# The In-vitro Antioxidant and Anti-inflammatory Action of *Aloe Vera, Chamomile,* and *Propolis*: A Formulation Analysis

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**Abstract**

**Aims & Background:**

The aim of this research is to evaluate the Antioxidant and Anti-inflammatory activity of *Aloe vera*, *Chamomile*, and *Propolis* formulation. Plant extracts have been widely used for their therapeutic properties for many centuries. Polyphenols are the primary compounds present in the *Aloe vera*, *Chamomile*, and *Propolis* which provides anti-oxidant and anti-inflammatory activity and are responsible for offering immunity against free radical tissue damage and are crucial in preventing various chronic diseases such as inflammation, anaemia, cardiovascular disorders and aging.

**Materials and Methods:**

The aqueous *Aloe vera, Chamomile, and Propolis* formulation was prepared. The antioxidant capacity of the formulation was determined using 2 different assays such as DPPH and $H_2O_2$ assays. The anti-inflammatory capability of the formulation was assessed using two assays: BSA and EA assays.

**Results:**

The inhibitory percentage of DPPH scavenging activity varied from 65%-90% at a concentration range between 10-50 µg/mL. The inhibitory percentage of $H_2O_2$ scavenging activity varied from 49%-85% at a concentration range between 10-50 µg/mL. The inhibitory percentage of heat-induced BSA denaturation ranged from 45%-80%. The protein denaturation inhibition ranged from 50%-80% at a concentration range between 10-50 µg/mL. The percentage of inhibition of egg albumin denaturation assay ranged from 55%-80%.

**Conclusion:**

The results obtained from the assays proved that *Aloe vera, Chamomile,* and *Propolis* formulations possess potential antioxidant and anti-inflammatory capacity and can be effectively used as an effective antioxidant agent and anti-inflammatory agent. In order to increase the efficacy, it is important to combine components that have increased therapeutic properties.

**Clinical significance:**

These findings support the present usage of *Aloe vera, Chamomile,* and *Propolis* formulation as an effective alternative for treating various diseases.

**Keywords:** Antioxidant, Anti-inflammatory, *Propolis, Chamomile, Aloe vera,* polyphenols

## Introduction:

Antioxidants are compounds that operate in cells which neutralizes free oxygen radicals and reactive oxygen species (ROS) [1]. The primary compound present in the plant extracts that are responsible for the antioxidant property are phenols which consist of an aromatic ring which makes it stable and allows it to relocate the unpaired electrons present in its structure, permitting the donation of hydrogen atoms and other electrons from their hydroxyl groups. The total content of phenol in plants changes according to the species, plant tissue, growing stage, and environmental factors such as water stress, temperature and light [2]. These antioxidants which provides anti-oxidant and anti-inflammatory activity are responsible for offering immunity against free radical tissue damage and are crucial in preventing various chronic diseases such as inflammation, anaemia, cardiovascular disorders and aging [1].

Inflammation is the reaction of our body's immune system to potentially damaging stimuli such as pathogens, impaired cells, UV irradiation, or toxic substances [3]. Plant extracts have been employed as natural anti-inflammatory agents, characterized by an excess of inflammatory mediators such as ROS and pro-inflammatory cytokines [4]. The compounds present in the plant extracts act by inhibiting tumour necrosis factor (TNF)-alpha production or pro-inflammatory interleukin (IL)-6, increasing IL10 production or inducible nitric oxide synthase expression or suppressing cyclooxygenase-2 [3].

*Aloe vera* is a fleshy xerophyte which consists of tissues that store water which allows it to sustain in dry places with less or inconsistent rainfall [5]. Polyphenols, anthroids and pyron derivatives, saponins, salicylic acid, flavonoids, steroids, fibres, minerals (calcium, chromium, zinc, natrium) and vitamins (E, C, beta-carotene, B1, B6, choline, B12, folic acid) are bioactive compounds present in aloe vera [6]. The skin leaf of *Aloe vera* has more polyphenolic chemicals than in the flower. Caechin predominates in the skin, although gentisic acid is more abundant in the flower [7]. The bioactive compounds present in *Aloe vera* contains anti-inflammatory properties and serve as a favourable medium for symbiotic bacteria [7].

*Chamomilla's* flower is a non-toxic and edible plant, the primary constituents of which are phenolics and other bioactive compounds such as alpha-bisabolol, a natural monocyclic sesquiteroene alcohol [8]. Many researchers have reported antimicrobial, anti-inflammatory, antioxidant, antisplasmodic, and antiviral activities owing to the presence of terpenoids, flavonoids (such as apigenin and luteolin), coumarins, and spiroethers present in the plant [8].

*Propolis,* a resin containing substance is collected by the honeybees from the leaves, buds, barks, and lacquers of numerous trees [9]. Propolis is made up of about 50% resins, 10% waxes and essential oils, 5% pollens and other miscellaneous organic compounds [4]. There are three major categories of propolis based on the shape, behaviour, and biological geography: Propolis from the temperate zone, propolis from the tropical zone, and propolis from the Pacific zone [4]. Compounds like flavonoids and phenolic acid present in propolis are responsible for its antioxidant properties [5]. The anti-inflammatory property of propolis comes from reducing IL-10 production, inhibiting cytokines and metabolically reprogramming LPS action in

macrophages [10]. Propolis has been demonstrated to be effective in treating and reducing dental/oral-related disorders and diseases due to its antibacterial, antioxidant, and anti-inflammatory qualities [10].

Consequently, this current research was planned to explore the effective oxidation inhibition and anti-inflammatory properties of aloe vera, chamomile, and propolis formulation. The primary aim of this study is to evaluate the oxidation inhibition capacity and Anti-inflammatory activity of *Aloe vera*, *Chamomile*, and *Propolis* formulation.

## Methods:

### Preparation of the Formulation:

To prepare the aqueous formulation of *Aloe vera*, *Chamomile*, and *Propolis* the following method was used: In 100 mL of distilled water, 1 g of *Aloe vera*, 1 g of *Chamomile*, and 1 g of *Propolis* was dissolved and boiled using heating mantle at 40º- 45º C. The solution was then filtered using a muslin cloth and the aqueous formulation was condensed to 5 mL and was placed in the refrigerator for further analysis (figure 1).

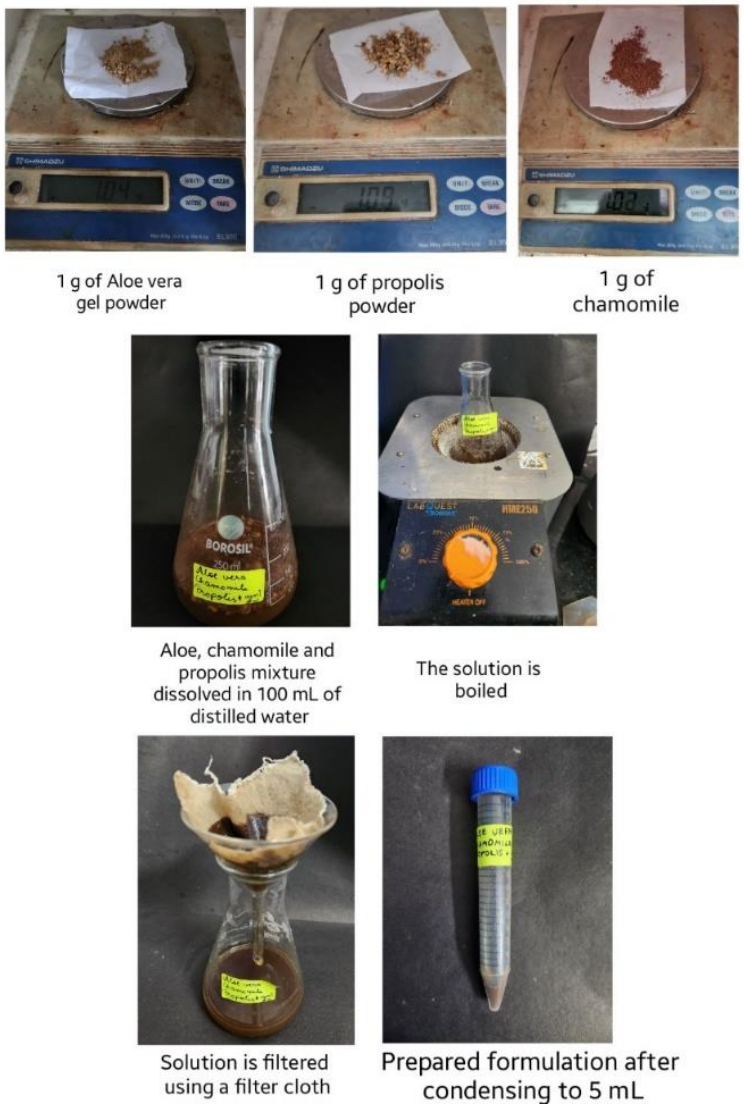


**Figure 1: Preparation of the formulation**

**Determination of Antioxidant Activity:**

**DPPH assay:**

A concentrated stock solution was prepared by adding of 0.1 mM of DPPH in methanol. For each assay, the initial stock solution was diluted in methanol to produce a fresh working DPPH solution with a concentration of 20 µM. In a 96-well plate, *Aloe vera*, *Chamomile*, and *Propolis* formulation were added in 5 concentrations (10 µg/mL - 50 µg/mL) to 200µL of the DPPH working solution. The incubation of the plate was done for about 30 minutes in a dark place at room temperature. A microplate reader was used to detect the absorbance at 517 nm. Methanol was used as a blank. The standard used in this study consisted of ascorbic acid (1 mg/mL). The scavenging activity (%) was established using the following formula:

% of DPPH Scavenging Activity = [(AB control – AB sample) / AB control] × 100

where AB control - ascorbic acid absorbance (DPPH solution without the sample)

AB sample - the sample (formulation) absorbance (DPPH solution with the extract)

**$H_2O_2$ assay:**

The Hydroxyl radical scavenging assay proposed by Halliwell et al was applied in this research to assess the antioxidant activity. Reaction mixture of 1 mL was made with 28 mM of 2-deoxy-2-ribose (100 µL) and various concentrations of *Aloe vera*, *Chamomile*, and *Propolis* formulation (10-50 µg/mL). In addition, 200 µL of 200 µm ferric chloride, 100 µL ascorbic acid and 200 µL of EDTA was added. The optical density was calculated at 532 nm against the blank solution after incubating it for 1 hour at 37 °C. The standard used was Vitamin E.

% of Hydroxyl radical scavenging activity = [(AB blank – AB sample)/AB blank] × 100

Where AB blank - the absorbance of standard (vitamin E) (without sample)

AB sample - the absorbance of the formulation (with the sample).

**Determination of Anti-inflammatory activity:**

**Bovine serum albumin denaturation assay:**

The anti-inflammatory activity of the formulation was assessed using two assays: Bovine serum albumin denaturation assay and Egg albumin denaturation assay. 0.05 mL of the formulation in various concentrations (10-50 µg/mL) was added to 0.45 mL of bovine serum albumin. The pH was altered to 6.3. After placing it outside at room temperature for 10 mins, incubation was done at 55°C in a water bath for 30 minutes. The standard in this assay was Diclofenac sodium. The samples were then spectrophotometrically measured at 660nm.

The protein denaturation (%) was determined utilizing equation,

$$\% \text{ inhibition} = \frac{\text{control absorbance - Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

**Egg Albumin denaturation assay:**

0.2mL of fresh egg albumin was mixed with 2.8 mL of phosphate buffer to perform the Egg albumin denaturation assay. The prepared formulation was mixed with to reaction mixture at different concentrations (10-50 µg/mL). The pH was moderated to 6.3. After placing it outside at room temperature for 10 mins, incubation was done at 55°C in a water bath for 30 minutes. The standard group used was Diclofenac sodium. The samples were then spectrophotometrically measured at 660 nm.

Percentage of protein denaturation was determined utilizing following equation,

% of inhibition = control absorbance - Absorbance of sample × 100

Absorbance of control

## Results:

### DPPH radical scavenging activity:

At 10 µg/mL, the percentage of inhibition of the standard ascorbic acid and the aqueous formulation was 70% and 65%. At 30 µg/mL, the inhibitory percentage of the standard and the formulation was 90% and 85%. The inhibitory percentage of DPPH scavenging activity of the formulation was dose-dependent ranging between 65% at 10 µg/mL and 90% at 50 µg/mL (figure 2).

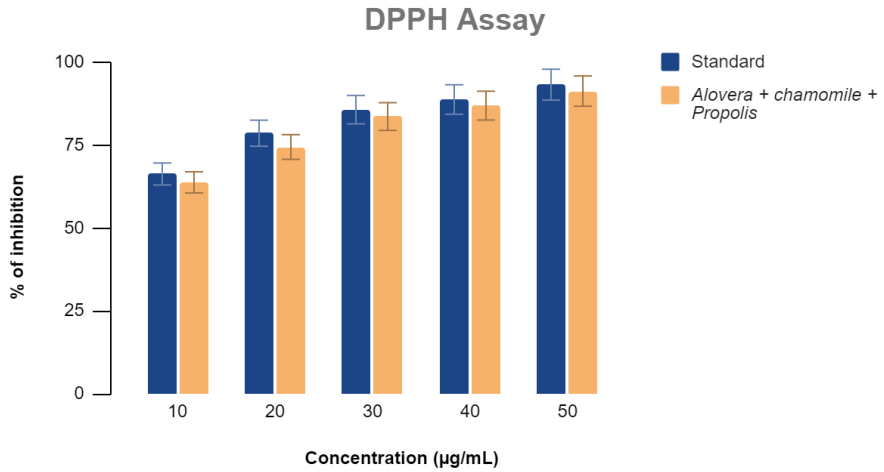


**Figure 2: Antioxidant activity of *Aloe vera, Chamomile* and *Propolis* formulation using DPPH assay**

### Hydrogen peroxide scavenging activity:

At 10 µg/mL the percentage of inhibition of the standard vitamin E and the aqueous formulation was 51% and 49%. At 30 µg/mL the inhibitory percentage of the standard and the formulation was 65% and 60%. According to our results, the formulation was shown to scavenge the $H_2O_2$ radicals with an excellent scavenging activity with an exhibition of 85% at 50 µg/mL (figure 3).

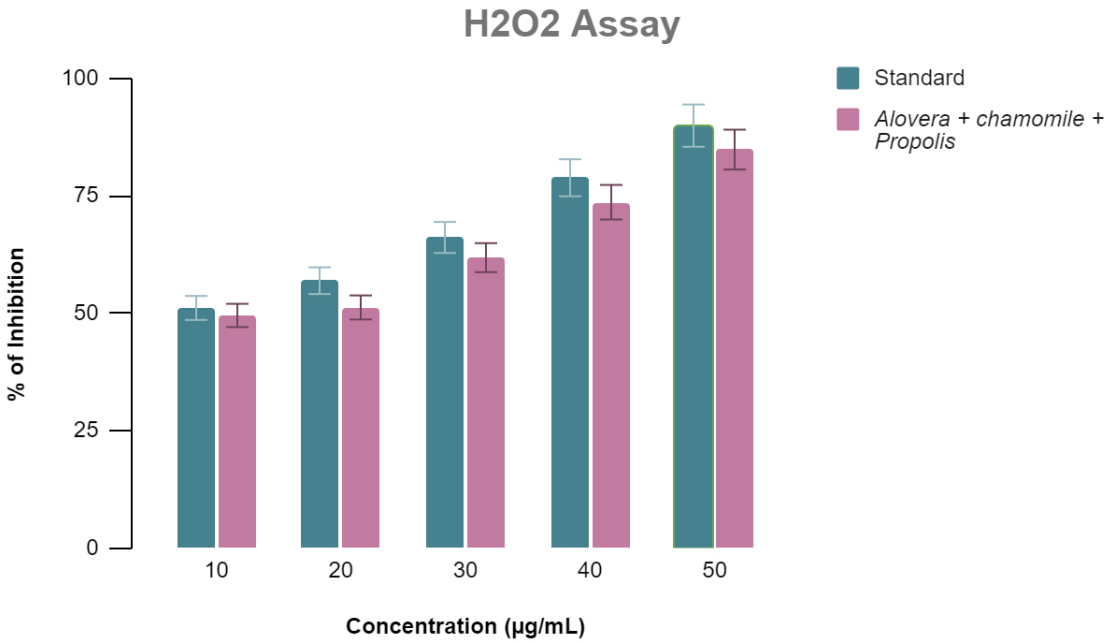


**Figure 3: Antioxidant activity of *Aloe vera, Chamomile* and *Propolis* formulation using $H_2O_2$ assay**

**Bovine serum albumin denaturation assay (BSA assay):**

At 10 µg/mL the inhibitory percentage of the standard diclofenac sodium and the aqueous formulation was 46% and 45%. At 30 µg/mL the inhibitory percentage of the standard and the formulation was 70% and 65%. The percentage of inhibition of the standard and the formulation was highest at a rate of 83% and 80% at the concentration of 50 µg/mL. Aloe vera, Chamomile, and Propolis formulation significantly exhibited an increased inhibition of heat-induced BSA protein denaturation at increased concentrations (figure 4).

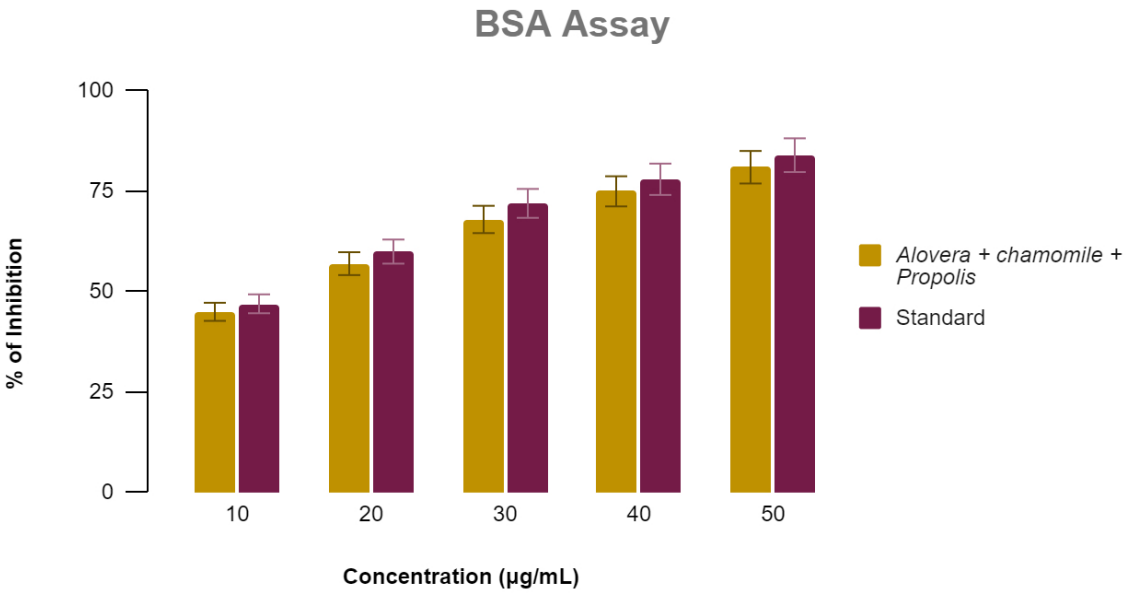


**Figure 4: Anti-inflammatory activity of *Aloe vera, Chamomile* and *Propolis* formulation using BSA assay**

**Egg albumin denaturation assay (EA assay):**

At 10 µg/mL, the inhibitory percentage of the standard diclofenac sodium and the aqueous formulation was 55% and 50%. At 30 µg/mL the inhibitory percentage of the standard and the formulation was 70% and 65%. The highest inhibitory rate of 80% was observed in a concentration of 50 µg/mL (figure 5).

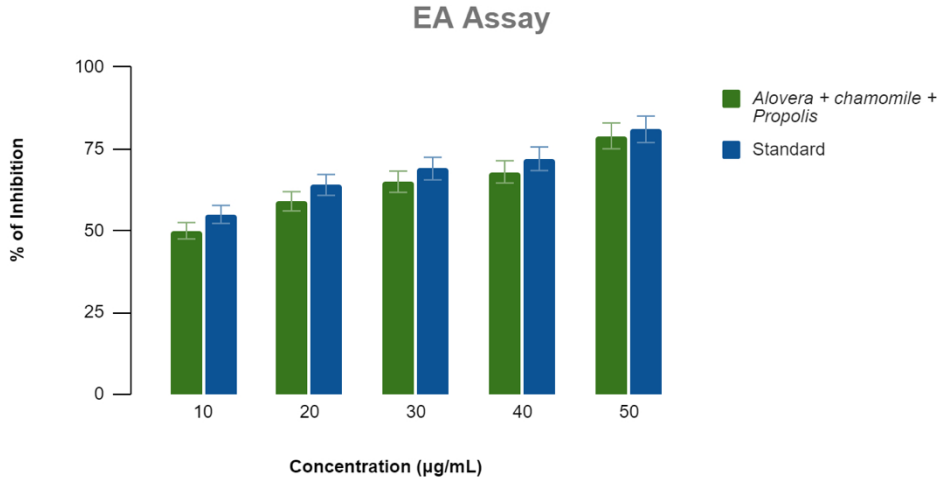

**Figure 5: Anti-inflammatory activity of *Aloe vera, Chamomile* and *Propolis* formulation using Egg Albumin denaturation assay**

## Discussion:

The percentage of inhibition of DPPH scavenging activity varied from 65%-90% at a concentration range between 10-50 µg/mL. The inhibitory percentage of $H_2O_2$ scavenging activity varied from 49%-85% at a concentration range between 10-50 µg/mL. The inhibitory percentage of heat-induced BSA denaturation ranged from 45%-80%. The inhibitory percentage of egg albumin protein denaturation ranged from 50%-80% at a concentration range between 10-50 µg/mL.

In one study, the antioxidant property of *Aloe vera* gel powder was analysed by DPPH assay with different solvents, and the results obtained from the study showed maximum antioxidant activity of 51.09% in ethanol solvent [11]. In another study, the anti-oxidant and anti-inflammatory activity was assessed for carbohydrate enriched fraction from aloe vera extract [13]. The highest percentage of inhibition in the DPPH assay was 70% and 75% in the $H_2O_2$ assay at 2 mg/mL. In heat-induced albumin denaturation assay, the carbohydrate fraction (CF) extract of *Aloe vera* exhibited a maximum inhibition of 93.2% at concentration of 2 mg/mL. The formulation prepared in our study exhibited a maximum inhibition of 90% and 85% in DPPH and $H_2O_2$ assay at the highest concentration of 50 µg/mL. In the BSA assay, the formulation prepared in our study showed a maximum inhibition of 80%. This shows the increase in efficacy of *Aloe vera* when combined with *Chamomile* and *Propolis*.

The oxidation inhibition potential of propolis was evaluated by DPPH assay in another study [9]. The ethanolic-extracted propolis showed a higher percentage of inhibition (94.45%+0.85) than the water extract propolis (90.01% + 0.18) which is similar to the results obtained from our study. The anti-inflammatory potential of Sonoran propolis extract was evaluated by BSA assay and the percentage of inhibition was evaluated between the range of 81.67% to 100% at the concentration of 6.25 to 50 µg/mL in another study [14] which is similar to the results obtained in our study.

In another study, assessment of the oxidation inhibition potential of *Chamomile* extract was done. The DPPH scavenging activity of the extract in their study was in the range of 94.8% at 1.50 mg/mL to 84.2% at 0.15 mg/mL [12]. In our study, 90% of inhibition was exhibited by the extract at 0.05 µg/mL. It was found in a study that *Chamomile* reduces production of NO and Inducible nitric oxide synthase (iNOS) expression in macrophages and that these effects are mediated by the NF-B/Rel transcription factor [15]. Nitric oxide is critical in the pathophysiology of several inflammatory disorders including cancer. *Chamomile*'s inhibitory effect on iNOS gene expression implies that it is one of the mechanisms behind its anti-inflammatory properties.

Researchers have become increasingly interested in the usefulness of polyphenols during the last decade. This is mostly due to their antioxidant qualities and participation in the prevention

of disorders associated with oxidative stress such as cancer, neurodegeneration, and diabetes. Polyphenols are a wide class of compounds that include a lot of phenolic hydroxyl groups connected to ring structures, allowing them to be antioxidants. There are no studies stating the antioxidant potential of *Aloe vera, Chamomile*, and *Propolis* as a formulation. Thus, the usage of herbal plants as raw materials for drug formulation can be a cheap and effective alternative for treating diseases [16].

## Conclusion:

There are various studies in which the oxidation inhibition potential and the anti-inflammatory activity of *Aloe vera, Chamomile*, and *Propolis* were evaluated separately. The results obtained from the assays proved that *Aloe vera, Chamomile*, and *Propolis* formulation possesses antioxidant and anti-inflammatory capacity and can be effectively used as an anti-oxidant and anti-inflammatory agent. In order to increase the efficacy, it is important to combine components that have increased therapeutic properties.

## Clinical significance:

These findings support the present usage of *Aloe vera, Chamomile*, and *Propolis* formulation as an effective alternative for treating various diseases.

## List of abbreviations:

DPPH – 2,2-diphenyl-1-picrylhydrazyl

$H_2O_2$ – Hydrogen peroxide

BSA – bovine serum albumin denaturation assay

EA – egg albumin denaturation assay

ROS – reactive oxygen species

UV – ultraviolet

TNF – tumour necrosis factor

IL – interleukin

LPS – lipopolysaccharide

EDTA – ethylenediaminetetraacetic acid

INOS – inducible nitric oxide synthase

NF – nuclear factor

% – percentage

µg – microgram

C – celcius

mM - millimolar

g – gram

nm – nanometer

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