WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 1, 141-156. Research Article ISSN 2277– 7105

IN VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY PROFILES OF BIOACTIVE FRACTION FROM OPILIA CELTIDIFOLIA (GUILL. & PERR.) ENDL. EX WALP (OPILIACEAE)

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Article Received on
27 October 2018,
Revised on 17 Nov. 2018,
Accepted on 07 Dec. 2018
DOI: 10.20959/wjpr20191-11667

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ABSTRACT

Polyphenol contents, antioxidant and anti-inflammatory activities of bioactive fraction (ethyl acetate fraction) of (leaves, leafy stems and roots) from *Opilia celtidifolia* were investigated in this study in oder to provide a scientific basis for the traditional use of plants in Burkina Faso. Folin-ciocalteu, aluminium nitrate using rutin as the standard methods respectively were used to access phytochemical composition. The antioxidant potential of the samples was evaluate using three separate methods, hydroxyl radical scavenging assay, hydrogen peroxide scavenging assay and phosphomolybdate assay for the total antioxidant capacity of ethyl acetate fraction. For anti-inflammatory activity, the Human Red Blood Cell (HRBC) membrane stabilization method, Membrane stabilization test by heat induced hemolysis, proteinase inhibitory activity and lypoxygenase inhibition methods were used. In this study, the ethyl acetate fraction of leaves of *O. celtidifolia* exhibited the best results.

KEYWORDS: Polyphenol, antioxidant, anti-inflammatory, bioactive fraction.

INTRODUCTION

Traditional medicine, having a long history, is defined as the total sum of the knowledge, skills and practices based on the theories, believes and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses. The terms Complementary/alternative/non conventional medicines are used interchangeably with traditional medicine in some countries.[1] As it is increasingly believed now that traditional medicines become more popular worldwide, there is accumulating evidence suggesting medicinal plants are unlimited reservoirs of drugs. The amazing structural diversity among their active components makes them a useful source of novel therapeutics. Researchers with interest in natural products have intensified their efforts towards scientific evaluation of traditional medicines. The World Health Organization (WHO) estimates that herbal medicine is still the most common source for primary health care of about 75-80% of the world's population, mainly in the developing countries, because of better cultural acceptability, better compatibility with the human body and fewer side effects.[2] Therefore, the use of plants as source of remedies for the treatment of diseases date back to prehistoric times and people of on all continents have are used to this old tradition. In many countries plants are used as a source of medicines to treat infections and other disorders and some of the most powerful and potent drugs used nowadays were derived from plants.[3] Various parts of plant (root, fruit, stem, flower, modified plant organs and twigs exudates) having different therapeutic properties are used as herbal medicines. They are collected on a minute scale to utilize by folk healers and local communities, while several others are collected as raw material in larger amounts to trade them in the market for numerous herbal industries.[4]

Traditional Burkinabe medicine has been widely used to treat chronic diseases and its therapeutic benefits have been recognized for centuries. Although there is still a lack of evidence on the efficacy of many medicinal plants remedies, they are still widely accepted by the population, the majority of which are poor.[5] Our study focused on *Opilia celtidifolia*, a woody climber, spreading, heavily branched shrub or tree up to 10 m high, common in fringing forest and savanna. It is widespread in the region from Senegal to Nigeria (West Africa) and dispersed over the dried part of tropical Africa as Burkina Faso.[6;7] Ethnobotanical investigations have shown that *O. celtidifolia* was mostly identified by

traditional healers as an antimalaria, appetizer, and antidermatitis plant, constipation an abdominal pain killer and an intestinal worm cure and other diseases as well.[8] Some pharmacological studies revealed that a saponin fraction of a methanol extracts of the stem bark of *O. celtidifolia* possess the following properties: intestinal antispasmodic, uterine stimulant, hypotensive and depression of the coronary out flow, but has no effect on renal outflow. It activated to a great extent the activities of certain dog parasites (Taenia pisiformis and Toxoscaris leonani). It produced a fall in the blood pressure and an increase in respiratory rate. Moreover, the root powder is used in the treatment of constipation, jaundice, liver cirrhosis, anorexia, worming. The root decoction is purgative, diuretic. The decoction of the leaves and roots is aperitif, used in abdominal pain, edema, leprosy, meningitis.[9] Selon,[10] leaves of *O. celtidifolia* possess some healing properties. In Burkina Faso, *Opilia celtidifolia* Guill. & Perr. *(Opiliaceae)* are traditionally used against fever and malaria attacks.[7; 11; 12] It is well know that natural bioactive compounds in plants play a key role in plants defense system and are also well known for their unambiguous physiological action on human body. Amino acids, proteins common sugars and chlorophyll are plants primary metabolites whereas secondary metabolites comprise of flavonoids, alkaloids, tannins, saponins, and terpenoids. In view of the tremendous importance of secondary metabolites as therapeutic agents; they are becoming parts of the integrative health care system as alternative or supportive medicines.[13] Phytochemical analysis of the Opiliaceae specie study has mainly demonstrated the presence of saponosides, coumarins, steroids, tannins, polyphenols and flavonoids, alkaloids and active polysaccharides.[7; 10] The present work aimed to evaluate the phytochemical constituents and to investigate the antioxidant and inflammatory capacities. To do this, we undertook this study of bioactive fraction from this Opiliaceae in order to provide a scientific basis for the traditional use of this plant in Burkina Faso. These activities were determined in of bioactive fraction from roots, leaves and leafy stems for their medicinal importance in Burkina Faso.

MATERIALS AND METHODS

Plants material

The vegetable materials of *Opilia celtidifolia* Guill. & Perr. *(Opiliaceae)* were collected in August 2014 in Dedougou, 230 Km West of Ouagadougou, capital of Burkina Faso. This plant was botanically identified by Dr. Traoré Lassina from the plants Biology Department of the University of Koudougou.

Extraction

The field grown fresh samples were washed with tap water followed by distilled water to remove the adhering dust particles. After blotting, samples were air dried in shade. The dried plant materials (roots, leaves and leafy stems) were ground to fine powder and stored in clean air tight containers. A sample of 50 g was placed in the soxhlet and run by using 80% aqueous acetone (500 ml) in 1/10 ratio (w/v) for 24 h under mechanic agitation at room temperature. After filtration all the extracts were dried in vacuum rotary evaporator at 40°C under reduced pressure. Each of these extracts were weighed and stored at 4°C for further analysis.

Fractionation

Fifty grams (50g) of powdered plant materials were extracted with 80% aqueous acetone (500 ml) in 1/10 ratio (w/v) for 24 h under mechanic agitation at room temperature. After filtration, ethanol was removed under reduced pressure in a rotary evaporator at approximately 40°C. The aqueous extracts were subjected to sequential liquid-liquid extraction with oil ether, dichloromethane, ethyl acetate and n-butanol. Each fraction was then collected and concentrated to dryness under reduced pressure to obtain oil ether fraction (OEF), dichloromethane fraction (DCMF), ethyl acetate fraction (EAF) and n-butanol fraction (n-BF). The different fractions were freeze-dried by Telstar Cryodos 50 freeze-dryer. The fraction residues were packed in waterproof plastic flasks and stored at 4°C until use. After qualitative analysis of phytochemical constituents, it revealed that ethyl acetate fraction contents the most secondary metabolites than the other.

Determination of the phytochemical constituents of ethyl acetate fraction

Total Phenolic content

Total phenolic content was determined by the Folin- Ciocalteau method. Stock solution (1 $mgmL^{-1}$) of gallic acid was prepared in distilled water.[14] Different aliquots of extracts ranging from 10 to $100\mu gmL^{-1}$ were prepared. Ethyl acetate fraction weighing 100 mg in 1 mL of distilled water, from the above stock 0.1 mL was pipetted out into test tube. A volume of 1.5 mL FC reagent was added in each test tube and kept aside for 5 min and 4 mL of 1 M sodium carbonate solution was added and made up to 10 mL with distilled water. The mixture was allowed to stand at room temp for 30 min and the absorbance was measured at 738 nm. Gallic acid was used as reference and the results were denoted as µg gallic acid equivalent.

Total flavonoid content

Total soluble flavonoid content of the extracts was determined with aluminium nitrate using rutin as the standard.[15] 1 mg of the bioactive fraction was added to 1 mL of 80% ethanol. An aliquot of 0.5 mL of sample was added to test tubes containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 4.3 mL of 80% ethanol. The absorbance of the supernatant was measured at 415 nm after incubation at room temperature for 40 min. The total flavonoid content in the bioactive fraction was determined as rutin equivalent per gram by using the standard rutin graph.

Antioxidant activity

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured by the ability of the ethyl acetate fraction to scavenge the hydroxyl radicals generated by the Fe^{3+}- ascorbate - EDTA - hydrogen peroxide system.[16;17] The reaction mixture (1 mL) contained 100 µL of 2- deoxy -2 -ribose (28 mM in 20 mM KH2PO4 buffer, pH 7.4), 500 µL of the fraction (roots, leaves and leafy stems) at various concentrations (100 - 500 μgmL^{-1}) in buffer, 200 µL of 1.04 mM EDTA and 200 µM ferric chloride (1:1 v/v), 100 µL of 1.0 mM hydrogen peroxide (H_2O_2) and 100 µL of 1 mM ascorbic acid. Test samples were kept at 37°C for 1 h. The free radical damage imposed on the substrate deoxyribose was measured using the thiobarbituric acid test. About 1 mL of 1% thiobarbituric acid (TBA) and 1 mL 2.8% trichloroacetic acid (TCA) were added to the test tubes and were incubated at 100°C for 20 min. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Quercetin (100-500 μgmL^{-1}) was used as a positive control.

Hydrogen peroxide scavenging assay

Hydrogen peroxide solution ($2mML^{-1}$) was prepared with standard phosphate buffer (pH 7.4). Various concentrations of the ethyl acetate fraction (roots, leaves and leafy stems) (100-500 μgmL^{-1}) in distilled water were added to 0.6 mL of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the extracts was determined and the IC_{50} values were compared with the standard, α-tocopherol.[18]

Phosphomolybdate assay

The total antioxidant capacity of the ethyl acetate fraction (roots, leaves and leafy stems) was determined by phosphomolybdate method using α-tocopherol as the standard.[19] An aliquot of 0.1 mL of the ethyl acetate fraction (100 µg) solution was combined with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in boiling water bath at 95°C for 90 min. The samples were cooled to room temperature and the absorbance was measured at 695 nm against the blank. The total antioxidant capacity was expressed as gallic acid equivalent per gram.

Anti-inflammatory activity of ethyl acetate fraction

The Human Red Blood Cell (HRBC) **membrane stabilization method**

Fresh whole human blood (5 mL) was collected and transferred to the centrifuged tubes containing Heparin or EDTA or Sodium citrate to prevent clotting. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.[20] The reaction mixture consists of 1.0 mL of test sample of different concentrations (100µg –300 µg) in normal saline and 0.5 mL of 10% HRBC suspension, 1 ml of 0.2 M phosphate buffer, 1 ml hyposaline were incubated at 37^{0}C for 30 min and centrifuged at 3,000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as standard and a control was prepared without extracts. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following formula.[21]

% Hemolysis = (Optical density of Test sample/Optical density of Control) x 100

% Protection =100–[(Optical density of Test sample/Optical density of Control) x100]

Membrane stabilization test by heat induced hemolysis

The reaction mixture in heat induced hemolysis consists of 1.0 mL of test sample of different concentrations (100µg – 300µg) in normal saline and 1.0 mL of 10% RBC suspension, instead of test sample, only saline was added to the control. Diclofenac sodium was taken as a standard drug. All the tubes containing reaction mixture were incubated in a water bath at 56^{0}C for 30 min. After incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates. The

percentage of heat induced HRBC hemolysis and membrane stabilization or protection was calculated by using the following Formula.[22]

% Hemolysis = (Optical density of Test sample / Optical density of Control) x 100

%Protection =100–[(Optical density of Test sample/Optical density of Control) x100]

Proteinase inhibitory activity

The reaction mixture contains 1.0 mL of test sample of different concentrations (100µg – 300 µg), 1.0 mL of 20 mM Tris HCl buffer (pH 7.4) containing 0.06 mg trypsin, and the mixture was incubated at 37^0C for 5 min and then 1.0 mL of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. Diclofenac sodium was used as standard drug. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was determined.[23]

Lipoxygenase inhibitory assay

Lipoxygenase inhibiting activity of plant extracts with linoneic acid as a substrate was measured with a UV/visible light spectrophotometer as described by[24] with some modifications. Fractions were screened for lipoxygenase inhibitory activity at some final concentrations (100µg – 300µg/ml). The mixture assay consisted of 150 µl phosphate borate (1/15 M, pH 7.5), 50 µl fraction solution and 50 µl enzyme solutions (0.28 U/ml in phosphate borate). The reaction was initiated by adding 250 µl of substrate solution (0.15 mM in water). Enzymatic kinetic was recorded at 234 nm for 02 min. Negative control was prepared and contained 1% methanol solution without extract solution. All experiments were performed in triplicate. Lipoxygenase inhibitory activity was expressed as the percentage inhibition of lipoxygenase, calculated as (%) inhibition following equation:

(%) inhibition = (E - S/E) × 100, where E is the change in absorbance of the assay without the plants extracts and S is the change in absorbance of the assay with the plants extracts.

Statistical analysis

The data were expressed as Mean±Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $p<0.05$ and linear regression) was carried out with XLSTAT 7.1.

RESULTS

Polyphenols contents

Total phenolic content of the different extracts were expressed as µg gallic acid equivalent. The content of the total phenols in the ethyl acetate fraction decreased in the order of leaves > leafy stems > roots (Table 1). The total flavonoid content of the ethyl acetate fraction of leaves, leafy stems and roots were 28.32±0.01mg RE/g, 23. 62±0.68 mg and 5.31±0.54 mg-rutin equivalent per gram respectively (Table 1).

Antioxidant activity

The measures of antioxidant activity were obtained using three described methods. Hydroxyl radical scavenging activity was quantified by measuring the inhibition of the degradation of deoxyribose by the free radicals generated by the Fenton reaction. The Hydroxyl radical scavenging activity of the ethyl acetate fraction of leaves was very similar to the (quercetin, 0.30mgmL^{-1}). The IC_{50} values of the leafy stems and roots fractions were 0.33 and 0.37 mgmL^{-1} respectively (Table 2).

For scavenged hydrogen peroxide, *Opilia celtifolia* extracts scavenged hydrogen peroxide in a concentration - dependent manner. The ethyl acetate fraction of leaves exhibited hydrogen scavenging activity (IC_{50}= 0.17 mgmL^{-1}) whereas the standard, α-tocopherol had potent scavenging activity with 0.065 mgmL^{-1}. The leafy stem and root extracts showed moderate scavenging activities in comparison with standard (Table 2).

The phosphomolybdate method is quantitative, since the total antioxidant capacity is expressed as gallic acid equivalents. Among the extracts tested, the leaves extract contained 47.12 mg GAE/g. The antioxidant activity increased in the order of leaves > leafy stems > roots (Table 2).

Anti-inflammatory capacity of bioactive active fraction

The human red blood cell (HRBC) membrane stabilization method

The ethyl acetate fraction of (leaves, leafy stems and roots) *O. celtidifolia* was studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. Among all the concentrations, 300 µg/ml of ethyl acetate fraction showed significant anti-inflammatory activity and 80.18% protection of HRBC in hypotonic solution. Results were compared with standard diclofenac which showed 81.45% protection and presented in Table 3.

Hence, all the fractions and particularly leaves and leafy stems fractions had potential for anti-inflammatory activity when compared with Diclofenac used as standard (Table 3).

Membrane stabilization test by heat induced hemolysis

The percentage of hemolysis and protection of fractions extracts is represented in Table 4. The maximum percentage protection was recorded as 73.61 and minimum percentage 61.90 at the concentrations of 300µg/ml and 100µg/ml from plant extracts respectively. The maximum percentage protection was recorded 77.53 and minimum percentage 64.24 at the concentrations of 300µg/ml and 100µg/ml from STD respectively.

Proteinase inhibitory activity

Fraction extracts of *O. celtidifolia* have exhibited proteinase inhibitory activity at various concentrations significantly. The maximum inhibition of fraction was observed at 300µg/ml concentration as 67.45% and minimum at 100µg/ml concentration as 64.28% respectively and standard has shown maximum inhibitory activity at 300µg/ml concentration as 79.67% and minimum at 100µg/ml concentration as 65.17% respectively (Table 5).

Lipoxygenase inhibitory assay

The fraction extracts tested for *in vitro* inhibition of the enzyme soybean 15-lipoxygenase had varying activity presented as percentage inhibitions in Table 5. The most activity was obtained by leaves fraction with 83.45 µg/ml followed by roots fraction with 80.6 µg/ml at 300µg/ml concentration. The activity of the fractions was concentration-dependent and the percentage inhibition ranged from 83.45 to 46.99 µg/ml (Table 5).

Table 1: Polyphenols contents of ethyl acetate fraction.

Ethyl acetate fraction	Total phenol (mg GAE/g)	Total flavonoid (mg RE/g)
Leaves fraction	62.01 ±0.62[a]	28,32±0.01[a]
Leafy stems fraction	53.23±0.18[b]	23,62±0.68[b]
Roots fraction	39.45±0.01[c]	5,31±0.54[c]

Values are Mean ±SD (n=3). Different in the same column indicate significant difference ($P<0.05$) for our different extracts. mg GAE/g= mg equivalent Gallic acid for 1g dried extracts; mg RE/g= mg equivalent Rutin for 1 g dried extracts.

Table 2: Antioxidant Properties of ethyl acetate fraction.

Extracts	Hydroxyl radial scavenging (IC_{50} mg/mL)	Hydrogen peroxide scavenging (IC_{50} mg/mL)	Total antioxidant assay (mg GAE/g)
Leaves fraction	0.35±0.96[b]	0.17±0.01[a]	47, 12±0.01[a]
Leafy stems fraction	0.33±0.48[a]	0.36±0.01[b]	42, 23±0.02[b]
Roots fraction	0.37±0.54[c]	0.50±0.01[c]	23, 33±0.03[c]
Quercetin	0.30±0.01	---------------------	----------------
A-tocopherol	---------------------	0.07±0.62	----------------

Values are Mean ±SD (n=3). Different in the same column indicate significant difference (P<0.05) for our different extracts. mg GAE/g= mg equivalent Gallic acid for 1g dried extracts.

Table 3: Anti-inflammatory activity: The human red blood cell (HRBC) membrane stabilization method.

Extracts and reference compound	%Haemolysis			%Protection		
	100 µg/mL	**200 µg/mL**	**300 µg/mL**	**100 µg/mL**	**200 µg/mL**	**300 µg/mL**
Leaves fraction	41.23±1.53	34.31±1.15	20.63±1.31	60.29±1.00	66.12±1.02	80.18±1.84
Leafy stems fraction	47.26±1.00	35.22±0.60	22.89±1.84	52.94±1.01	64.02±1.00	79.03±1.00
Roots fraction	56.11±0.58	45.51±1.00	38.36±0.58	44.84±1.15	55.27±0.58	62.01±0.00
STD	32.42±1.84	25.29±0.58	18.31±1.00	67.14±0.00	74.26±0.60	81.45±1.53

STD= Diclofenac

Values are Mean ±SD (n=3). Different in the same column indicate significant difference (P<0.05) for our different extracts.

Table 4: Heat induced HRBC membrane stabilization test of fractions and diclofenac.

Extracts and reference compound	%Haemolysis			%Protection		
	100 µg/mL	**200 µg/mL**	**300 µg/mL**	**100 µg/mL**	**200 µg/mL**	**300 µg/mL**
Leaves fraction	44.30±1.00	35.02±1.53	22.18±0.60	55.19±1.15	63.79±1.00	73.61±1.00
Leafy stems fraction	50.13±1.01	40.03±0.60	31.45±0.58	49.82±1.31	58.94±1.10	66.61±0.00
Roots fraction	49.24±0.00	43.27±1.00	36.52±1.53	54.60±1.00	56.12±1.31	61.90±0.58
STD	34.22 ±0.58	26.17±1.22	19.42±1.84	64.24±0.00	72.44±1.84	77.53±0.60

STD= Diclofenac

Values are Mean ±SD (n=3). Different in the same column indicate significant difference (P<0.05) for our different extracts.

Table 5: Proteinase inhibitory and Lipoxygenase inhibitory activities of fractions and diclofenac or quercetin.

Extracts and reference compound	%Inhibition of Proteinase			%Inhibition of Lipoxygenase		
	100 µg/mL	200 µg/mL	300 µg/mL	100 µg/mL	200 µg/mL	300 µg/mL
Leaves fraction	54.13±0.16	61.01±0.02	67.45±0.31	47.82±0.24	58.82±0.16	81.35 ±1.65
Leafy stems fraction	46.35±1.22	59.21±1.65	64.28±1.01	46.85±0.22	56.17±1.72	79.15±0.31
Roots fraction	45.34±0.24	57.27±1.22	65.31±1.65	46.99±1.84	52.38±0.50	80.6±0.31
STD	65.17±0.22	72.29±1.02	79.67±0.22	----------	-------------	------------
Quercetin	----------	----------	-----------	52.74±0.22	59.87±1.65	72.31±1.01

STD= Diclofenac

Values are Mean ±SD (n=3). Different in the same column indicate significant difference (P<0.05) for our different extracts.

DISCUSSION

A characteristic feature of higher plants is their capacity to synthesize an enormous variety of organic molecules. The production and accumulation of a wide variety of organic chemicals is one major mechanism by which plants defend themselves against herbivores, and attacks by microbial pathogens and invertebrate pests. Most of these chemicals are products of the secondary metabolism, originally thought to be waste product not needed by the plants for primary metabolic functions. It is, however, well known that their presence in different parts of the plant (root, bark, leaves etc) deters feeding by slugs, snails, insects and vertebrates, as well as attacks by viruses, bacteria and fungi.[25] However, it was the potential use of plant secondary metabolites in health care and personal care products, and as lead compounds for development of novel drugs, that lead to a huge interest in their isolation and characterization from major plant species. At present, the total number of identified secondary metabolites exceeds 100,000. These can be grouped into three main chemical classes: Phenolic, Nitrogen containing compounds and terpenes.[26] Our results showed that ethyl acetate fraction of leaves from *O. Celtidifolia* presented the highest amount of polyphenol content than the other types of fractions in the study. The abundance of polyphenols in our extracts may explain their utility the inflammatory and infectious diseases treatment.[7] Some secondary metabolites such as flavonoids, are used to treat viral diseases.[27] Then, flavonoids attenuate the infection or affect the intracellular replication of other viruses such as respiratory syncytial virus, herpes simplex virus and adenovirus. The abundance of fraction extracts in polyphenol content should also explain the antioxidant activity results. It is well known that, phenolics constitute one of the major groups of compounds antioxidants.[28] A recent study show that flavonoids however are phytoconstituents which consist of large group of

polyphenolic compounds having a benzo – y – prone structure and are abundantly present in plants. Flavonoids are proved for its high antioxidant activity *in vitro* & *in vivo*.[29] Regarding the antioxidants properties, we have tested three methods for a best appreciation of our results; because a recent study demonstrates that there are differences between the test systems for the determination of the antioxidants properties.[30,31] Analysis of our results shows a linear relationship between polyphenol content and antioxidants properties.[32,33,34]

Concerning antioxidant activity by the reducing ability of a compound generally depends on the presence of reductones, which exert antioxidant activity by breaking free radical chain culminating in donating a hydrogen atom.[18] The antioxidant principle present in the fraction extracts of *O. celtidifolia* caused the reduction of Fe^{3+}/ ferricyanide complex to the ferrous form, and thus proved the reducing ability.[35] The hydroxyl radical scavenging activity of leaves fraction was effectual than other fractions. Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but it is an intracellular precursor of hydroxyl radicals which is very toxic to the cell.[36] The leaves fraction extract scavenged hydrogen peroxide, which may be attributed to the presence of phenolic group that donate electrons to hydrogen peroxide there by neutralizing it into water, as opined by.[37] The antioxidant activity by phosphomolybdenum method exhibited higher antioxidant activity of leaves fraction in comparison with other fractions.

About anti-inflammatory activity, we noticed that fraction extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of RBC membrane. The RBC membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is play an important role in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release.[38]

The mechanism of anti-inflammatory activity of ethyl acetate fraction extracts of *O. celtidifolia* was studied by RBCs membrane stabilization at various concentrations. All the concentrations were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This anti-inflammatory effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation.[39] The plant extract exhibited the presence of cardiac glycosides are known to possess potent anti-inflammatory activities.[39]

Ethyl acetate fraction extracts of *O. celtidifolia* have exhibited proteinase inhibitory activity at various concentrations significantly. Neutrophils are most important source for proteinases which carries in their lysosomal granules and are involved in arthritic reactions. Proteinases have been implicated in arthritic reactions. It was already reported that leukocytes proteinase play important role in the development of tissue damage during in inflammatory reactions and significant level of protection was provided by proteinase inhibitors. Recent studies have shown that many flavonoids and related polyphenols contributed significantly to the anti-inflammatory activities of many plants.[39] Due to the presence of bioactive compounds such as flavonoids, saponins, phenols, tannins and cardiac glycosides in the extract certainly may contribute in its anti-inflammatory activity. Therefore, we could say that *O. Celtidifolia* has inflammatory activity because this extract has an inhibition of more than fifty percent. [24] The results allowed us to make a link between flavonoids and anti-inflammatory activity by the lipoxygenase inhibition method. Anti-inflammatory activity of our fraction extracts could be explained by the abundance of the extracts in polyphenol content because, polyphenol as flavonoids have anti-inflammatory properties.[40]

CONCLUSION

We should keep that among our fraction extracts, leaves fraction presented the best results in polyphenol contents, antioxidants properties and anti-inflammatory activity. Antioxidant and anti-inflammatory activities may be due to the presence of many phytochemical in the extract. However, further studies are required to identify the lead molecule in the extract and to study the action of mechanism.

DECLARATION OF INTEREST

The authors report no declarations of interest.

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