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EVALUATION OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITIES OF DICHLOROMETHANE LEAF EXTRACTS OF *FICUS TRICHOCARPA* AND *LASIANTHERA AFRICANA*

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ABSTRACT

This study evaluated the bioactive compounds present in the dichloromethane leaf extracts of Ficus trichocarpa and Lasianthera africana and also assessed the antioxidant activities of the same leaf extracts. The plant materials were washed under running water to remove foreign matters, air-dried at ambient temperatures under a shade for up to 2 weeks, and afterwards pulverized into coarse powders. The bioactive compounds were analysed using Gas Chromatography-Mass Spectrometry (GC-MS) while the antioxidant activities were assessed by ferric reducing antioxidant power (FRAP) assay, DPPH radical scavenging assay, and hydroxyl radical assay. The results of phytochemical content showed that the leaves are rich in secondary metabolites such as alkaloids, terpenes, flavonoids and non-flavonoid phenolics. Quantitatively, alkaloids were the most abundant secondary metabolites (55.73%) present in the leaf of F. trichocarpa. On the other hand, terpenes were the most abundant secondary metabolites present in the leaf of L. Africana (55.45%). The results of antioxidant assay revealed that both F. trichocarpa and L. africana leaves exhibited high antioxidant activities in a dose-dependent manner: 69.52% and 52.08% respectively at 100 µg/ml for DPPH-scavenging activities; 73.18% and 65.65 % respectively at 200 µg/ml for FRAP assay; and (86.25% and 67.08 % respectively at 200µg/ml for hydroxyl radical activities. In conclusion, the dichloromethane leaf extracts of F. trichocarpa and L. africana are rich in secondary metabolites such as alkaloids, terpenes, flavonoids and non-flavonoid phenolics. The leaf extracts also have high antioxidant property.

Keywords: Bioactive Compounds, *Ficus trichocarpa*, *Lasianthera africana*, Phytochemical content, Antioxidant, Secondary Metabolites

INTRODUCTION

The term "secondary plant metabolites" refers to a variety of chemical compounds produced by plant cells through metabolic pathways derived from the primary metabolic pathways (Rehab and El-Anssary, 2017). Albrecht Kossel, who won the 1910 Nobel Prize in physiology or medicine, was the first to develop the term "secondary metabolite." After thirty years, Czapek referred to them as final goods. He claimed that these compounds are produced from nitrogen metabolism through processes he referred to as "secondary alterations," including deamination. The development of the field of phytochemistry was based on the increasing recovery of these compounds by the middle of the 20th century thanks to improvements in analytical techniques like chromatography (Bjeldanes and Shibamoto, 2009). The biological effects of secondary metabolites have been demonstrated to vary, which support many

ancient civilizations' usage of herbs in traditional medicine a scientific foundation. They are able to defend plants against diseases since they are antibiotic, antifungal, and antiviral, according to descriptions (Pichersky and Gang, 2000). Additionally, they contain significant ultraviolet (UV) absorption chemicals that protect the leaves from severe light damage. Some herbs used as feed grasses, such as clove or alfalfa, have been found to express estrogenic characteristics and interact with animal fertility. Traditional herbalists in Southern Nigeria have used tropical shrubs as ornamental and medicinal plants to manage and treat a variety of ailments (Onyegeme-Okerenta and Essien, 2021).

A substance with some biological action is referred to as a bioactive substance (Walia *et al*., 2019). A bioactive compound (or substance) affects a living creature directly through its physiological or cellular effects, as its name implies (the Greek word "bios" means "life" and the Latin word "activus" means dynamic or full of energy). Depending on the substance's nature, its dose, and its bioavailability, such impacts might be advantageous or harmful (Walia *et al.*, 2019). In light of this, bioactive food components are substances found in foods or dietary supplements that, in addition to meeting fundamental nutritional requirements, have the potentials to alter the health of both humans and animals that consume them (Bernhoft, 2010). Nutrients are typically not considered to be "plant bioactive compounds" in plants. Typically, bioactive substances in plants are created as secondary metabolites that are not essential for the plant's everyday operations (like growth), but they are crucial for competition, defence, attraction, and signalling (Bernhoft, 2010). Then, these substances can be referred to as secondary plant metabolites that have pharmacological or toxicological impacts on both people and animals. A wealth of available scientific evidence demonstrates that natural bioactive compounds render a number of diversified biological effects, such as antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic and vasodilatory actions, as well as anti-mutagenicity, anti-carcinogenicity and antiaging effects (Chandrasekara and Shahidi, 2018). About 80% of the population in Africa relies on herbal medicine for their main healthcare, and phytomedicine has been around for a while (Okigbo and Mmeka, 2006). Although modern medicine may be accessible, traditional medicine is still preferred for treating illnesses, possibly for historical or cultural reasons (Balogun *et al*., 2016). With renewed interest in phytochemicals, a flood of new research has been conducted to examine the antimicrobial activities of medicinal plants in Nigeria, which are possible sources of new antibiotics (Anyanwu and Okoye, 2017).

It has long been known that naturally occurring substances in plants have antioxidant activity (Sunday *et al.*, 2016). Interest in oxygen-containing free radicals in biological systems and their posited roles as causal agents in the aetiology of a range of chronic disorders has grown recently (Sunday *et al.*, 2016). The therapeutic potentials of medicinal plants as sources of antioxidants in preserving biochemical processes in the cells of the organisms containing them are thus being given consideration. Antioxidants contribute to the maintenance of the pro/antioxidant balance by neutralizing the oxygen-derived radical and nitrogen species (reactive oxygen species [ROS] and reactive nitrogen species [RNS]), which are accountable for the harmful processes in biological systems (Gautam *et al.*, 2011). ROS/RNS initiate radical reactions with

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membrane lipids, for instance, leading to oxidative stress conditions, which may include cellular defunctionalization and decompartmentalization ultimately leading to cell death (Gautam *et al.*, 2011). A number of illnesses' occurrence and development have been linked to the presence of free radicals in the human body.

In Nigeria, especially in the southeast, *L. africana* is a widespread plant used as food and medicine to manage a variety of human diseases (Sidiqat *et al.*, 2017). It is a 10–12-foot glabrous shrub. Submembraneous, obovate or oblanceolate-oblong, delicately and frequently very abruptly acuminate, constricted to the petiole, and generally cuneate at the base are the characteristics of the leaves. A species of climbing fig found in the Moraceae family is called *F. trichocarpa*. About 850 species belong to the family Moraceae genus Ficus (Nawaz *et al.*, 2019). Similarly, in the forests of tropical and subtropical areas, there are about 200 different types of woody ficus trees, shrubs, and vines. There are about 500 different species of ficus can be found in Asia and Australia. Some ficus species are grown both indoors and outdoors as decorative plants. Ficus species are utilized as a source of food and are high in nutritional value. Ficus species have been discovered to be abundant sources of phenolic acid and flavonoids, making them capable of defending against oxidative stress illnesses (Nawaz *et al.*, 2019). These plants' extracts have reportedly shown promise in the treatment of cancer, diabetes, piles, ulcers, dysentery, inflammation, and oxidative stress. This study evaluated the bioactive compounds and antioxidant activities of the dichloromethane leaf extracts of *F. trichocarpa* and *L. africana*.

MATERIALS AND METHODS

Sample collection and identification

The leaves of *F. trichocarpa* and *L. africana* (Figure 1) were collected from Etche Local Government of Rivers State. The leaf of *F. trichocarpa* was authenticated by Mr Ezekiel Tamunodiepiriye of the Department of Plant Science and Biotechnology, Rivers State University and deposited at the Rivers State University Herbarium with the Voucher number RSU/F2/126 while that of *L. africana* was authenticated by Dr. Ekeke Chimezie of the Department of Plant Science and Biotechnology Herbarium, University of Port Harcourt, and given the Voucher number UPH/P/373. They were air-dried at room temperature (29±1°C) for 3 weeks and then pulverized with the aid of Marlex Excellent grinder (Mumbai, India). The ground samples were then passed through a sieve of 0.5 mm pore size to obtain a fine uniform powder. The powdered samples were kept in an airtight container until required.

Ficus trichocarpa *Lasianthera africana*

Figure 1: Photographs of *Ficus trichocarpa* and *Lasianthera africana*

Sample Extraction:

Ten grams (10 g) of each of the powdered samples were weighed into a well-stoppered bottle and 20 mls of dichloromethane was added. The mixtures were vigorously agitated and were left to stand for 5 days. Each crude extract was collected by filtering into a quartz beaker and the process was repeatedly carried out for two more consecutive times. The combined aliquots of each extract collected were concentrated on a steam bath to about 5 ml. This was purified by passing through a Pasteur's pipette packed with silica gel and anhydrous sodium sulphate on a membrane and air-dried to about 2 ml for gas chromatographic analysis.

GC-MS Analysis of the leaf extracts of *F. trichocarpa* and *L. africana*

Extracts for phytochemical analysis were subjected to a sequential methylene chloride-n-hexane (1:1) clean up specifically for these analytes. One microliter (1µl) of the sample was injected into a gas chromatograph equipped with both a narrow- or wide-bore fused-silica capillary column and an electrolytic conductivity detector (GC/ELCD). GC–MS analysis was carried out using an Agilent 6890 gas chromatograph with a 5975 MS detector equipped 30-m x 0.25 mm or 0.32 mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1µm film thickness. (Agilent). The following temperature ramp was used: injector at 250 °C, oven initially at 200 °C, held for 1 min and heated to 230 °C (1.5 °C min^{-1}, then held for 10 min). The characterization and identification of phytochemicals, from the sample was completed in the SCAN mode with the m/z range varied from 35 to 450. The flow rate of the helium as carrier gas was 1 ml min^{-1}; manual injection; the injection volume was 1 µl. Interpretation of mass spectrum of GC-MS was done using database of National Institute Standard and Technology (NIST). The mass spectrum of unknown component was compared with the spectrum of the known component stored in the NIST library. Major components were identified with authentic standards recorded from computerized libraries film thickness (Ukwubile *et al*., 2019).

Identification of chemical constituents

Bioactive compounds present in the different extracts were identified based on GC retention

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time on HP- 5 column and matching of the spectra with computer software using the Chem-software attached to the MS library. Detection of compounds present in each leaf sample was confirmed using the database of the National Institute of Standards and Technology (NIST) which houses more than 62,000 patterns. The spectrum of the unidentified component was compared with the spectrum of the identified components stored in the NIST library. The name, molecular weight, structure of the components in the test material were then ascertained (Ukwubile *et al*., 2019).

Antioxidant Activities Assay

For FRAP assay, 150 µl of the standard solution (Trolox) and 150 µl of sample extract were allowed to react with 2850 µl of FRAP solution in different test tubes for 30 min in the dark. Thereafter, a coloured solution (ferrous tripyridyltriazine complex) was formed. Readings of the coloured solution of the standard and the sample were then taken at 593 nm. The concentration of FRAP content in the extract was reported as mg trolox equivalent (TE)/g extract (Oyaizu, 1986).

For DPPH radicals scavenging activity, 1mM solution of DPPH in ethanol and also 1mg/ml extract solution in ethanol were prepared, and 1.5ml of these solutions was added to 1.5 ml of DPPH (Mensor *et al*., 2001). The absorbance was measured at 517nm against the corresponding blank solution. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by the equation below:

$$\text{DPPH scavenged (\%)} = \frac{(\text{Acon} - \text{A test}) \times 100}{\text{Acon}}$$

Acon = the absorbance of the control reaction

A test = the absorbance in the presence of the sample of the extracts.

In the case of hydroxyl radical scavenging assay, different concentrations of extract (250, 500, 750 and 1000µg) were taken and 1ml of iron EDTA solution, 0.5ml of EDTA solution, 1ml of DMSO and 0.5ml of ascorbic acid were added to it. The mixture was incubated in a boiling water bath at 80 to 90°C for 15 min. After incubation, 1ml of ice cold Trichloroacetic acid (TCA) and 3 ml of Nash reagent were added and the reaction mixture was incubated at room temperature for 15 min. The absorbance was read at 412 nm. The % hydroxyl radical scavenging activity was calculated using the formula:

$$\text{\% HRSA} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100$$

where, HRSA is the Hydroxyl Radical Scavenging Activity, Abs control is the absorbance of control and Abs sample is the absorbance of the extract (Barry *et al*., 1987)

RESULTS AND DISCUSSION

The bioactive compounds present in the leaf extracts *of F. trichocarpa* and *L. africana* with their molecular weight, Retention Time (RT), Peak area (%), are shown in Tables 1-2 respectively. The most abundant bioactive compounds in the leaf of *F. trichocarpa* were Lobeline (28.67), Carpaine (27.81%), Squalene (27.62%), and Lupenone (27.30%) followed by Pelletierine, Sparteine and Campesterol (26.73%, 26.36% and 26.93% respectively). Other relatively abundant bioactive compounds were: Anabasine, Benzylisoquinoline, Pelargonidin, Malvidin, Eugenol, Phytol, phytic acid, Myrcene, Beta-Caryophyllene etc.

Similarly, phytochemical screening assessed on the leaf of *L. africana* has shown that the

leaf is rich in secondary metabolites such as alkaloids, terpenes, flavonoids and non-flavonoid phenolics. *L. africana* leaf contains more alkaloids than other secondary metabolites quantitatively. However, the leaf contains more of terpenes than other secondary metabolites qualitatively. The most abundant bioactive compounds in the leaf *L. africana* were Pelletierine (25.89%), Sparteine (25.53%) and Beta-Caryophyllene (25.17%) followed by Lobeline and Myrcene (24.83% and 24.27% respectively). Other bioactive compounds present are: Phytol, phytic acid, Anabasine, Benzylisoquinoline, Squalane, Resorcinol, bis(tert-butyldimethlsilyl),

Table 1: Bioactive Compounds in *F. trichocarpa* Leaf

Compound	R.T (min)	M.W (g/mol)	Peak Area (%)	Dev. (min)
2,2-Dimethoxybutane	8.845	118	1.78	39
3-Furaldehyde	9.357	96	3.36	51
Neocurdione	9.769	236	2.16	78
Maltol	10.874	122	0.17	48
1,2,3-propanetriol, 1-acetate	12.662	134	1.45	56
Caryophyllene	16.895	204.3	0.11	78
bata-Amyrene	19.422	410	3.01	52
Phytol	23.202	128.17	2.10	35
phytic acid	23.575	660.04	0.18	87
Myrcene	23.793	136.23	6.02	79
Beta-Caryophyllene	24.773	204.36	0.92	35
Lupenone	27.305	424.7	3.63	27
Squalene	27.624	410.7	2.18	42
Quinoline,3-methyl	13.429	143	6.47	64
Copaene	15.573	204	3.69	45
Delphinidin	16.341	303.24	5.13	62
Cyanidin	18.764	287.24	10.48	37
2-Pyrazoline, 1-isopropyl-5-methyl	20.338	126	4.13	48
Peonidin	21.473	301.27	8.72	23
Anabasine	22.256	162.3	1.55	24
Benzylisoquinoline	22.638	219.28	0.72	18
Pelargonidin	24.218	271.24	8.17	42
Sparteine	26.356	234.385	3.45	52
Pelletierine	26.733	141.21	1.11	24
Carpaine	27.813	478.7	1.59	39
Lobeline	28.674	337.455	0.52	51
Campesterol	26.931	400.7	0.72	38
Malvidin	22.951	331.2968	0.66	24
Eugenol	21.822	164.2	6.29	58
Resorcinol, bis(tert-butyldimethlsilyl)	20.731	246	3.44	45
Coumaric acid	14.954	164	1.28	28
Ethyl2,2-diethoxypropinate	10.313	190	4.83	33

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Table 2: Bioactive Compounds in *L. africana* Leaf

Compound	R.T (min)	M.W (g/mol)	Peak Area (%)	Dev. (min)
Borneol	8.254	154.25	4.73	38
Neocurdione	9.286	328.27	9.13	42
Maltol	10.325	122	3.19	49
Caryophyllene	13.628	204.3	5.47	93
Lupenon	14.789	424	1.92	22
bata-Amyrene	16.337	410	0.83	75
Squalane	18.869	422	6.76	76
Adonisine	21.698	1131.26	6.42	42
Phytol	22.374	128.17	0.64	83
phytic acid	22.782	660.04	4.18	89
Myrcene	24.270	136.23	2.39	42
Beta-Caryophyllene	25.174	204.36	9.79	19
Sparteine	25.526	234.385	1.45	46
Lobeline	24.826	337.455	6.91	13
Anabasine	19.748	162.3	2.18	82
Benzylisoquinoline	21.254	219.28	5.19	51
2-Pyrazoline, 1-isopropyl-5-methyl	17.764	126	1.43	72
Etoposide	14.341	588.557	1.44	42
Quinoline,3-methyl	10.985	143	0.26	17
Agrimophol	8.749	474.54	1.54	21
Bergenin	9.674	190	2.86	86
Coumaric acid	12.327	164	6.71	82
Aesculetin	12.695	204	1.62	43
Resorcinol, bis(tert-butyldimethlsilyl)	18.353	246	7.51	45
Eugenol	19.205	164.2	4.69	13
Pelletierine	25.884	141.21	0.76	53

The result of DPPH- scavenging activities showed that both *F. trichocarpa* and *L. africana* leaves exhibited high DPPH- scavenging activities in a dose-dependent manner (Figure 2). The DPPH-scavenging activity was more than 50% at a concentration of 100 µg/ml. However, aqueous leaf extract of *F. trichocarpa* exhibited a higher DPPH-scavenging activity than that of *L. africana* (69.52% and 52.08% respectively) at 100 µg/ml.

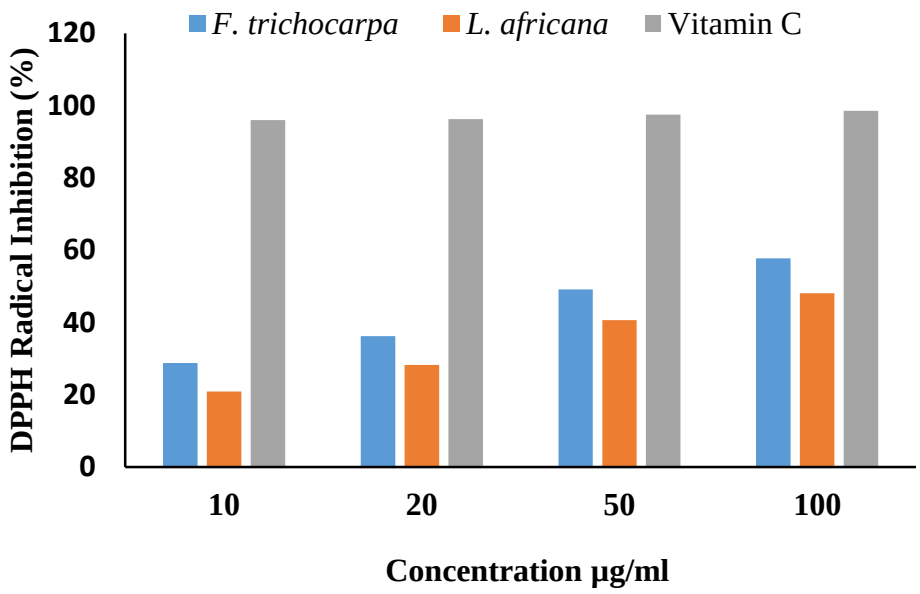

Figure 2: 2,2-DPPH activities of leaf extracts of *F. trichocarpa* and *L. africana*

The result of Ferric Reducing Antioxidant power (FRAP) assay showed that both the leaf of *F. trichocarpa* and *L. africana* exhibited high FRAP activities which were above 50% at the concentration of 100µg/ml and 200µg/ml: 67.84% and 51.69% respectively at 100µg/ml and 73.18% and 65.65% respectively at the concentration of 200µg/ml (Figure 3). As in the case of DPPH-scavenging activities, *F. trichocarpa* leaf exhibited a higher FRAP activity than *L. africana* leaf at all concentrations.

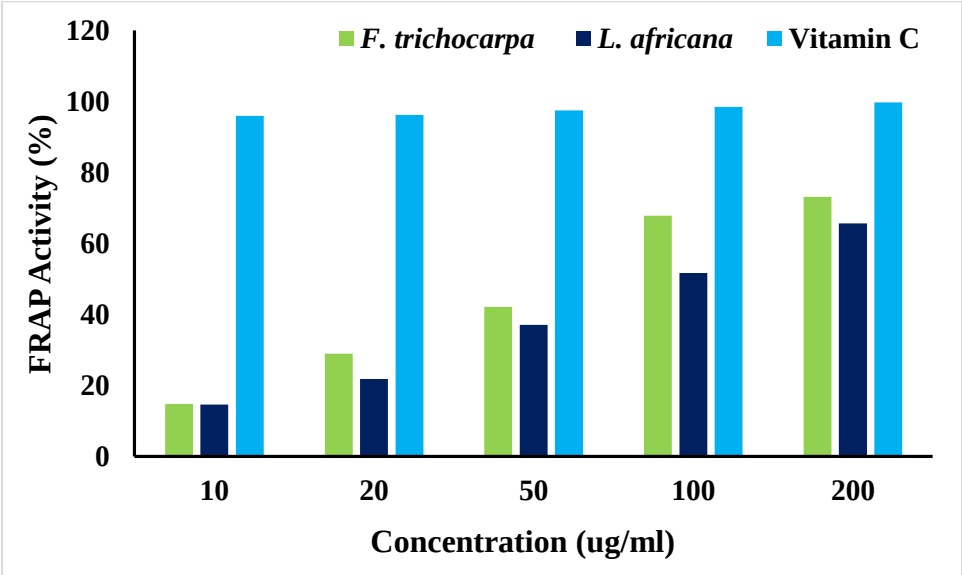

Figure 3: FRAP of leaf extracts of *F. trichocarpa* and *L. africana*

The result of Hydroxyl radical scavenging activity of the leaf extracts of *F. trichocarpa* and *L. africana* as presented in Figure 4 showed that both leaves exhibited high hydroxyl radical scavenging activities: 78.22% and 50.34% respectively at the concentration of 100µg/ml and 86.25% and 67.08 % respectively at the concentration of 200µg/ml.

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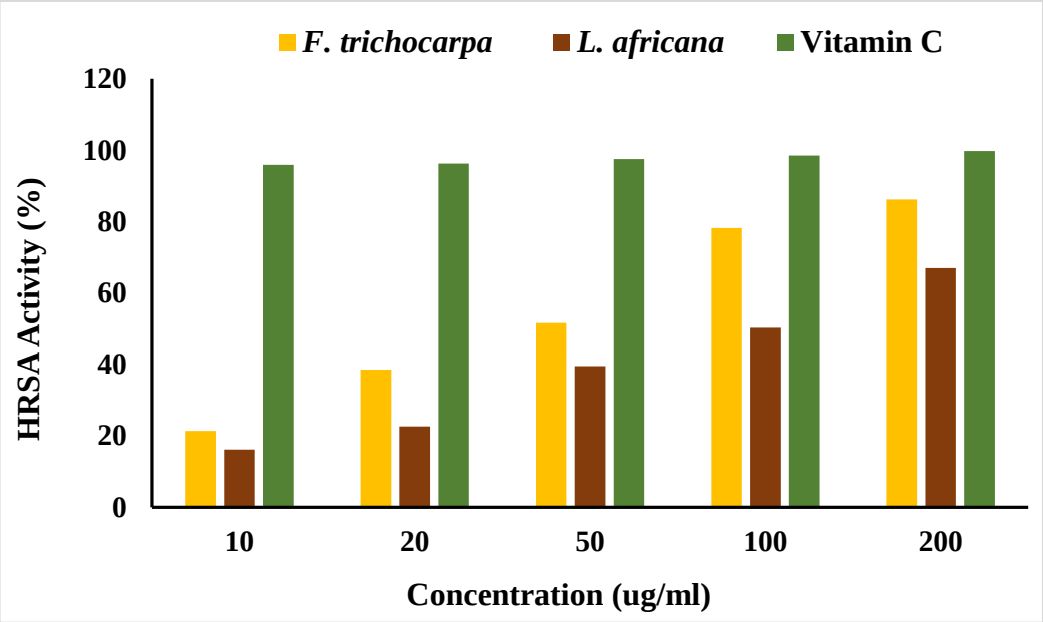

Figure 4: Hydroxyl radical scavenging activity of leaf extracts of *F. trichocarpa* and *L. africana*

DISCUSSION

Secondary plant metabolites are organic compounds produced by plants during secondary metabolism (Pagare *et al.*, 2015). They are generally the products of primary metabolites, and are produced from biosynthetic modifications, including methylation, glycosylation and hydroxylation (Kaisarun *et al.*, 2016). These compounds are often involved in plants protection against biotic or abiotic stresses, and a good number of them are used as chemicals such as drugs, flavours, fragrances, insecticides, and dyes and thus have a great economic value (Kaisarun *et al.*, 2016). The leaves of *F. trichorcarpa* and *L. africana* were subjected to phytochemical screening, which revealed that the leaves are abundant in secondary compounds like alkaloids, including Pelletierine, Sparteine, and Lobeline, terpenes, flavonoids, and non-flavonoid phenolics. Alkaloids were more in number than other secondary metabolites in the *F. trichocarpa* leaf both qualitatively and quantitatively. The second and third most prevalent secondary compounds were discovered to be terpenes and flavonoids, respectively. Similar to this, the quantitative amount of alkaloids in *L. africana* leaf is higher than that of other secondary compounds. Terpenes, however, are significantly more abundant in the leaf than other secondary metabolites.

Pelletierine (*Punica granatum*), lobelanine (*Lobelia inflata*), and piperine (*Piper nigrum*), alkaloids with the piperidine nucleus, have a typical biosynthetic route (Neugebauer *et al.*, 2007). Tanret first isolated *Pelletticine tannate* from pomegranates in 1878, and it has been used to treat human tapeworm infections with different degrees of success. The biosynthesis of many of the more complex lycopodium alkaloids begins with the parent lycopodium alkaloid, pelletticine (Wang *et al.*, 2020). Despite having a seemingly straightforward structure, the compound has received a lot of synthetic attention and has undergone multiple total synthesis. Quinolizidine rings are a common component of the natural product structures for alkaloids, such as lycopodium and other alkaloids. Similarly, sparteine is an

alkaloid and a sodium channel blocker that belongs to a family of antiarrhythmic drugs. It is believed to bind the bivalent cations calcium and magnesium and is the main alkaloid in *Lupinus mutabilis* (Golebiewski and Spenser, 1988). Also, The Indian tobacco plant alkaloid lobeline is a nicotinic receptor antagonist, a VMAT-2 function inhibitor, and it has a minimal affinity for the dopamine and serotonin transporters (Neugebauer *et al*., 2007). Another major alkaloid present in the leaf extracts of *F. trichorcarpa* and *L. africana* is Carpaine. Carpaine is reported to be one of the key alkaloid components of papaya leaves which have been studied for its cardiovascular effects. Circulatory effects of carpaine were studied in Wistar and it was reported that carpaine affects the myocardium directly. These effects may be related to its macrocyclic dilactone structure, a possible cation chelating structure (Burdick, 2006).

There are many different types of triterpenes in plants, and lupenone is one of these triterpenoids (Xu *et al*., 2018). Asteraceae, Balanophoraceae, Cactaceae, Iridaceae, Musaceae, Urticaceae, Leguminosae, Bombacaceae, etc. are among the many plant families that contain lupenone. Human life has a significant consumption of lupenone in vegetarian diets (Xu *et al.*, 2018). Lupenone showed a variety of pharmacological actions during pharmacological testing, including anti-inflammatory, antiviral, anti-diabetes, anti-cancer, and improving Chagas disease without significant toxicity. Squalene is also a triterpenoid with the formula C_3OH_5O. It is a colourless oil, although impure samples appear yellow. It was originally obtained from shark liver oil (hence its name, as Squalus is a genus of sharks). An estimated 12% of bodily squalene in humans is found in sebum. Squalene has a role in topical skin lubrication and protection (Micera *et al.*, 2020). The leaf extracts of *F. trichorcarpa* and *L. africana* contain squalene and can be used as a pharmacological agent.

A bicyclic sesquiterpene compound callad Beta-caryophyllene (BCP) is widely found in the plant kingdom. It gives essential oils a distinctive aroma and is important to the survival and development of higher plants (Scandiffio *et al*., 2020). It was discovered to be present in the leaf extracts of *F. trichorcarpa* and *L. africana* evaluated in this study. Recent research demonstrated the protective functions of BCP in animal cells, emphasizing its potential application as a cutting-edge therapeutic tool. According to experimental data, BCP has the ability to decrease pro-inflammatory mediators like tumour necrosis factor-alfa (TNF-), interleukin-1 (IL-1), interleukin-6 (IL-6), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-B), thereby improving chronic pathologies marked by inflammation and oxidative stress, especially neurological and metabolic diseases. BCP has been shown to have a beneficial effect on obesity, non-alcoholic fatty liver disease/non-alcoholic steatohepatitis liver diseases, diabetes, cardiovascular diseases, pain and other nervous system disorders. Beta-caryophyllene has strong anti-inflammatory, antimicrobial, antibacterial, and antioxidant effects because of its special capacity to bind with CB2 receptors. It has been effective in the treatment of seizures and osteoporosis (Scandiffio *et al.*, 2020).

Plants are known to contain a variety of natural antioxidants that protect and preserve their physical and metabolic integrity as well as their heredity by way of their seeds. Many of these extracts and compounds from plants are emerging as candidates for moderating the effects of the aging process on skin by limiting

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biochemical consequences of oxidation (Chaves *et al.*, 2020). DPPH is a free radical, stable at room temperature, which produces a violet solution in methanol. It is reduced in the presence of antioxidant molecules, making the colour of the solution to turn yellow (Al-Rimawi *et al.*, 2016). The use of DPPH provides an easy and rapid way to evaluate antioxidants. DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. The result showed that both *F. trichocarpa* and *L. africana* dichloromethane leaf extracts exhibited high DPPH-scavenging activities in a dose-dependent manner. This finding agreed with an earlier report by Motalleb *et al.* (2005) that the scavenging effects on the DPPH radical increase sharply with increasing concentration of the samples and standards. Several studies have reported that phenolic compounds are responsible for the DPPH scavenging activities of plant materials as evidenced by the positive correlation between DPPH activities and phenolic content (Sunday *et al.*, 2016).

Ferric Reducing Antioxidant (FRAP) assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and producing a coloured ferrous tripyridyltriazine ($Fe2^{+}$-TPTZ). The free radical chain breaking takes place through donating a hydrogen atom (Nilima and Hande, 2011). At low pH of about 3.6, reduction of Fe^{3+}-TPTZ complex to blue coloured Fe^{2+}-TPTZ takes place, which has absorbance at 593 nm (Bibhabasu *et al.*, 2008).

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage (Aurelia *et al.*, 2016). They were produced in this study by incubating ferric-EDTA with ascorbic acid and H_2O_2 at pH 7.4, and reacted with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with TBA at low pH. High antioxidant activities exhibited by aqueous leaf extracts of *F. trichocarpa and L. africana* may not be unconnected with their richness in phenolic compounds. Phenolic compounds constitute one of the major groups of compounds known to act as primary antioxidants or free radical terminators, which is why it is important to quantify the amount of these compounds in the selected species.

CONCLUSION

In conclusion, the results of the study have demonstrated that the dichloromethane leaf extracts of *F. trichocarpa and L. africana* are rich in secondary metabolites such as alkaloids, terpenes, flavonoids and non-flavonoid phenolics. The leaf extracts also have high antioxidant properties.

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