CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.3360753

Available online at: http://www.iajps.com *Research Article*

EVALUATION OF IN VITRO ANTI-INFLAMMATORY AND ANTIOXIDANT POTENTIALS OF METHANOL LEAF EXTRACT OF FAGARA ZANTHOXYLOIDES

Enechi Osmund Chukwuma[1], Okeke Emmanuel Sunday[1,2*], Abonyi Uchenna Collins[1], Nwankwo Nicodemus Emeka[1,2], Ndimele Ugochi Joy[1], Benneth Moses Ebere [1].

[1]Department of Biochemistry, University of Nigeria, Nsukka, Enugu State Nigeria, [2]Natural Science Unit, School of General Studies, University of Nigeria, Nsukka, Enugu State, Nigeria.

Article Received: June 2019 **Accepted:** July 2019 **Published:** August 2019

Abstract:

Background: *Fagara zanthoxyloides is a medicinal plant used in traditional medicine for the treatment of elephantiasis, toothache, sexual impotence, malaria, dysmenorrheal and abdominal pain among rural dwellers.*

Objective: *To study the In vitro anti-inflammatory and antioxidant activities of methanol extract of Fagara zanthoxyloides leaves.*

Materials and Methods: *Methanol extract of Fagara zanthoxyloides leaves were used for this study. In vitro anti-inflammatory studies were performed for the extract using proteinase inhibition activity and albumin denaturation inhibition assays, while antioxidant activities were performed by determination of DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging assay and hydrogen peroxide scavenging activity. Aspirin was used as a standard drug for the anti-inflammatory activity while vitamin C and E were used as standard drugs for the determinationof DPPH scavenging assay and hydrogen peroxide scavenging activity respectively.*

Results: *At the concentration of 75 and 100 µg/ml, the methanol extract of Fagara zanthoxyloides showed significant (p < 0.05) inhibition of 39 and 44% of proteinase inhibitory action and, 42 and 63% of albumin denaturation inhibition activity. In addition, the extract had effective DPPH radical scavenging and hydrogen peroxide scavenging activities. At a concentration of 80 µg/ml, the extract showed an inhibition of 53 and 77% of DPPH radical scavenging and hydrogen peroxide scavenging activities respectively.*

Conclusion: *The present study showed In vitro antioxidant and anti-inflammatory activities of the methanol extract of Fagara zanthoxyloides which scientifically prove the ethnomedicinal claims.*

Keywords: *Fagara zanthoxyloides, anti-inflammatory, antioxidant, protease inhibition, albumin denaturation, DPPH , hydrogen peroxide.*

Corresponding author:

Okeke Emmanuel Sunday,

Department of Biochemistry, University of Nigeria, Nsukka.

Natural Science Unit, School of General Studies, University of Nigeria, Nsukka.

Tel: +2348035277554 Email: emmanuel.okeke@unn.edu.ng

QR code

Please cite this article in press Okeke Emmanuel Sunday et al., ***Evaluation of in Vitro Anti-Inflammatory and Antioxidant Potentials of Methanol Leaf Extract of Fagara Zanthoxyloides.,*** *Indo Am. J. P. Sci, 2019; 06(08).*

INTRODUCTION:

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. Protein denaturation is a process in which proteins lose their tertiary and secondary structure by application of external stress or compounds such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological activities or functions when denatured. Denaturation of protein is a well documented cause of inflammation [1] [2]. Also, neutrophils are known to be a rich source of serine protease and it was previously reported that leukocytes protease play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by protease inhibitors [3]. Non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin, are among the most co mmonly recommended and prescribed drugs for treating anti-inflammation in the world. Antioxidants are chemical compounds that can scavenge free radicals that are formed in the body due to normal physiological process. These free radicals initiate a chain reaction which leads to the formation of various other free radicals leading to oxidative stress which in turn results in the production of reactive oxygen species and reactive nitrogen species causing lipid peroxidation (LPO) and cellular damage. These free radicals are also capable of disintegrating various biomolecules such as nucleic acids, proteins and lipids. Compounds showing scavenging activities towards these ROS may have therapeutic potentials towards inflammatory diseases.

Medicinal plants play an appreciable role in the development of modern herbal medicines as many diseases like cancer, liver diseases and arthritis find no complete cure in allopathy. The bioactive compounds of medicinal plants are used as anti diabetic, chemotherapeutic, anti inflammatory, anti arthritic agents, where no satisfactory cure is present in modern medicines. Medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases without proper knowledge of their function. Although physiotherapy continues to be used in several countries, few plants have received scientific or medical scrutiny [4]. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the World. Among these 2,500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as Botanical garden of the World [5].

Fagara zanthoxyloides, which is the plant of interest in this study, is a medicinal plant that belongs to the family of Rutaceae. It is commonly called 'toothache bark' or 'candle wood' (English), Orin ata (Yoruba). It is widespread in the West Tropical Africa occurring in Savanna and dry forest areas. The plant is also found in Coastal areas. *Fagara zanthoxyloides* is known for its antioxidative, anti-inflammatory, antisickling, antibacterial, antiviral, antihepatotoxicity, antiallergic, antitumoral and antihypertensive properties [6] [7]. The present study involves the evaluation of the *In vitro* anti-inflammatory and anti-oxidant activities of the ethanol extract of *Fagara zanthoxyloides* leaves.

MATERIALS AND METHODS:

Collection of plants:

Fresh leaves of *F. zanthoxyloides* were collected, from Ugwu-Awgbu in Orumba North Local Government Area of Anambra State, Nigeria. The fresh leaves were authenticated by Mr. Alfred Ozioko of the Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State.

Preparation of extract:

The plant material was shade-dried with regular turning to avoid decaying, until crispy. The dried leaves were pulverised into powdered form using a mechanical grinder. A known weight of the pulverized leaves (1.5kg) was macerated in 95% methanol using a maceration flask. The mixture was left for 72 hours with occasional stirring, after which it was filtered into a flat bottomed flask. The filtrate were concentrated using a rotary evaporator at 45°C to obtain the crude methanol extract. The concentrated extract was stored in a labeled sterile screw-capped bottle at 2-8°C.

Assessment of *In vitro* anti-inflammatory activity:

Inhibition of albumin denaturation:

According to previously reported protocol [8, 9], with slight modification. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction was adjusted using small amount of 1N HCl. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Aspirin was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

Protease inhibition assay:
The test was performed according to the method of Oyedepo and Femurewas [10] and Sakat *et al.* [9]. The reaction mixture was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (10 – 100 µg/ml). The mixture was incubated at room temperature for 5 min and then 1 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 arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

$$\%\ Inhibition = \left(\frac{Abs\ control - Abs\ sample}{Abs\ control}\right) X\ 100$$

Determination of antioxidant efficacy:
DPPH scavenging assay:
The DPPH free radical scavenging assay was performed by the method of Liyana- Pathirana and Shahidi [11], 200 µL of 0.1 mM DPPH prepared in methanol was added to 100 µL of the plant extract. The resulting mixture was incubated at room temperature in the dark for 15 minutes. Absorbance was observed at 517 nm. The experiment was carried out in triplicates and percentage inhibition of the DPPH radical scavenging activity was calculated;

$$\%\ Inhibition = \left(\frac{Abs\ control - Abs\ sample}{Abs\ control}\right) X\ 100$$

Hydrogen peroxide scavenging activity:
This activity of the plant was evaluated by the method of Ruch *et al.* [12]. 850 µL of the aqueous plant extract was added to 150 µL of 4 mM hydrogen peroxide solution prepared in phosphate buffer (0.1 M, pH-7.4). This was incubated for 10 minutes, and absorbance was read at 230 nm. The reaction was carried out in triplicates. Percent inhibition of the assay was calculated;

$$\%\ Inhibition = \left(\frac{Abs\ control - Abs\ sample}{Abs\ control}\right) X\ 100$$

RESULTS:

Effect of methanol extract of *F. zanthoxyloides* leaves on Inhibition of albumin denaturation:
The methanol extract of *F. zanthoxyloides* leaves was effective at inhibiting albumin denaturation. At a concentration of 100 µg/ml, there was a non-significant ($p > 0.05$) difference between the % inhibition of the extract and the standard drug which showed 63% and 68% respectively. However, a significant ($p < 0.05$) difference was observed between the standard drug at 200 µg/ml and the extract at 100 µg/ml which showed 83% and 63 % respectively.

Table 1: Effect of methanol extract of *F. zanthoxyloides* leaves on albumin denaturation

Treatments	Concentrations (µg/ml)	Absorbance at 660nm	% inhibition of albumin denaturation
Control	…….	0.103 ± 0.006^{f}	…….
Extract	10	0.090 ± 0.002^{e}	13
	25	0.082 ± 0.002^{d}	20
	50	0.067 ± 0.002^{c}	35
	75	0.060 ± 0.001^{c}	42
	100	0.038 ± 0.004^{b}	63
Standard drug	100	0.033 ± 0.007^{b}	68
(Aspirin)	200	0.018 ± 0.003^{a}	83

Data are represented in means ± SD; (n=3). Values with different letters as superscripts are significantly different at $P < 0.05$.

Effect of methanol extract of *F. zanthoxyloides* leaves on Proteinase inhibition:
The methanolic extract of *F. zanthoxyloides* leaves was effective at inhibiting protease activity. At a concentration of 100 µg/mL, the extract showed a higher % inhibition of protease activity, 44% when compared to the standard drug which showed a % inhibition of 42% at the same concentration.

Table 2: Effect of methanol extract of *F. zanthoxyloides* leaves on protease inhibition

Treatments	Concentration (µg/mL)	Absorbance At 660nm	% inhibition of Protease activity
Control	…….	2.170 ± 0.600^{f}	…….
Extract	10	1.442 ± 0.002^{e}	34
	25	1.372 ± 0.002^{d}	37
	50	1.345 ± 0.004^{d}	38
	75	1.325 ± 0.002^{c}	39
	100	1.207 ± 0.002^{a}	44
Standard drug (Aspirin)	100	1.250 ± 0.001^{b}	42
	200	1.227 ± 0.006^{a}	43

Data are represented in means ± SD; (n=3). Values with different letters as superscripts are significantly different at $P < 0.05$.

DPPH Radical Scavenging Activity of Leaf Extracts:
From the table below, the plant extract at different concentrations (5, 10, 20, 40, 80 µg/ml) inhibited significantly ($p < 0.05$) oxidative stress caused by DPPH radicals when compared to the control. There was a decrease in the mean absorbance value and increase in the percentage inhibition of DPPH radical scavenging activity with increased extract concentration. A standard antioxidant (Vitamin C) followed a similar trend.

Table 3: DPPH Radical Scavenging Activity of Leaf Extracts

Treatments	Concentration (µg/ml)	Absorbance At 517nm	% inhibition
Control	…….	0.979 ± 0.600^{e}	…….
Extract	5	0.776 ± 0.006^{d}	20
	10	0.756 ± 0.009^{d}	23
	20	0.656 ± 0.027^{c}	33
	40	0.548 ± 0.007^{b}	44
	80	0.461 ± 0.004^{a}	53
Standard drug (Vitamin C)	100	0.534 ± 0.003^{b}	45

Data are represented in means ± SD; (n=3). Values with different letters as superscripts are significantly different at $P < 0.05$.

Hydrogen peroxide scavenging activity of the leafe extract:

Table 4 represents the hydrogen peroxide scavenging activity of the methanol extract of *Fagara zanthoxyloides*. The plant extract of different concentrations (20, 40, 60, 80, 100 µg/ml) protected significantly ($p < 0.05$) against H_2O_2 radicals when compared to the control. A concomitant increase in percentage inhibition as observed as the concentration of the extract increased with a decrease in absorbance. A standard antioxidant followed a similar trend.

Table 4: Hydrogen peroxide scavenging activity of the leafe extract

Treatments	Concentration (µg/ml)	Absorbance At 230nm	% inhibition
Control	…….	0.772 ± 0.002^{F}	…….
Extract	20	0.337 ± 0.003^{e}	56
	40	0.217 ± 0.008^{c}	72
	60	0.209 ± 0.004^{c}	73
	80	0.139 ± 0.017^{b}	77
	100	0.099 ± 0.002^{a}	61
Standard drug (Vitamin E)	80	0.299 ± 0.022^{d}	87

Data are represented in means ± SD; (n=3). Values with different letters as superscripts are significantly different at $P < 0.05$.

DISCUSSION:

In recent years, the search for medicinal plants possessing antioxidant and anti-inflammatory properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging [13]. Due to risk of adverse effects encountered with the use of synthetic agents, medicinal plants may offer an alternative source for agents with significant anti-inflammatory and antioxidant activities.

Denaturation of proteins is a well documented cause of inflammation. Agents that can prevent protein denaturation, therefore, would be possible candidate for anti-inflammatory drug development. In the present study, the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of methanol extract of *F. zanthoxyloides* with a wide range of dose concentrations. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the extract throughout the concentration range of 10-100 µg/ml). Aspirin (at the concentration range of 100-200 µg/ml) was used as the standard drug, which also exhibited concentration dependent inhibition of protein denaturation. The increased absorbance in both the extracts and the standard drug with respect to control indicates the protein stabilizing activity (denaturation is inhibited) with increased dose. The plant leaf extracts (at100 µg/ml) and standard drug (at 100 µg/ml) exhibited 63% and 68% inhibition respectively. This shows a close relationship between the potency of the extract and the standard drug as an anti-inflammatory agent. Hence it is obvious, that if these crude extract is purified, the pharmacological activity will increase significantly and might even match those of the standard drug.

As earlier stated in the introduction, neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors [3]. Methanol extract of *F.zanthoxyloides* exhibited significant anti-proteinase activity at different concentrations as shown in Table 2. It showed maximum inhibition of 44% at 100µg/ml, which from the result was greater than the inhibition offered by aspirin which showed % inhibition of 42 and 43% at 100µg/ml and 200µg/ml respectively. Though not reflected in this study, the anti-inflammatory effect of methanol extract of *F.zanthoxyloides* may be due to the presence of active phytochemicals such as flavonoids and polyphenols [14].

Furthermore, the result of the antioxidant study

showed that the extract could be an effective antioxidant from the DPPH and hydrogen peroxide scavenging activities models. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity study shows *that the* methanol extract of *F. zanthoxyloides* leaves has good antioxidant properties, as there was decrease in the mean absorbance value and increase in the percentage inhibition of DPPH radical scavenging activity with increased extract concentration. The essence of DPPH method is that the antioxidants react with DPPH (1, 1-diphenyl-2-picrylhydrazyl) and convert it to 1, 1-diphenyl-2-picryl hydrazine with discoloration. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. The experimental data of the extract revealed that the extract is likely to have the effects of scavenging free radicals. The reducing properties are generally associated with the presence of reductones [15] whose antioxidant action is based on breaking of the free radical chain by donating one hydrogen atom [16]. Reductones also react with certain precursors of peroxide, thus preventing peroxide formation. The results indicated that the absorbance value of the methanol extract of *F. zanthoxyloides* leaves may be due to presence of polyphenols, which may act similar to reductones by donating the electrons and reacting with free radicals to convert them to more stable product and terminate free radical chain reaction; the presence of such compounds in the methanol extract of *F. zanthoxyloides* leaves endows it with anti-oxidant activity, making *F. zanthoxyloides* leaves a good antioxidant plant. The antioxidant activity of phenolics (polyphenols) is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers [17]. This observation on the DPPH radical scavenging activity of the methanol extract of *F. zanthoxyloides* leaves is in agreement with that Agarwal *et al.* [18].

H_2O_2 is highly important because of its ability to penetrate into biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radicals (OH^-) in the cell [19]. Scavenging of H_2O_2 by the extract may be attributed to the phenolics, which can donate electrons to H_2O_2, thus neutralizing H to water (H_2O) [20]. The results show that the methanol extract of *F. zanthoxyloides* leaves had potent H_2O_2 scavenging activity which may be due to the antioxidant compounds. As the antioxidant components present in the extracts are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O. The results show an excellent inhibition percentage which increases as the concentration of the extract increased, with the standard drug having a similar increase.

CONCLUSION:

The extract was found to possess radical scavenging and anti-inflammatory activities as determined by albumin denaturation, protease inhibition assay, scavenging effect on the DPPH, and hydrogen peroxide. Further research on isolating the responsible components may be undertaken and they may be incorporated into existing anti-inflammatory herbal compositions to improve their efficacy.

Acknowledgement:
The authors are thankful to the Head of department and lecturers of the department of biochemistry, University of Nigeria, Nsukka for their constant help and support in conducting this work to full satisfaction.

Conflict of Interest:
None declared.

REFERENCES:

1. Leelaprakash G, Mohama, D S. *In vitro* anti-infmammatory activity of methanol extract of enicostemma axillare. Int J Drug Dev Res, 2010; **3**: 189-196.
2. Ingle PV, Patel DM. C- reactive protein in various disease condition- An overview. Asian J Pharm Clin Res, 2011; 4(1): 9-13.
3. Das SN, Chatterjee S. Long term toxicity study of ART-400. Indian Indg Med, 1995; **16** (2): 117-123.
4. Dinesh K, Mitra A, Manjunatha M. *In vitro* and *In vivo* studies of antidiabetic Indian plants. A review J Herb MedToxicol, 2009; **3**(2): 0973-4643
5. Clifford J, Caroline BD. 1989. Traditional Plant Medicines as Treatments for Diabetes. Department of Pharmaceutical Sciences, Aston University, Birmingham B4 7ET, UK;**12**: 8553-564
6. Sofowara EA, Isaac-Sdeye WA, Ogunkoya LO. Isolation and characterization of antisickling agent from *Fagara zanthoxyloides* root. Lloydia, 1975*;* **38**: 169-174.
7. Adesina SK. The Nigerian zanthophylum; chemical and biological values. Afr J Trad CAM, 2005; **2(**3):282-351.
8. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol, 1968;**20**(3):169-73.
9. Sakat S, Juvekar AR, Gambhire MN. *In vitro*

antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci, 2010;**2**(1):146-56.
10. Oyedepo OO, Femurewas AJ. Anti-protease and membrane stabilizing activities of extracts of *Fagra zanthoxiloides, Olax subscorpioides* and *Tetrapluera tetraptera*. Ln. J Pharm, 1995;**33**:65-9.
11. Liyana-Pathirana CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. J Agric Food Chem, 2005; **53**: 2433-2440.
12. Ruch RJ, Cheng SJ, Klaunig JE. (). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 1989; **10**(6):1003-1008.
13. Halliwell B. Antioxidants in human health and disease. Annu Rev Nutr, 1996; **6**: 33-50.
14. Godhandaraman S, Ramalingam V. In vitro anti-infllammatory activity of different parts of *Pedalium murex.* Int J Herb Med, 2016; **4**(3): 31-36
15. Pin-Der D. Antioxidant activity of Budrock (*Arctium lappa Linn*); Its scavenging activity on free radical and active oxygen. J Am Oil Chem' Soc, 1998; **75**: 455-461.
16. Gardon MF. The mechanism of antioxidant action *in-vitro*. In: Food antioxidant, Hudson BJF. (ed). Elsevier Applied Science, London, 1960;1-18.
17. Akula US, Odhav B. *In vitro* 5-lipoxygenase inhibition of polyphenolic antioxidants from undomesticated plants of South Africa. J Med Plants Res, 2008; **2**(9): 207-212.
18. Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. The effects of oxidative stress on female reproduction: A review. Reprod Biol Endocrinol, 2012; 10: 49.
19. Gülçin I, Huyut **Z**, Elmastaş M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. Arab J Chem, 2010; **3**(1): 43-53.
20. Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxidant activities of methanol extract of *Sambucus ebulus L*. flower. Pak J Biol Sci, 2009; **12**:447-50.