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In-Vitro Evaluation of Ethanolic Extracts of Zingiber Officinale, Sygzium Aromaticum and their 1:1 Extracts Blend on Protein Denaturation during Acute Inflammation

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Abstract

Zingiber officinale and Sygzium aromaticum are well known and widely used herbs, which possesses health promoting properties as well as several other interesting bioactive constituents. The use of flavonoids and other natural antioxidants, as a therapeutic option, is found desirable and increasingly being practiced. This study aimed to evaluate and compare the anti-inflammatory effects of ethanolic extracts of Zingiber officinale and Sygzium aromaticum and a 1:1 blend of the two extracts against the denaturation of protein in vitro. The test extracts of varying concentrations were incubated with egg albumin under controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. A standard anti-inflammatory drug, Ibuprofen, was used as reference drug. The results obtained exhibited a concentration- dependent inhibition of protein (albumin) denaturation by both extracts, including the 1:1 blend of the extracts. From the present findings it can be concluded that both Zingiber officinale and Sygzium aromaticum possessed marked anti-inflammatory effect against the denaturation of protein, in vitro; with Zingiber officinale extract being more effective than the Sygzium aromaticum extract.

Keywords: Zingiber officinale; Sygzium aromaticum; Ibuprofen; anti-inflammatory; denaturation; egg albumin.

1. Introduction

Inflammation, usually characterized by redness, swelling, pain and a sensation of heat, is one of the body's self-defense systems. This biological response is a protective mechanism of organisms for defense against noxious physical or chemical stimuli. However, chronic inflammation has been reported to be involved in the development of various diseases such as allergic rhinitis [1], atopic dermatitis [2], rheumatoid arthritis [3,4], cancer [5,6], multiple sclerosis [7, 8], inflammatory bowel disease[9], bronchial asthma [10] and atherosclerosis [11 - 17] and increase of protein denaturation and membrane alterations[18], etc. Inflammation can be initiated by complex processes triggered by microbial pathogens or by the release of several soluble mediators of inflammation, reactive oxygen species (ROS), lipid mediators, host proteins such as proteases, and cytokines [11, 19, 20] or by the release of chemical mediators from injured tissue and migrating cells [21]. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes /macrophages. The commonly used drugs for management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation and ulcer. As a result, a search for other alternatives seems necessary and beneficial. For quite some times now, traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles [22-34]. The major merits of herbal medicine seem to be their perceived efficacy and low incidence of serious adverse effects. This explains the reason for which this work was undertaken.

2. Review of Literature

Flavonoids are well known pro-inflammatory mediators in the pathogenesis of inflammation [35-37] and in inhibiting protein oxidation [38]. Various flavonoids, some of which are found in zingiber officinale and sygzium aromaticum, have been found to possess anti-inflammatory activity [39-42] by inhibiting cycloxygenase-2 (COX2) and inducible nitric oxide synthase[43], cytosolic and tyrosine kinase[44,45] and neutrophil degranulation [44, 46] and others [47-49] also demonstrated the Connections between antioxidant activities of flavonoids to their anti-inflammatory activities. This study is carried out to investigate and compare the possible anti- inflammatory effects of aqueous extracts of zingiber officinale and sygzium aromaticum against the denaturation of protein in vitro. Studies have shown that both selected plants are rich in large quantities of phenolic compounds and flavonoids [39, 50-52].

Ginger is an herb as well as a spice, it belongs to the family of Zingiberceae, and its botanical name is Zingiber officinale. Ginger is one of the most naturally occurring medicinal plants which are grown in different countries like India, China, South East Indies, Mexico [53-55] and other parts of the world including Nigeria. Its spicy aroma is mainly due to the presence of ketones, especially the gingerol which happens to be the most important component of ginger studied in much health related scientific research. The rhizome, which is the horizontal stem from which the roots grow, is the main portion of ginger that is consumed.

At least 115 constituents in fresh and dried ginger varieties have been identified by a variety of analytical process. Gingerol is the constituent of fresh ginger and are found slightly reduced in dry ginger whereas shagoals which are the major gingerol dehydrating product are more abundant in dry ginger than in fresh ginger [56]. The portion of each individual component in a sample depends on the country or origin, commercial processor, and whether the ginger is fresh, dried or processed [57].

One of the many health claims attributed to ginger is its purported ability to decrease inflammation, swelling, and pain [58]. Ginger is also known to treat related ailment such as sore throats, constipation, vomiting, hypertension, dementia, fever, infectious diseases amongst others. The main pharmacological actions of ginger and compounds isolated from it include; immune-modulatory, anti-tumorigenic, anti-apoptic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions [59].

Cloves are aromatic dried buds of a tree in the family myrtaceae; its scientific name is Syzium aromaticun, belonging to the genius syzygium. The clove tree is an evergreen tree that grows to a height ranging from 8-12m, with large leaves. Cloves are native to Maluku Island in Indonesia and are used as spice in cuisines all over the world [60]. Cloves are used in Indian Ayurvedic medicine, Chinese medicine and western herbalism and dentistry where essential oil is used as an anodyne for dental emergency. Cloves are used as carminative to increase hydrochloric acid in the stomach to improve peristalsis. Cloves are also said to be a natural anthelminic [61]. The essential oil is used in aromatherapy when stimulations and warming are needed, especially for digestive problem [62].

One of the most active compounds found in clove is eugenol. It comprises of 72-90% of the essential oil extracted from clove, and is the compound responsible for the cloves' aroma. The other important essential oil constituents of clove oil include acetyl eugenol, bêta-caryophyllene vanillin and tannins [63,64]

3. Materials and Methodology

3.1 Chemicals and Drugs

The standard reference drug, Ibuprofen, was purchased from the Sabon Gari drug market, Zaria. Other reagents used are analar grade from BDH, M&B, Sigma or, Fluka.

3.2 Plant Materials

The Zingiber officinale plant material and Sygzium aromaticum were purchased from Sabon Gari market in Zaria, Kaduna state. Both samples were identified at the herbarium, Department of Biological Sciences in Faculty of Sciences, Ahmadu Bello University Zaria, Kaduna state of Nigeria.

3.3 Preparation of Extracts

The Zingiber officinale plant material was washed, peeled and chopped into bits and air-dried for 5days. The Sygzium aromaticum was purchased dried as it was difficult to obtain fresh clove buds in this part of the country. The two samples were separately grounded using a mortar and pestle and about 500grams of each sample were macerated respectively in distilled ethanol for 3 days. These were then filtered using a what man filter paper and the filtrates concentrated on a rotary evaporator. The concentrated filtrates were transferred to open pre weighed beakers and put on water bath until they were evaporated to dryness to yield the dry extracts of *Zingiber officinale*

(yield: 12.49%) and Sygzium aromaticum (yield: 10.52%) The dry extracts were kept in a vacuum desiccator until when ready for use.

3.4 Estimation of total phenolic compounds and flavonoids

The total phenolic contents of the two extracts were determined spectrophotometrically by applying the Folin-Ciocalteu assay with gallic acid as standard [12, 49]; while a slight modification of the method of Chang et al [65] was used for estimation of flavonoids contents of the test extracts with rutin as standard.

3.5 In-vitro anti-inflammatory activity

The screening for anti-inflammatory activity was carried out according to a modification of the in-vitro protein denaturation bioassay methods of Jagtap et al [47]and Shallangwa et al [49]. The standard reference drug, Ibuprofen, and extracts of Zingiber officinale and Sygzium aromaticum were dissolved in minimum quantity of distilled water and diluted with phosphate buffer (0.2 M, pH 7.4). The test mixtures (5 mL each) made up of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS,pH 7.4) and 2 mL of varying standard solutions of Zingiber officinale and Sygzium aromaticum extracts so that final concentrations became 50.0, 100.0, 200.0, 400.0, 800,1600 µg/mL of Zingiber officinale and Sygzium aromaticum respectively were prepared. Respective test solutions were incubated at 37° +1° C in Corsair Heating & Catering Limited incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60° +1° C in waterbath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible U2800 Spectrophotometer, Hiatachi Ltd). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average taken. The percentage inhibition of denaturation was calculated by using following formula.

% Inhibition = 100 X [Vt / Vc – 1]

Where,

Vt = Mean absorbence of test sample.

Vc = Mean absorbence of control

The results are described in Table 1. The extract concentration for 50% inhibition (EC_{50}) was determined by the dose-response curve using Graphppad Prism 5.00.

4. Results.

Table 1: Anti-inflammatory data Z. officinale, S. aromaticum, Extracts blend of Z. officinale and, S. aromaticum (1:1) and, Ibuprofen

Concentration (µg/mL)	Z. officinale (% inhibition)	S. aromaticum (% inhibition)	Extracts blend 1:1 (Z. officinale / S.aromaticum) (% inhibition)	Ibuprofen (% inhibition)
Control	--	--	--	--
100.0	46.62±15.5	15.72±10.2	125.26±13.1	64.69±7.3
200.0	118.56±3.1	27.58±5.2	146.13±1.2	82.47±2.2
400.0	188.40±2.2	37.88±6.2	172.42±10.3	109.27±9.1
600.0	298.19±5.6	69.58±1.2	184.53±13.2	115.97±4.3
800.0	329.89±3.2	73.00±5.7	196.90±12.5	143.04±8.2
1600.0	371.39±8.2	96.39±15.2	225.77±25.4	271.64±2.2

(Values are expressed as SEM of 3 readings)

Table 2: EC_{50} values for Z. officinale , S. aromaticum, Extracts blend and Ibuprofen				
	Z. officinale	S. aromaticum	Extracts blend 1:1 (Z. officinale / S.aromaticum)	Ibuprofen
IC_{50} (µg/mL	10.0±2.2	382.7±15.5	10.0±4.7	14.6±7.5

(Values are expressed as SEM of 3 readings)

5. Discussion

In the present study, the evaluation of anti-inflammatory effects was undertaken using the effect of separate extracts of Zingiber officinale, Sygzium aromaticum and a 1:1 blend of the two extracts on protein denaturation. Denaturation of proteins is well documented and is caused by inflammation process, mostly in conditions like rheumatoid arthritis [21, 47-49]. Therefore, using agents that can prevent protein denaturation would be worthwhile for anti-inflammatory drug development. Several anti-inflammatory drugs have shown dose dependent ability to inhibit heat induced protein denaturation [66]. Protection or inhibitory effect against protein denaturation, which is the main mechanism of action of NSAIDS, plays an important role in the anti-inflammatory activity of NSAIDS [67]. There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated [18,21,47,48], or lack of presence of functional offices of constituted research ethic committees in most cities in places like Nigeria.

The ability of Zingiber officinale and Sygzium aromaticum extracts to inhibit protein denaturation may contribute to their anti-inflammatory properties. In the present investigation, the in vitro anti-inflammatory effect of Zingiber officinale and Sygzium aromaticum extracts, including a 1:1 blend of their extracts, were evaluated against denaturation of egg albumin. The results are as presented in Table 1. The study showed a concentration-dependent inhibition of protein (albumin) denaturation by Zingiber officinale and Sygzium aromaticum and the 1:1 blend of the two extracts, within the concentration ranges of 100 to 1600 µg/mL studied. The reference drug, Ibuprofen, also exhibited concentration-dependent inhibition of protein denaturation [Table 1]. Denaturation of proteins is a well-documented cause of inflammation in conditions like rheumatism and arthritis [18, 21, 47-49].Therefore, any substance that can prevent or inhibit protein denaturation will be a good anti-inflammatory agent. Table 1 showed that at low concentration of 100 µg/mL the 1:1 blend of the two extracts was more effective than the reference drug, the Zingiber officinale extract and the Sygzium aromaticum respectively, and in that order, in preventing protein denaturation. But as the concentration increased (from 200 - 800 µg/mL), the Zingiber officinale extract became more effective than the blend, Ibuprofen and Sygzium aromaticum, in that respective order too. However, at a higher concentration of 1600 µg/mL, the Zingiber officinale extract was most effective, followed by references drug, Ibuprofen, then the blend, with the Sygzium aromaticum extract being the least effective. The EC50 values were presented in Table 2. In this study the EC50 values showed that the Zingiber officinale extract was more effective or comparable with the blend, followed by the Ibuprofen reference drug; with the Sygzium aromaticum being the least effective.

There were increments in the absorbance of the test samples, with respect to the control, which indicated that there was stabilization of protein. That is, there was inhibition of protein (albumin) denaturation, which was also a measure of the anti-inflammatory effect of the test extracts, the blend and the reference drug, Ibuprofen [18, 21, 47-49].

Both Zingiber officinale and Sygzium aromaticum contain varying amounts of polyphenols particularly flavonoids [39-42, 50, 52,68-70] . In the present study, the high anti-inflammatory effect of both Zingiber officinale and Sygzium aromaticum may possibly be attributed to their high flavonoids contents.

6. Conclusion

It has been reported that one of the features of several non-steroidal, anti-inflammatory drugs, was their ability to prevent or inhibit denaturation of protein (albumin) [18, 21, 47-49]. In conclusion, the results of this study, showed that both Zingiber officinale and Sygzium aromaticum extracts possessed marked anti-inflammatory effect as they can limit the denaturation of protein process in vitro. This can contribute to the validation of the anti-inflammatory activity of these plants and may provide some evidence for their folk uses and further exploitations as therapeutic anti-inflammatory agents

7. References

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