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In vitro evaluation of *Aloe vera* and *Camellia sinensis* aqueous extracts effect on protein denaturation during acute inflammation

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ABSTRACT

Aloe vera L. (syn.: *Aloe barbadensis* Miller) and Green tea (*Camellia sinensis*) are well known and widely used herbs, which contain several interesting bioactive constituents and possesses health promoting properties. Oxidative stress contributes to the pathogenesis and progression of many diseases. The use of natural antioxidants, as a therapeutic option, is desirable and is increasingly being practiced. The aim of the present study is to evaluate and compare the *in vitro* anti-inflammatory effects of aqueous extracts of *Aloe vera* and green tea against the protein denaturation . The testedextracts of varying concentrations were incubated with egg albumin under controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property, by comparison with thestandard anti-inflammatory drug, Diclofenac sodium, used as reference drug. The obtained results showed a concentration- dependent inhibition of protein (albumin) denaturation by both extracts. From the present findings it can be concluded that both *Aloe vera* and green tea possessed a marked anti-inflammatory effect against the in vitro protein denaturation ,. Green tea was found to be more active than *Aloe vera*, possibly due to the higher flavonoid contents.

Keywords: *Aloe barbadensis*, *Camellia sinensis*, denaturation, inflammation, oxidative stress

1. INTRODUCTION

Many diseases are caused by oxidative stress [1]. The morbidity and mortality due to various cardiovascular diseases are on the rise. Accelerated cell oxidation contributes to cardiovascular diseases such as hypertension, atherosclerosis, heart attacks, ulcer, cancer, arthritis, wrinkled skin, chronic fatigue syndrome, Parkinson´s and Alzheimer's disease, diabetes[2-5] and even a decline in energy and endurance [6,7] by the free radicals that are resulting asbyproducts of the cellularmetabolism[8-17]. Short-term oxidative stress (OS) and the unbalance between the formation and scavenging of the reactive oxygen species (ROS), may be important in the prevention of aging due to the triggering of the process known as mitohormesis [19-24]. In average, about 70 % of the world population is excessively impacted by OS caused by free radicals (FRs) and more than 80% of the population relies on traditional medicine for their primary health care needs [25]. Therefore, ROS

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monitoring may have a protective role to play in preventing certain conditions such as those degenerative health conditions mentioned above [19, 22, 23, 26-34].

Some plants, vegetables and fruits are reported to be rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds, flavonoids and hydrolysable tannins, which can significantly destroy the free radicals responsible for degenerative diseases [35] or prevent free radical damage, thus reducing the risk of chronic diseases.These plants enclude *Ocimum basilicum* (basil), *Prunella vulgaris* (self-heal) *Cinnamomum cassia*, *Ginkgo biloba L.*, *Camellia sinensis Lin.*, *Aloe vera,* and quite a number of others. This study was carried out to investigate and compare the possible anti- inflammatory effects of aqueous extracts of *Aloe vera* and green tea against the *in vitro* protein denaturation. Previous studies have shown that both selected plants are rich in large quantities of phenolic compounds and flavonoids. Major plant antioxidants are secondary metabolites of the shikimic acid pathway and phenylpropanoid metabolism,including phenolics, coumarins, tannins, chalcone, flavonoids etc. Flavonoids-flavones, flavanones, flavonols, isoflavones, anthocyanin, chalcone-also inhibit cytotoxic low density lipoproteins (LDL) formation [36, 37].

Aloe vera L. (syn.: *Aloe barbadensis* Miller) is a perennial succulent plant belonging to the Aloeaceae family (sub-family of the *Asphodelaceae*) [38]. From about 400 *Aloe* species, *Aloe vera* is most widely accepted and used for various medical and cosmetic purposes [39-41]. The plant is made of turgid green leaves joined at the stem in a rosette pattern. Each leaf consists of two parts: an outer green rind (skin) and an inner clear pulp (gel). The plant contains a large amount of phenolic compounds [42-47]. It also has a high content of 1, 8-dihydroxyanthraquinone derivatives (aloe emodin) and their glycosides (aloins), which are used as cathartic [42, 47, and 48]

Green tea, which is obtained from the plant *Camellia sinensis* leaves, of the family *Theaceae*, is a widely consumed beverage in the world, and contains antioxidants such as catechins, ascorbic acid, α-tocopherol and β-carotene [49-50]. The prominent flavonoid in tea is the flavon-3-ols, catechin, epicatechin, epicatechin gallate, epigallocatechin gallate, and their fermentative products: theaflavins, thearubigin [49, 51-55]. Dry green tea contains much of flavonoid and catechin, while on fermentation, catechin decreases but flavones, quercetin, kaempherol, and myricitin are not affected [49, 56.57].

It has been reported that one of the features of several non-steroidal, anti-inflammatory drugs, is their ability to prevent denaturation of protein (albumin) [59-61, 65], so the purpose of the present study was to investigate this property for extracts obtained from two plants, *Aloe vera* and gren tea, already recognized for theitr antioxidant effects.

2. EXPERIMENTAL SECTION

2.1. Chemicals and drugs. The standard reference drug, Diclofenac sodium, was purchased from the Sabon Gari drug market, Zaria. Other reagents used are analytical grade from BDH, M&B, Sigma or, Fluka.

2.2. Plant Materials. The *Aloe vera* plant leaves were obtained from a cultivated garden in Hayin Danyaro, Samaru, Zaria, and were identified by the Department of Botany, Faculty of Sciences, Ahmadu Bello University, Zaria, Kaduna state of Nigeria, while the green tea (*Camellia sinensis*) leaves were from a farm in Gembu plateau in Taraba state of Nigeria. The tea leaves were also identified by the Department of Botany, Faculty of Sciences, Ahmadu Bello University.

2.3. Preparation of Extracts. The crushed plant materials (50g) were extracted with distilled water (350 mL) by boiling under reflux for 30minutes. The extracts were filtered and evaporated to

dryness to yield the dry extracts of Aloe vera, (yield: 32.28%) and green tea, (yield: 52.51%). The dry extracts were kept in a vacuum desiccator until when ready for use. The total phenolic contents of the two extracts were determined spectrophotometrically by applying the Folin-Ciocalteu assay with gallic acid as standard [12].

2.4. *In-vitro* anti-inflammatory activity. The screening for anti-inflammatory activity was carried out according to the adapted *in-vitro* protein denaturation bioassay method of Jagtap *et al* [58] and Sangita Chandra *et al* [59].

The standard reference drug, Diclofenac sodium, and extracts of *Aloe vera* and green tea 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 *Aloe vera* and green tea so that final concentrations became 31.25, 62.5, 125, 250, 500,1000 µg/mL. The 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 protein denaturation inhibition was calculated and compared with a 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.

$$\% \text{ Inhibition} = 100 \text{ X } [V_t / V_c - 1]$$

Where, V_t = Mean absorbence of test sample; V_c = Mean absorbence of control

The results are described in Table 1. The extract concentration for 50% inhibition (IC 50) was determined by the dose-response curve.

3. RESULTS SECTION

In the present study, the evaluation of anti-inflammatory effects was undertaken using the effect of *Aloe vera* and green tea extracts on protein denaturation. Denaturation of proteins is well documented and is caused by inflammation process, mostly in conditions like arthritis [58-63]. Therefore, using agents that can prevent protein denaturation would be worthwhile for anti-inflammatory drugs development.

Table 1: Anti-inflammatory data of *Aloe vera*, Green tea and Diclofenac sodium

Concentration (µg/mL)	***Aloe vera* (% inhibition)**	**Green tea (% inhibition)**	**Diclofenac sodium (% inhibition)**
Control	--	--	--
31.25	243.8±8.0	187.8±32.1	10.2±4.1
62.5	408.5±5.1	315.7±4.4	20.3±1.3
125	804.0±4.3	621.3±1.3	40.7±1.7
250	1517.6±7.1	1172.5±72.5	81.3±2.8
500	3215.9±6.2	2485.0±5.0	163.0±2.9
1000	6444.7±10.5	4985.0±5.0	406.3±7.7

The ability of *Aloe vera* and green tea to inhibit protein denaturation may contribute to their anti-inflammatory properties. In the present investigation, the *in vitro* anti-inflammatory effect of *Aloe vera* and green tea were evaluated against denaturation of egg albumin. The results are as presented inTable 1. The study showed a concentration-dependent inhibition of protein (albumin) denaturation

by both *Aloe vera* and green tea extracts, within the concentration ranges of 31.25 to 1000 µg/mL. The reference drug, Diclofenac sodium, also exhibited concentration-dependent inhibition of protein denaturation [Table 1]. The IC 50 values are summarized in Table 2. In this study green tea was found to be more effective than *Aloe vera*; but the effect of diclofenac sodium was found to be lower when compared with both the test extracts. Their IC 50 values comfirmed this.

Table 2: IC_{50} values of *Aloe vera*, Green tea and diclofenac sodium

	Aloe vera	**Green tea**	**Diclofenac sodium**
IC_{50} (µg/mL)	8.8±2.0	6.8±1.2	605±15.5

There were increments in the absorbance of the test samples, with respect to the control, suggesting the inhibition of protein (albumin) denaturation, which is also a measure of the anti-inflammatory effect of the test extracts and of the reference drug, diclofenac sodium [59-61].

Both *Aloe vera* and green tea contain varying amounts of polyphenols, particularly flavonoids [42-47, 49-51, 64]. Polyphenols are a class of compounds that possess potent microbial and biological properties [64]. In the present study, the high anti-inflammatory effect of both *Aloe vera* and green tea may possibly be attributed to their high flavonoids (catechin) contents.

4. CONCLUSIONS

In summary, from the results of this study, it can be concluded that both *Aloe vera* and green tea possess a marked anti-inflammatory effect as they can limit the denaturation of protein process *in vitro*. This test 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. However, it is pertinent to suggest that identifying the potent fractions or components of these extracts is essential and remains to be investigated in further studies.

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