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Drying Temperature Effect on the Anti-Inflammatory Activities of *Moringa Oleifera* Leaves Extract

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Abstract There is an increasing trend in the study of the anti-inflammatory properties and phytochemical content of herbal medicines worldwide. Literature is however lacking on the effects of heat treatment during the processing of medicinal plants of their bioactivities and phytochemical content. This study seeks to fill this gap by investigating how temperature affects the anti-inflammatory properties of *Moringa oleifera* leaves. The plants were harvested and ground into a powder, after which aqueous and ethanol extracts were conducted. The extract was freeze-dried for further examination. The anti-inflammatory assay activity shows that the IC_{50} values of moringa leaves at room temperature, 60℃, and 90℃ were 0.1313±0.0269, 0.0939±0.0901l, and 0.0757±0.0162 respectively. Diclofenac sodium, a standard anti-inflammatory drug showed an IC_{50} value of 0.1022±0.0204. The study also showed that temperature influenced the phytochemical components in the moringa leaf extract. Phytochemical components such as reducing sugars, saponins, phenols, flavonoids, triterpenes, and phytosterols were all present.

Keywords: *herbal medicine, anti-inflammatory, phytochemicals, temperature*

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1. Introduction

Herbal plants have been the centerpiece of herbal medicine amongst indigenous people across the world for many years [1]. Because of their high therapeutic efficacy, several herbs and spices have been discovered to have antibacterial [2], antimicrobial, toxicity of sick states, anticonvulsant, anti-inflammatory [3], and hypotensive properties. *Moringa oleifera* Lamarck *(Moringaceae)* is a fast-growing perennial species native to northwestern India, which is now cultivated in many areas worldwide [4]. Moringa is known as very useful in the treatment of inflammatory conditions[4] The *Moringaceae* family of flowering plants has only one genus, Moringa. It grows to a height of 10 to 15 meters and is a small to medium-sized tree. This plant can be found in East Asia, Polynesia, and the West Indies [5].

Phytochemicals are bioactive non-nutrient plant components. Fruits, vegetables, grains, and other foods contain them. Plant foods that have been related to a reduced risk of cardiovascular disease and chronic diseases are important, the name "*phyto*" comes from the Greek word *phytos*, which means "to grow." The Greek term *phyto* means "plant" [6]. The presence of these bioactive components is thought to give resistance against bacterial, fungal, and pesticidal pathogens. These bioactive components are thought to be responsible for plant extracts' antibacterial properties in vitro [7].

Indicators of inflammation can be primarily grouped into four. These are pain, redness, heat or warmness and swelling. When there is an injury to any part of the human body, the arterioles in the encircling tissue dilate. This increases blood circulation towards the affected area (redness) [8]. When situations of this nature arise, chemicals known as anti-inflammatory drugs are used to suppress and control the crisis.

In general, there is an increasing trend in the study of the anti-inflammatory properties and phytochemical content of herbal medicines worldwide. Literature is however lacking on the effects of heat treatment during the processing of medicinal plants of their bioactivities and phytochemical content. This study seeks to fill this gap by investigating how drying temperature affects the anti-inflammatory properties of *Moringa oleifera* leaves extract.

2. Materials and Methods

2.1. Collection of Plant Material

Fresh leaves of Moringa were collected from a backyard garden. Leaves were identified and authenticated

at the Center of Plant Medicine Research, Mampong by a Botanist. Fresh leaves of Moringa leaves collected were washed and rinsed with distilled water.

2.2. Drying of Plant Sample

Moringa leaves were air-dried for five days on cotton sheets in a well-ventilated room until they were crisp and brittle to the touch. Some of the leaves were also dried for four hours in a hot oven at 60℃ and 90℃ respectively. The dried leaves were grounded into a powder in a high-powered blender and stored in airtight containers for further testing.

2.3. Preparation of Crude Extracts

Maceration was used to make crude extracts. Powdered plant materials (20g) were steeped overnight in 1L of 70% v/v ethanol for the ethanol extract and 1L distilled water for the aqueous extract. The acquired extracts were separated into two parts, each of which was freeze-dried and the other kept in a container. A rotary evaporator was used to evaporate ethanol for freeze-dried extracts. The resultant extracts were kept refrigerated for further phytochemical and anti-inflammatory studies.

2.4. Phytochemical Analysis of Crude Extract

2.4.1. Test for Flavonoids

A few drops of sodium hydroxide solution were added to the crude extract (2mg diluted in 5ml distilled water). The presence of flavonoids was shown by the formation of a bright yellow color that faded to colorlessness when dilute acid was added [9].

2.4.2. Test for Alkaloids

Two drops of Mayers reagent were placed along the walls of the test tube to around 2mg of crude extract. The presence of alkaloids was shown by the appearance of a white creamy precipitate [10].

2.4.3. Test for Glycosides

3ml chloroform was added to 2ml filtered hydrolysate and agitated, the chloroform layer was separated, and a 10% ammonia solution was added. The presence of glycosides was indicated by a pink color [10].

2.4.4. Test for Phytosterols

The crude extract (50mg) was dissolved in 2ml of acetic anhydride solution. 1 - 2 drops of concentrated sulfuric acid were progressively applied along the walls of the test tube. Phytosterols were detected by a variety of color changes [10].

2.4.5. Test for Phenolic Compounds

3-4 drops of ferric chloride solution were added to the crude extract (2mg diluted in 5ml distilled water). The presence of phenols was indicated by the formation of a bluish-black tint [9].

2.4.6. Test for Polyuronides

2ml Sample was mixed with 5ml acetone. The presence of polyuronides is indicated by the appearance of a precipitate [11].

2.4.7. Test for Reducing Sugars

2mg of crude extract was diluted in 5ml of distilled water and added to freshly made Fehling's solution A and B to test reducing sugars, according to Fehling's method. The solution was then heated for 15 minutes. The presence of reducing sugars is indicated by the brick-red color [11].

2.4.8. Test for Triterpenes

5ml sample was poured into a separating funnel after 30 minutes of refluxing with 10% HCl, and 3ml diethyl ether was added. The funnel was covered and gently shaken, with occasional openings to release pressure. After allowing the mixture to separate for a while, the diethyl ether layer was collected in three dishes and evaporated to dryness. To dissolve the residue, equal parts chloroform and acetic anhydride were used. A drop of conc. H_2SO_4 was added to each test tube after splitting the mixture into two test tubes. The presence of a red color is indicated by its appearance [11].

2.5. In-Vitro Anti-Inflammatory Analysis on Crude Extracts

The capacity of different concentrations of Moringa extracts of various drying temperatures to suppress albumen denaturation was carried out using the method of [12] with minor changes. The reaction mixtures were made up of 0.5ml (1.5mg/ml albumen) and various quantities of extracts, which were then incubated at 37℃ for 20 minutes. 2.5ml of 0.5M phosphate buffer, pH 6.3, was added to the reaction mixtures after they were heated at 70℃ for 10 minutes. In triplicates, 1cc of each reaction mixture was pipetted into clean-dried test tubes. The tubes were cooled, and the turbidity was measured using a UV-Spectrophotometer against a reagent blank at 660nm. Diclofenac sodium, a standard anti-inflammatory drug was used as a positive control, and water was used as a negative control.

The quantity of protein left was calculated using the expression:

$$\left[\frac{\text{Abs of sample} - \text{Abs of blank}}{\text{Abs of standard} - \text{Abs of blank}}\right] \times 100$$

The percentage inhibition was calculated using the expression, percentage inhibition.

$$= \left[\frac{\text{Abs of control} - \text{Abs of sample}}{\text{Absorbance of control}}\right] \times 100$$

The IC_{50} values were established by plotting a linear graph. On the graph, % inhibition was plotted for control against treatment concentration.

2.6. Statistical Analysis

Statistical analyses were carried out in GraphPad Prism 8 software using one-way analysis of variance. Significance was set at $P < 0.05$. Data are presented as the mean ± standard error of the mean (SEM).

3. Results and Discussion

Table 1. Phytochemical Screening of Moringa Leaf Extract

Phytochemical screening	Room temperature	60℃	90℃
Reducing Sugars	+	+	+
Saponins	+	+	+
Polyuronides	-	-	-
Phenolic Compounds	+	+	+
Alkaloids	+	-	-
Flavonoids	+	+	+
Triterpenes	+	+	+
Phytosterols	+	+	+
Glycosides	-	-	-

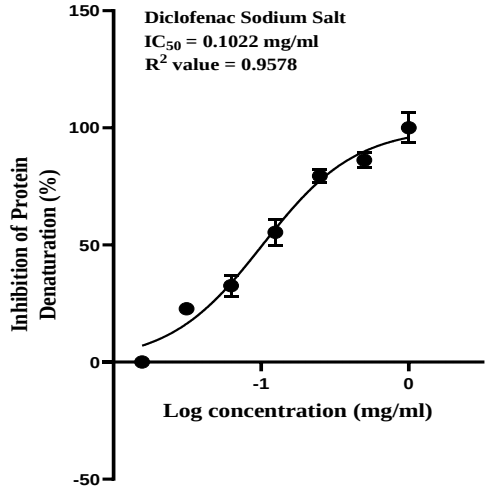

Figure 1. Graph of log concentration against Inhibition of protein denaturation for Diclofenac sodium salt

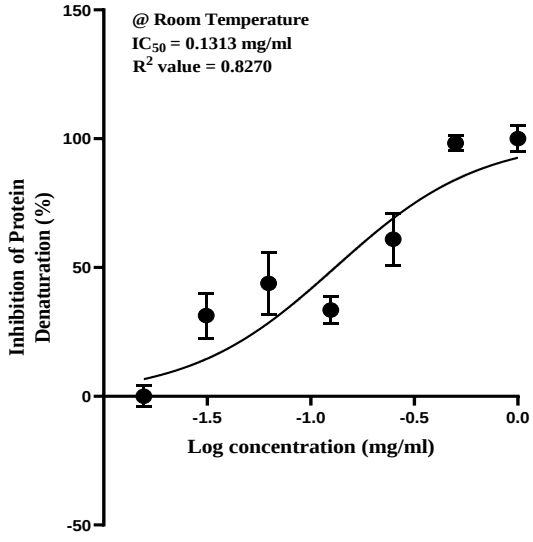

Figure 2. Graph of log concentration against Inhibition of protein denaturation for moringa at room temperature

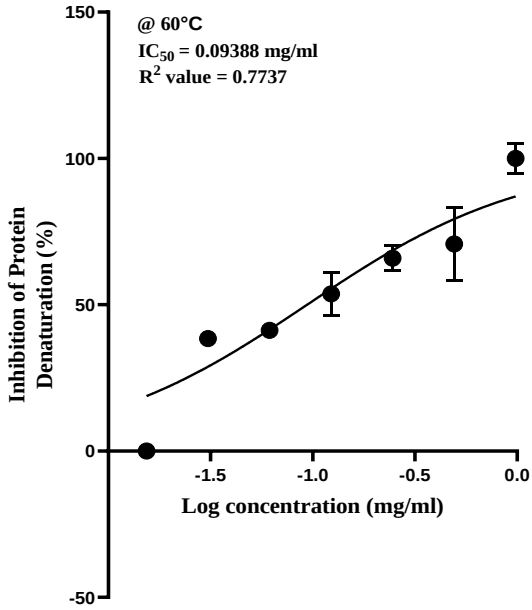

Figure 3. Graph of log concentration against Inhibition of protein denaturation for moringa at 60℃

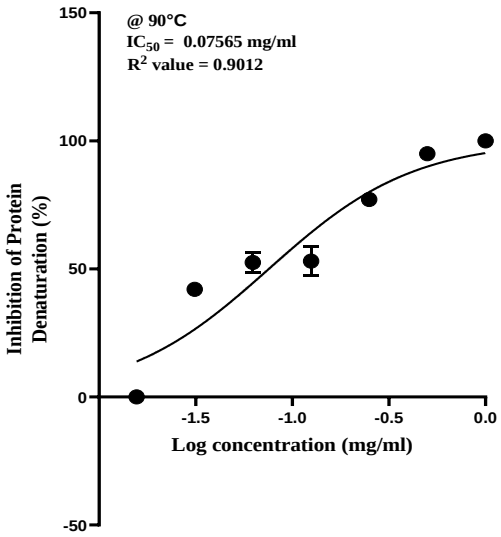

Figure 4. Graph of log concentration against Inhibition of protein denaturation for moringa at 90℃

The results in Table 1 indicated the presence of phytochemical constituents such as saponins, flavonoids, phytosterol, reducing sugars, phenols and triterpenes in the leave extract of Moringa. Flavonoids are antioxidants that help with the absorption of Vitamin C. [13]. Physiologically, they are also known to fight liver poisons, tumors, viruses, and other microbes [14]. Red blood cell hemolysis is caused by saponins [15]. Sugar reduction reduces the risk of being overweight or obese, as well as the risk of developing diabetes. Phytosterol also prevents the absorption of cholesterol in the body. Throughout the day, the average human consumes roughly 300mg of phytosterols. Phytosterols are incapable of lowering LDL cholesterol at that concentration. However, preliminary

evidence suggests that including more phytosterols in our diets can lower LDL cholesterol levels in as little as two to three weeks if proper dietary routine is maintained [16]. Triterpenes have been described as anti-inflammatory, antiviral, antimicrobial, antitumoral, and immunomodulator substances, as well as anti-inflammatory, antiviral, antimicrobial, and antitumoral agents [17]. Several of them have been linked to the resolution of immunological illnesses, however, their effects are not always evident.

The anti-inflammatory studies began with extract concentrations of 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.625mg/ml, 0.3125mg/ml, 0.15625mg/ml, 0.078125mg/ml and 0.0390625mg/ml. The anti-inflammatory activities of moringa extract at various drying temperatures were determined based on their ability to inhibit protein denaturation. The turbidity of the various temperatures was measured and their IC_{50} value was determined. The IC_{50} for the moringa at 60℃ was found to be 0.0939±0.0901, and that of 90℃ was 0.0757±0.0162, the IC_{50} for the moringa at room temperature was also found to be 0.1313±0.0269. Diclofenac sodium was used as a positive control the IC_{50} value was found to be 0.1022±0.0204. The lower the IC_{50} value the better the protein inhibition, the drying temperature at 90℃ was found to have a lower IC_{50} as compared to the other temperatures. This shows that drying at a higher temperature is essential for maximum inhibition of protein denaturation by the constituents of *Moringa Oleifera* leaves. One way analysis of variance was used to determine the significant difference in the anti-inflammatory activity of moringa leaves at the various drying temperatures. The values in the samples were $P<0.05$ hence there were no significant differences in the samples.

4. Conclusion

The extracts of the herbal plant *(Moringa oleifera)* were found to be good source of phytochemicals such as triterpenes, flavonoids, phytosterols, phenols, and saponins. Variations in temperature influenced the phytochemical constituents found in the moringa oleifera leaf. The IC_{50} for the various extracts at different drying temperatures was determined. The moringa dried at 90℃ showed a strong anti-inflammatory activity.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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