its derivatives (Table 2). Ascorbic acid is a sugar acid lactone. It is synthesized in plants from glucose or other simple carbohydrates (Benites et al., 2015). Ascorbic acid is an essential micronutrient and antioxidant needed for normal metabolic function of the body. It plays an important role as a component of enzymes involved in the synthesis of collagens and carnitine. Ascorbic acid plays an important role in a number of metabolic functions including the activation of the B vitamin, folic acid, the conversion of cholesterol to bile acids and the conversion of the amino acid, tryptophan, to the neurotransmitter, serotonin (Naidu, 2003;Chambial et al., 2013).

21 f) Determination of Carotenoid: Lycopene and ?-carotene

The carotenoid content (lycopene and ?carotene) of the extract was as shown in Table 3. Carotenoids are very potent natural antioxidants. Carotenoids are powerful antioxidants and are obtained primarily from fruit and vegetables. Different carotenoids, such lycopene and ?-carotene have high potentials to decrease risk of disease. Carotenoids are important natural isoprenoid pigments synthesized in plants and have essential roles in protecting against excess light energy and oxidative damage. Their provitamin A activities and antioxidant properties were their most attractive functions. ?-carotene is the major and most effective vitamin A precursor among carotenoids, and plays a crucial role in human health, protecting against age-related degenerative diseases, cardiovascular disease, certain cancers and vitamin A deficiency **??**Fiedor and

22 g) Free Radical Scavenging and Antioxidant Activities

The percentage inhibitions of the extract at various concentrations (2000, 1000, 750, 500 and 100 µgml -1) were 90.91, 90.22, 86.03, 84.64 and 72.07%, respectively. The methanolic leaf extract of L. taraxacifolia IC 50 valueof 0.75 ?gml -1 was twelve-fold lower than that of the reference compound ascorbic acid, which had an IC 50 value of 9.0 ?gml -1 and the AAI 4), while the related species such as L. indica with IC 50 12.2 ?gml -1 for hot water extract **??**Wang et al., 2003) and leaf methanolic and aqueous extracts of L. sativa var. longifolia reported to have the DPPH antioxidant activities with IC 50 of 3.5 and 4.1 ?gml -1 respectively. Therefore, the leaf extract of L. taraxacifolia investigated in this study had higher Antioxidantpotential than the reference compound and related species.

23 h) Phosphomolybdate Total Antioxidant Capacity (PTAC)

The PTAC of leaf extract of L. taraxacifolia was found to be moderately high as shown in Table 4. The phosphormolybdenum method is quantitative since the PTAC is express as ascorbic acid equivalents. Natural products had become the target of a great number of studies in finding the sources of potentially safe, effective and cheap antioxidants because accumulation of free radicals causes pathological conditions (Lu et al., 2010). On the basis of the results obtained in the present study, it was concluded that methanolic extract of L. taraxacifolia exhibited potent free radical scavenging activities which might be helpful in preventing the progress of various oxidative stress mediated disorders (Anil and Suresh, 2011

24 i) Anti-Arthritic and Anti-Inflammatory Potentials

Leaf methanolic extract of L. taraxacifolia at different concentrations showed considerably high (14-80%) anti-arthritic/anti-inflammatory potential with IC 50 0.25 mgml -1 against the denaturation of bovine serum albumin, as compared to the synthetic drugs (aspirin) (Table 5). This result is similar to what was obtained from the in vivo anti-arthritic test on Ulva lactuca from Mediterranean Sea shores in Alexandria (Ahmed et al., 2017). Synthetic drugs for rheumatoid arthritis have certain shortcomings and side effects. Natural products are being preferred over conventional drugs nowadays due to their easy and continuous availability, better compatibility, cost effectiveness, less potential of toxicity and side effects, higher safety, and improved efficacy (Ekor, 2014;Alamgeer et al., 2017). Denaturation of proteins is the cause of inflammation, lipodystrophy, hyperlipidaemia, vasomotor rhinitis, rheumatoid arthritis, atherosclerosis, cardiovascular diseases, cancer, kidney stones and diabetes mellitus **??**Kumar et al., 2011; **??**rabhu et al., 2014; **??**raore et al., 2014). Phytochemicals from plants that can prevent denaturation of protein inhibition therefore, would be useful for the development of anti-arthritic, anti-inflammatory and analgesic drug (Garcia-Garcia et al., 2014; **??**rivedi et al., 2017). Therefore, this study showed that the leaf extract of L. taraxacifolia is capable of preventing and controlling the denaturation of protein and thereby it inhibited the denaturation of protein and its effect was compared with the standard drug. The mechanism of denaturation involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Arya et al., 2014;Elisha et al., 2016; **??**umathi and Anuradha, 2017).

25 j) Bactericidal Potentials

The antibacterial screening of the leaf extract of L. taraxacifolia gave wide range of zones of inhibition against the tested strains of bacteria. The zones of inhibition of the leaf extract of L. taraxacifolia (11.0-30.0 mm) extract showed high bactericidal activities from sensitive to ultra-sensitive as compared to synthetic antibiotic (gentamicin) (Table **??**). In this study extract demonstrated antibacterial activities which may explain anonymous claim on the traditional uses of L. taraxacifolia for treatment of bacteria infections. The antibacterial properties of the extract investigated in this study were more active than the extract of other Lactuca species such as leaves aqueous and methanolic extract of L. sativa from Saudi Arabia which showed moderate inhibitions against S. aureus, S. pyogenes, B. subtilis, E. coli and P. aeruginosa between 9.0-14.0 mm (Bhat and Al-Daihan, 2014). Likewise, methanolic extract of L. have synergistic potential to tackle these problems, in that they possess antibiotic properties, safer than synthetic drugs, offering profound therapeutic benefits and more affordable treatment **??**Aiyegoro and [1] [2]

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Figure 1: Table 1 :

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[2] Phytochemical, Antioxidant, Anti-Arthritic, Anti-Inflammatory and Bactericidal Potentials of the Leaf Extract of Lactuca teraxacifolia © 2017 Global Journals Inc. (US)

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TPC	TFC	TAA
3,041.50 ±0.00 µgmg -1 GAE	59.05±0.00 µgmg -1 QE	47.88±0.00 µgmg -1 AAE

Data are presented as the mean value ± S.D. of triplicate

Figure 2: Table 2 :

Figure 3:

3

Carotenoid	Concentration (mgg -1)
?-carotene	0.50
Lycopene	0.20

Figure 4: Table 3 :

4

Extract and Reference Drug	DPPH IC 50 µgml -1	AAI	PTAC µgmg -1 AAE
Extract	0.75	53.33	903.85±0.00

Figure 5: Table 4 :

5

	Reference Drug		
Conc. µgml -1	% Inhibition	IC 50 mgml -1	% Inhibition of Aspirin 3000 µgml -l
1000	80		
500	40	0.25	40
250	40		
100	14		

Figure 6: Table 5 :

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Leaf Extract	Synthetic Antibiotic GEN

[Note: B Volume XVII Issue II Version I © 2017 Global Journals Inc. (US)]

Figure 7: Table 3 :

Key note: Resistant (–), not sensitive (<8 mm), sensitive (9-14 mm), very sensitive (15-19 mm) and ultrasensitive (>20 mm)

.1 IV. Conclusion

This study had demonstrated the medicinal properties of the methanolic leaf extract of L. taraxacifolia and showed that this therapeutic effect could be attributed to the active secondary metabolites such as phenolic and flavonoid compounds in the plant. Leaves of the plant contain wide range of health-promoting phytochemicals. This work also contributed to the appreciation of the nutritional and medicinal values of the plant. The characteristics of the leaf as a dietary source of antioxidant and antibiotic were also pointed out. The leaves of the plant possessed high antioxidant activity which might be helpful in preventing or slowing the progress of various oxidative stress related disorders and therefore can be used in food and pharmaceutical industries.

.2 Conflict of Interest Statement:

The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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