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In Vitro Anti-Inflammation Studies of *Jakaranda mimosifolia*Extract Using Protein Denaturation Technique.

J. D. Habila[1*]., Y. Isah[2] ., M. M. Adeyemi[3]., A. Y. Mahmoud[1]., A. J. Habila[4] and M. H. Hamza[1]

[1]Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Chemistry, Federal University Lokoja, Kogi State.
[3]Department of Chemistry, Nigeria Defense Academic (NDA), Kaduna.
[4]Department of Science Laboratory Technology, Nigeria Institute of Leather and Science Technology (NILEST), Samaru-Zaria, Nigeria.
*Correspondent author: habilajames2005@yahoo.com, +2348060359397

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Abstract

The present study investigates the anti-inflammation potential of *Jacaranda mimosifolia*, which is reported in traditional medicine. Microwave energy was used for the extraction of plant material and, the anti-inflammatory activity was evaluated using protein denaturation method. In-vitro anti inflammation test on different concentrations of the extracts solution was conducted. The highest percentage inhibition of protein denaturation of the lowest concentration (31.25 µg/ml) of the extracts was observed as 86. 21 % in the chloroform extract (CE), which is highly significant and comparable to that of the standard drug (Diclofenac) shown as 82.76 %. The hexane extract (HE) and ethylacetate extract (EE) both inhibited protein denaturation at 68.97 % and that of methanol extract (ME) was observed as 68.97 %. The result of our investigation reveals that *Jacaranda mimosifolia* has potentials to be explored in the search for a potent anti-inflammatory drug from natural origin. Therefore our studies support the use of the plant in the treatment of inflammation in traditional medicine.

Key-words:*Jacaranda mimosifolia*, Protein denaturation, Anti-inflammatory, Microwave Assisted Extraction.

INTRODUCTION

Inflammation is a common risk factor in the pathogenesis of conditions such as infections, arthritis, type-2 diabetes mellitus, obesity, cancer and so on. [1]. Report has shown that, inflammation is a potential hazard that causes life threatening hypersensitivity reactions and progressive organ damage [2]. It is fundamentally a protective response, the ultimate goal of which is to get rid of the organism of both the initialcause of cell injury (for example microbes and toxins) and the consequences of such injuries[3][4]. Various medicinal plants provide relief from symptoms comparable to that obtained from allopathic medicines [5]. The majority of clinically important medicines belong to steroidal (SAIDs) or nonsteroidal anti-inflammatory drugs (NSAIDs) [6]. The NSAIDs are reported to possess abilities to prevent denaturation of proteins, which act as antigens and leads to auto-immune diseases.Denaturation of proteins is a well-documented cause of inflammation and rheumatoid arthritis. Though these drugs have potent activity, they have a number of severe adverse effects such as gastrointestinal disturbances and body fat redistribution. Hence, there is a need to develop safe and new anti-inflammatory agents with minimum side effects. In this scenario, use of plant derived products to treat inflammation and related condition becomes a viable and valid approach [7].

Unlike modern therapeutic drugs which are single active components that target one

specific pathway, herbal medicine works in a way that depends on an orchestral approach [8]. Plants with alleged traditional uses as anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search for new anti-inflammatory drugs.

Jacaranda mimosifolia among many other plants have long been recognized as important source of therapeutically effective medicines [12] [16]. It is traditionally used in the treatment of inflammation [9].However, there is no published scientific report indicating utility of *Jacaranda mimosifolia* material in the treatment of inflammation, hence the present study.

MATERIALS AND METHODS.

Sample Collection and preparation.

Jacaranda mimosifolia stem bark, roots and leaves were obtained in May 2015 from biological science garden, faculty of science, Ahmadu Bello University Zaria, Kaduna state, Nigeria. It was identified first at the field using a standard key and description. Its botanical identification was further confirmed and authenticated at herbarium section of the botanical unit of the department of biological Sciences, Ahmadu Bello University Zaria where the voucher number was given as 2503. The leaves, roots and stem bark were reduced to smaller pieces then dried at room temperature for fourteen days after which they were pounded into powder using a wooden mortar and pestle and finally stored in a polythene bags until they were needed for extraction.

Extraction

The plant leaves powder (150 g) in a container was added 250 ml of n-hexane. This was allowed to stand for 5 min, then placed inside a conventional microwave oven set at defrost and microwaved at 3 min interval for thirty minutes with cooling at each interval[13][14][17]. This process was repeated 4 times, after which it was transferred to a sieve cloth and washed down with different solvent base on increasing polarity, to give the corresponding solvent extracts; n-hexane (HE), chloroform (CE), ethyl acetate (EE) and methanol (ME).

Preparation of solutions of different concentration

The plant extract (0.010g) of n-hexane, chloroform, ethyl acetate, and methanol extract were each dissolved in 10 ml of methanol to prepare a stock solution with a concentration of 1000 µg/ml. The control drug Diclofenac Sodium (50 mg) was dissolved in 50 ml of methanol to prepare a stock solution of the same concentration. Other concentrations (500, 250, 125, 62.5 and 31.25 µg/ml) were prepared using the serial dilution technique.

***In vitro* anti-inflammatory test**

This test was done according to the method described by Chandra *et al*., 2012. The reaction mixture (5 ml) consisting of 0.2 ml of egg albumin (from fresh hens egg), 2.8 ml of phosphate buffered saline (PBS, PH 6.4) and 2 ml of varying concentrations of the plant extracts so that final concentration will be 31.25, 62.5, 125, 250, 500, 1000 µg/ml. The mixtures were incubated at 37 ± 2^{o}C for 15 min and then heated at 70 ^{O}C for 5 min[18]. After cooling, their absorbance was measured at 660 nm (6405 UV/Vis. spectrometer) using methanol as blank. Diclofenac sodium at the final concentration of 31.25, 62.5, 125, 250, 500, 1000 µg/ml, was used as reference drug and treated for determination of absorbance [10][15]. The percentage inhibition of protein denaturation was calculated by using the formula:

Inhibition (%) = 100 x {1-Vt /Vc}

Where;

Vt = absorbance of test sample,

Vc = absorbance of control (consist: 0.2 ml of egg albumin, 2.8 ml of phosphate buffered saline, 2 ml methanol)

Table 1: Result of Absorbance of Declofenac and plant extracts

Conc. (µg/ml)	Declofenac	HE	CE	EE	ME
31.25	0.0050	0.0090	0.0040	0.0090	0.0070
62.5	0.0040	0.0050	0.0040	0.0030	0.0060
125	0.0020	0.0050	0.0020	0.0020	0.0040
250	0.0010	0.0190	0.0019	0.0010	0.0040
500	0.0009	0.0040	0.0020	0.0012	0.0020
1000	0.0001	0.0039	0.0050	0.0170	0.0010

Table 2: Percentage (%) Inhibition of Declofenac and plant extracts

Conc. (µg/ml)	Declofenac	HE	CE	EE	ME
31.25	82.76	68.97	86.21	68.97	75.86
62.5	86.21	82.76	86.21	89.66	78.31
125	93.10	82.76	93.10	93.10	86.21
250	96.55	34.48	34.48	96.55	86.21
500	96.90	86.21	93.10	58.62	93.10
1000	99.66	86.55	82.76	41.38	96.55

Key: He = hexane extract, CE = Chloroform extract, EE = Ethylacetate extract, ME = Methanol extract.

Results and Discussion

Protein denaturation is a documented cause of inflammation and rheumatoid arthritis, any agent that will inhibits the denaturation of protein will serve as a good candidate for anti-inflammation drug [11]. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. Ability of *J. mimosifolia* extract to greatly lower thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity. The results of the anti-inflammation studies (Table 2) showed that at a low concentration of 31.25 µg/ml, Percentage inhibition of 86. 21 % was observed for the chloroform extract (CE). As compared to the standard drug (Diclofenac) which showed inhibition of 82.76 % at 31.25 µg/ml. The other extracts; HE and EE both showed inhibition of 68.97 % and ME observed a 68.97 % inhibition. At a high concentration of 125 µg/ml CE and EE both showed 93.10 % inhibition same as the standard drug (diclofenac) at the same concentration. Sharp decreases in percentage inhibition were observed for HE and CE (34.48 % at 250 µg/ml) and EE (58.62 %) at 500 µg/ml. Indicative of optimal concentration for protein denaturation inhibition by the plant extracts, beyond which there is a decrease. After the concentration at which a decrease in inhibition was observed, the percentage inhibition increases with increase in concentrations of the extracts. The methanol extract (ME) was the only exception, showed a dose dependent increase in percentage inhibition same as the control drug.

The results of this investigation clearly shows that the plant *J. mimosifolia* has potentials that can be explore in the search for anti-inflammatory drug from nature.

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