

# In vitro evaluation of antioxidant and anti-inflammatory potentials of herbal formulation containing marigold flower (*Calendula officinalis* L) and green tea (*Camellia sinensis* (L.) extracts.

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## ABSTRACT

**Introduction:** Marigold flower (*Calendula officinalis* L) and green tea (*Camellia sinensis* (L.) extracts have antioxidant, anti-inflammatory, and antimicrobial properties. Various studies have assessed the properties of green tea and marigold tea extracts, but none have examined their combined antioxidant and anti-inflammatory actions.

**Aim:** To assess the anti-inflammatory and antioxidant properties of herbal formulation containing green tea (*Camellia sinensis* (L.) and marigold tea (*C. officinalis*) extract.

**Materials and methods:** Formulation of 2 grams of dried marigold flower petals and 2 grams of green tea leaves and 100 ml of distilled water were subjected to anti-inflammatory testing using albumin denaturation assay. Anti-protease activity and antioxidant testing by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay were also performed to assess their efficacy.

**Results:** Highest anti-inflammatory and antioxidant activities were exhibited at 50µL. The results of the present study showed that the formulation based on marigold and green tea extract had anti-inflammatory and antioxidant properties. The highest antioxidant and anti-inflammatory activities were present at 50ul.

**Conclusion:** It can be concluded that the formulation based on green tea and marigold tea showed anti-inflammatory and antioxidant properties

**Keywords:** Anti-inflammatory, Anti-oxidant, Calendula officinalis L and Camellia sinensis (L) Green tea, Marigold tea.

## INTRODUCTION

Tea is one of the most widely consumed beverages worldwide, and is available in various forms. Green tea is mainly consumed in Asia, some parts of North Africa, the United States, and Europe.<sup>1</sup> Green tea is richer in antioxidants compared to other forms of tea.<sup>2</sup> Green tea is produced from the leaves, buds, or even the stems of all the plants of the genus *Camellia*.<sup>3</sup> *Camellia sinensis* (L.) Kuntze is the most commonly used plant species for green tea usage both commercially and medically. Green tea has high polyphenol content, especially flavanols which make up 30% of fresh leaf dry weight. In green tea, flavanols have numerous health-promoting properties.<sup>4</sup> Green tea also has epigallocatechin-3-gallate (EGCG), which is a Catechin, a type of plant-based compound. EGCG is a potent antioxidant and acts against free radicals, thereby preventing cellular damage.<sup>5</sup> It has numerous health benefits such as reduction in blood pressure, cholesterol reduction, weight loss and also few studies suggest that the EGCG present in green tea can be beneficial in preventing degenerative brain disease.<sup>6</sup> It has been found that 250 ml of green tea contains about 50-100 mg of EGCG. While EGCG has multiple beneficial properties, high intake of EGCG (more than 800 mg) can increase transaminase levels in the blood, which is an indicator of liver damage.<sup>7,8</sup> Besides EGCG green tea pos-

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sesses additional antioxidants which make green tea extracts more stable than pure epigallocatechin gallate.<sup>8</sup>

*Calendula officinalis* L. (marigold) belongs to the Asteraceae/Compositae family, which is native to Central Europe and the Mediterranean.<sup>9</sup> *Calendula officinalis* is widely

grown in sunny areas and a variety of soils.<sup>10</sup> Medicinal properties of *C. officinalis* have been mentioned in Ayurvedic and Unani systems of medicine indicating that leaves and flowers are antipyretic, anti-inflammatory, antiepileptic and antimicrobial.<sup>11</sup> Few studies have demonstrated that *C. officinalis* have anti-inflammatory properties. Both Ayurvedic and Unani systems of medicine boast the various medicinal properties of *C. officinalis*, and it has been suggested that the leaves and flowers have antipyretic, anti-inflammatory, antiepileptic and antimicrobial properties.<sup>11</sup> In traditional homeopathic medicine, *C. officinalis* has been used to treat poor eyesight, menstrual irregularities, varicose veins, hemorrhoids, and duodenal ulcers.<sup>12</sup> Calendula flowers were also used for liver obstructions, and snake bites in olden times and were also used to strengthen the heart.<sup>13</sup>

In general, herbal medicines are complex mixtures of various compounds that frequently work together to achieve maximum efficacy.<sup>14</sup> Various studies have been conducted that assessed the various properties of green tea and marigold tea extracts, but there are no previous studies which assessed their combined action. Since, good properties have been exhibited by these two extracts in various individual studies, assessing the properties of the formulation based on the combination of marigold and green tea, might prove to be beneficial for the treatment of various diseases and conditions. The aim of the study was to assess the antioxidant and anti-inflammatory potentials of herbal formulation containing marigold flower and green tea extracts in an in vitro setting, as they have proven to have multiple health benefits.

## MATERIALS AND METHODS

This study was conducted in the Department of Nanobiomedicine, at Saveetha Dental College and Hospitals, Chennai. Dried marigold flowers and dried green tea leaves were obtained from a local market in Chennai. The extract was prepared and the antioxidant and anti-inflammatory properties was evaluated.

### Preparation of the extract:

The extract was prepared by dissolving 2 grams of dried marigold flowers and dried green tea leaves in 100 ml of distilled water separately. The mixtures were heated and filtered (Figure 1). The filtered extracts were reduced then to 10ml, and were mixed.

### Anti-inflammatory activity:

#### Albumin denaturation assay:

The anti-inflammatory activity for the formulation of green tea and marigold tea extract was tested by the following procedure proposed by Muzushima and Kabayashi with specific alterations by (Pratik Das et al.,2019).<sup>15</sup> 0.05 mL of the green tea and marigold tea formulation of various fixation (10µL,20µL,30µL,40µL,50µL) were added to 0.45 mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was adjusted to 6.3 using 1N hydrochloric acid. The samples were incubated at room temperature for 20 min and then heated at 55°C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. Dimeth-

yl Sulfoxide (DMSO) was utilized as a control.

The percentage of protein denaturation was determined utilizing the following equation,

$$\% \text{ inhibition} = (\text{Absorbance of control} - \text{Absorbance of sample} \times 100) / \text{Absorbance of control}$$

### Anti-proteinase activity:

Casein was used as a substrate and trypsin was used as aserine protease enzyme to cleave the peptide linkage of casein. 1mL of 20mM of Tris Hcl buffer (pH 7.4) was mixed with 0.06 mg of trypsin and 1mL of the test sample (100-500µg/mL). The reaction mixture was incubated at room temp (37°C) for 5 min. (0.8%) (W/V) casein was added to the reaction mixture and was further incubated for 20 more minutes. 2mL of 70% perchloric acid was added to the mixture to terminate the reaction. The solution was centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was recorded at 210nm. An equal volume of plant extract was replaced with DMSO solution and used as a control. Diclofenac sodium in different concentrations was used as a standard. The IC50 values were calculated. The experiment was performed in triplicate.

% inhibition was calculated using the following formulae:

$$\% \text{ inhibition} = \text{control OD} - \text{Sample OD} / \text{Control OD} \times 100$$

### Antioxidant activity:

#### 2,2-Diphenyl-1-picrylhydrazyl assay:

The DPPH free radical is a long-lived organic nitrogen radical with a deep purple color. When a DPPH solution is mixed with an antioxidant, its color turns from purple to yellow of the corresponding hydrazine. The reducing ability of antioxidants toward DPPH can be evaluated by monitoring the decrease of its absorbance at 515–528 nm. The results are expressed as IC50 or as % scavenging of DPPH at a fixed antioxidant concentration for all the samples.

#### Preparation of DPPH solution

DPPH solution was prepared by taking 7.89 mg of DPPH using a chemical balance, dissolving with 100 ml 99.5% ethanol, and finally kept in dark for 2 hr.

#### DPPH assay procedure:

DPPH solution of 1,000 µl was added with 800 µl of Tris-HCl buffer (pH 7.4) in a testing tube. And then 200 µl of testing sample solution was added and mixed quickly. The solution was kept at room temperature for 30 min. The absorbance of the solution at 517 nm was recorded. A mixed solution with 1,200 µl of ethanol and 800 µl of Tris- HCl buffer (pH 7.4) was used as the blank. The inhibition ratio (%) was obtained from the following equation:

$$\text{Inhibition ratio (\%)} = (A1 - A2) \times 100 / A1,$$

[where A1 is the absorbance of the addition of ethanol instead of testing sample and A2 is the absorbance of testing sample solution].

## RESULTS

The results of the present study showed that the formulation based on marigold and green tea extract had better anti-inflammatory activities at higher concentrations than the control. Highest anti-inflammatory activities were present at 50ul, while the lowest anti-inflammatory activity was seen at 10ul



(Figure 2). The antioxidant activity of marigold and green tea extract was greater in higher concentrations than the control and was lower than the control in lower concentrations. Highest antioxidant potential was seen at 50ul, whereas at 10ul the antioxidant properties of the formulation was lesser than the control. (Figure 3) (Table 1).

**DISCUSSION**

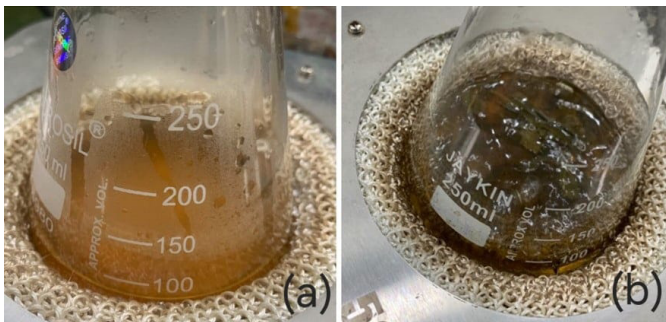
Green tea polyphenols suppress the activity of xanthine oxidase, increase the activity of various antioxidant enzymes such as CAT and GPx, EGCG, as one of the major and the most active components of green tea, has been shown to have protective effect against cellular damage, by the suppression of activ-

ity of transcription factors, NF-B and AP-1 thereby inhibiting expression of enzymes like COX 2.<sup>16,17</sup> It has been found that green tea extracts produced anti-inflammatory response by activating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway and increasing the level of antioxidant protein heme oxygenase-1 (HO-1).<sup>18</sup> In a previous study by A Hagiü et al, it was found that green tea extract had anti-inflammatory action by triggering the nuclear factor erythroid 2-related factor 2 pathway (Nrf2) and thereby increasing the levels of heme oxygenase 1 an antioxidant protein at higher concentrations, these results were synonymous with the results obtained in the present study.<sup>19</sup> K. Suzuki et al, had found evidence that in cancer patients oxidative stress is partially mediated by reactive oxygen species that are produced by neutrophils, it was found that green tea extracts had antioxidant potentials and caused reduction in reactive oxygen species. Higher antioxidant potentials were found at higher concentrations which were similar to the results obtained in the present study.<sup>20</sup>

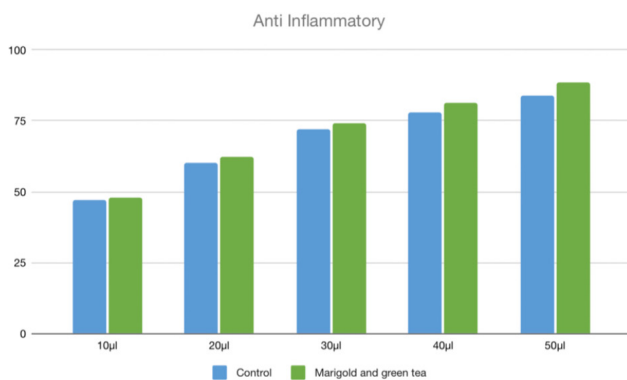
**Table 1:** The antioxidant and anti-inflammatory values obtained for Marigold and green tea extract

Concentration	Antioxidant		Anti inflammatory	
	Control	Marigold and green tea extract	Control	Marigold and green tea extract
10µl	76.56	75.56	47	48
20µl	78.52	77.12	60	62.2
30µl	85.63	89.4	72	74
40µl	88.68	92.1	78	81.4
50µl	93.15	96.3	84	88.6

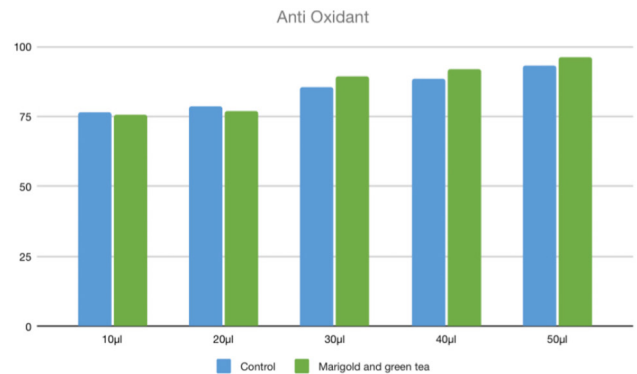
Marigold flower extract inhibits pro-inflammatory cytokines (IL-1β, IL-6, TNF-α and IFN-γ), COX-2, and subsequent prostaglandin synthesis.<sup>21</sup> It has antioxidant properties by inhibiting ROS and RNS, at low concentrations.<sup>22</sup> In a previous study it was reported that, higher anti-inflammatory effects of green tea were attributed to its higher flavonoids (catechin) content.<sup>23</sup> The effect may be due to the synergistic effect rather than a single constituent. The antioxidant and analgesic activities of *C. officinalis* were found to be comparable to the standard controls in previous studies hence the Aztec Marigolds has potential medicinal uses such as an anti inflammatory and analgesic agent.<sup>24</sup> In the present study, formulation of marigold and green tea extracts showed better anti-inflammatory and antioxidant activity when compared with the respective controls in higher concentrations. Previously in an in vitro study carried out by KM Alsaraf et al, antioxidant potential of marigold flower cream was assessed and it was found to have obvious antioxidant activities at 10% and at 15% (low concentrations).<sup>25</sup> In a previous silico study carried out by A. Bellal et al, it was found that benzopyran-4-one moiety, a constituent of *C. officinalis* is potent against MMP 8 and MMP 9, and hence it was suggested that Calendula can be used in the treatment of



**Fig. 1:** Dried Marigold flowers and dried green tea leaves, mixed with 100 ml of distilled water, and boiled.



**Fig. 2:** Anti inflammatory activity of control and marigold and green tea extract.



**Fig. 3:** Antioxidant activity of control and marigold and green tea extract.



diabetic foot ulcers.<sup>26</sup>

In the present study, the anti-inflammatory inhibition levels and the absorbance levels of antioxidant properties were better exhibited at higher concentrations. This could be due to the combined beneficial actions of both marigold and green tea. *Calendula officinalis* flower extract and Green tea extracts have been reported to possess several pharmacological activities, various homeopathic preparation of *Calendula officinalis* and *C. sinensis* have reported to possess antiviral and antibacterial activities.<sup>27</sup> Previously, similar studies were done to assess anti-inflammatory and antioxidant potentials of various herb extracts such as *A. muricata*, *Musa sapientum*, grape seed extract, formulation based on Lycopene, Raspberry and Green tea, Marine brown seaweed, it was found that the medicinal herbs and plant extracts had better anti-inflammatory and antioxidant activities than standard controls.<sup>28-32</sup> The limitations of the present study is that it was carried out in a controlled in vitro setting, hence the results obtained must be validated using cell lines and animal models can be carried out using the formulation based on marigold tea.

## CONCLUSION

Within the limits of the present study, it can be concluded that the formulation based on green tea and marigold tea showed anti-inflammatory and antioxidant properties. Further studies should be conducted to evaluate the phytochemical constituents and other properties of this formulation.

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