RESEARCH ARTICLE

Antioxidant, Anti-inflammatory and Anthelminthic Activities of Traditionally used *Ocimum basilicum* L., and *Ocimum tenuiflorum* L. Extract Loaded Emulsion

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ABSTRACT

The demand for cosmeceuticals is rapidly expanding. Various phytoconstituents are identified from *Ocimum basilicum* and *O. tenuiflorum* and have several pharmacological properties. The present study aimed to formulate a novel alternative herbal formulation for topical administration with natural antioxidant properties for the treatment of inflammatory conditions with the destructive capacity of parasitic worms. The egg albumin denaturation technique was used to evaluate the test formulations' and the standard's *in-vitro* anti-inflammatory properties, and the hydrogen peroxide assay was used to measure the extracts' antioxidant properties. Eudrilus eugeniae was the target of the investigation into *in-vitro* anthelmintic action. The pH of the cream was 6.8 (average), it was easily spreadable and washable, no gritty particles were found and the dye test confirmed that it was an o/w type of cream. The egg albumin denaturation technique was utilized to evaluate the anti-inflammatory properties. The results cleared those herbal formulations (F1 and F2) and protein denaturation was decreased by diclofenac sodium (Standard) in a dose-dependent manner. Test samples exhibited higher anti-inflammatory activities than the standard with their percentage inhibitions being 41.5 ± 0.5 (F2), 39.1 ± 0.4 mg/mL (F1), and 37.3 ± 0.3 mg/mL (F-Standard), respectively. Antioxidants are compounds that either completely stop or significantly reduce the harm that free unstable radicals. F2 showed a higher concentration-dependent anthelmintic activity than F1 and standard albendazole. Increasing the concentration decreases the paralysis and death time of earthworms. Finally concluded that F2 indicates higher anti-inflammatory and anthelmintic activities than others with high natural antioxidant power.

Keywords: *Ocimum basilicum, Ocimum tenuiflorum*, Anti-inflammatory activity, Anthelmintic activity, Antioxidant activity.

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INTRODUCTION

Numerous disorders exhibit inflammation as a symptom, which is commonly accompanied by discomfort. Inflammation is most commonly caused by infections, burns, trauma, and other immune reactions.[1] Non-steroidal anti-inflammatory medicines, which are frequently used to treat inflammatory disorders, have several side effects when taken as a standard treatment for these conditions, most notably stomach irritation that can result in the development of gastric ulcers.[2] One of the most typical diseases in the globe is helminthic infection.[3] The World Health Organisation (WHO, 2019) has estimated that helminthiasis affects about 1.5 billion people globally.[4]

Our bodies naturally produce reactive oxygen species (ROS) as a by-product of metabolism or as a result of exposure to toxins. The natural antioxidant system in our bodies is constantly on guard to keep their levels under control. However, external antioxidants are needed when ROS production is excessive and the body's own antioxidant system is unable to remove them. Synthetic antioxidants decrease oxidation but have a number of negative side effects. However, natural antioxidants fight off free radicals, lessening the negative impact.[5] Plants provide a rich source of secondary metabolites such as flavonoids, phenolics, alkaloids, saponins, and other compounds, which are rich in valuable bioactivities like antioxidant, anti-inflammatory, and

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anti-helmtics.[6,7] The anti-inflammatory properties of alkaloids and a number of inflammatory mediators, such as histamine, cytokines and inflammatory response enzymes, have their expression inhibited.[8] Saponins are thought to be possible natural anthelmintic substances.[9]

India and Southeast Asia are the primary producers of *Ocimum tenuiflorum* (family: Lamiaceae). The perennial herb *O. basilicum* (family: Lamiaceae), generally known as basil or sweet basil, is used for culinary purposes.[10,11] Whole plants are utilized as traditional medicines because they are therapeutically effective and exhibit anti-inflammatory, anti-helminthic properties and natural antioxidant activity.[3,12,13] Utilizing phytomedicine provides a number of benefits, such as the fact that it uses plant extracts that are non-toxic and have the least side effects to humans while also supplying the body with nutrients and other beneficial minerals that are simple to produce, inexpensive, and the least drug resistance and time- and labor-efficient.

The WHO, as well as our nation, has been promoting traditional medicine since it is affordable, widely accessible, and complete, particularly in poor nations.[14] The potential advantage of bypassing gastrointestinal first-pass metabolism associated with oral administration is offered by the delivery of active plant elements through the skin. The emulsion is a topical formulation that is a biphasic liquid dose form. Polyherbal emulsion is one type of topical formulation applied to the external surfaces of the body.[15] Using cosmetics not only helps one seem attractive on the outside but also prolongs good health by preventing skin issues.

The primary aim of this research is to explore new alternatives for treating inflammatory conditions and formulating effective anti-inflammatory, antioxidant and anthelmintic-based herbal emulsions using ethanolic extracts of both plants. Hence, an attempt is made in the ongoing study to analyze the potentiality of the *Ocimum* species to cure the different epidermal inflammatory conditions with the destructive capacity of parasitic worms.

MATERIALS AND METHODS

Materials

In December 2022, the study's plants – *O. tenuiflorum* and *O. basilicum* – were gathered from the Gopalpur, Balasore, neighborhood. We bought more excipients from Pallav Chemicals and Solvents PVT LTD.

Methods

Extraction of plant

Extraction was done by the percolation method. First, leaves from the plants were dried for four to five days at a temperature of around 30 to 35°C. Then crushed and powdered. Then maceration process was done for 30 to 35 hours using ethanol as the menstruum. Then, the percolator was set up by following the steps. Cotton, filter paper, and sand were used in the percolator apparatus for separating and filtering purposes. Wet macerated crude plant material packed intro percolator and ethanol were used as the menstruum. After 28 to 30 hours the drain valve was open and the extract was collected in a china disc. Then the residue was collected in a china disc and placed at a temperature of 75 to 80°C in the hot water bath. The residue was then kept in a refrigerator at 4°C to be used for further studies.[16]

Development of formulation

Four formulations were created and manufactured, namely F1, F2, F-control, and F-standard. On W/O kinds, it is based. Separately, an aqueous phase containing hydrophilic materials like borax and an oil phase comprising lipophilic materials including cetostearyl alcohol, beeswax, liquid paraffin, and petroleum jelly were heated to 80°C in a water bath. For test and standard formulations, different quantities of diclofenac sodium and plant extracts were added to the oil phase. After that, until the combination congealed at room temperature, the aqueous phase was progressively added to the oil phase while being constantly stirred.[17]

Evaluation of cream

The resulting cream was assessed for its organoleptic qualities, such as color, aroma, and condition. The cream's color and roughness were used to evaluate and assess its appearance. A digital pH meter was used to measure the pH of the dissolved cream. The spreadability was determined by the cream was placed between the two glass slides and compacted to an equal depth by inserting cream between the two glass slides for five minutes.

$$\text{Spredability} = \frac{\textit{Length of glass slide}}{\textit{Time taken to separatethe slides}} x\ \textit{Weight tide to the upper slide}$$

For confirmation of w/o type emulsion, the oil soluble colour was added in the cream and examined under the microscope. In contrast, the dispersed globules of cream became colorless.[18-21]

Spectroscopic methods

Plant samples at varying quantities (1–4 g/mL) were diluted in phosphate buffer at pH 5, and then subjected to UV-visible spectrometry scanning analysis LT-291 [LABTRONICS MODEL].[22]

Centrifugation test

The sample was centrifuged for 30 minutes at room temperature using a cycle of 3000 revolutions per minute. Phase separation, a sign of unstable cosmetic formulations, was looked for in the cosmetic formulations after the centrifugation time [Centrifugal Machine with a Digital Bench Top].[22]

Saponification value

Took the material and reacted it for 30 minutes with 0.5 N alcoholic KOH. Then an indicator, phenolphthalein, was added, and the 0.5 N HCL was used to titrate it.[19]

$$\text{Value of Saponification} = \frac{(\textit{ml of HCL used in blank titration} - \textit{ml of } 0.5\,(N)\ \textit{HCL solution used for titration})\ x\ 28.05}{\textit{Weight of substances in gm}}$$

Acid value

Carefully weighed the mixture of solvent ether and alcohol to dissolve the cream. The flask with the condenser was then attached, and the sample was slowly heated and refluxed until it was completely dissolved. After that, phenolphthalein was added, and the mixture was titrated with (0.1N) NaOH until a light pink color was observed, shaking for 20 seconds.[19]

$$\text{Acid Value} = \frac{Number\ of\ ml\ in\ NaOH\ required\ x\ 5.61}{Weight\ of\ the\ substances}$$

In-vitro anti-inflammatory activity

The primary concern with using animals as a paradigm in an experimental study is ethical. For the evaluation of the effectiveness of the anti-inflammatory study, the protein denaturation technique was utilized. Utilizing a protein (egg albumin) denaturation inhibition technique, *in-vitro* anti-inflammatory efficacy was assessed. It is a straightforward and practical way to assess anti-inflammatory activity. The managed solution of freshly made egg albumin (2 mL) was transferred to phosphate buffer saline of pH 6.4, and add distilled water (20 mL) to create the control solution. Standard approach freshly made egg albumin (2 mL) was mixed with 28 mL pH 6.4 phosphate buffer. Next, a 20 mL solution of diclofenac sodium in a range of concentrations from 10 to 2000 g/mL was added. Test response freshly made egg albumin (2 mL) was mixed with 28 mL pH 6.8 phosphate buffer and then (20 mL) solution of cream formulation with various concentration ranges from 10 to 2000 g/mL was added. All of the solutions were preheated for 5 minutes at 70°C on a water bath after being incubated at 37 2°C for 15 minutes. At room temperature, the solutions were allowed to cool. Then, using a vehicle as a blank, the absorbance was captured at 660 nm using a UV-visible spectrophotometer (LABTRONICS MODEL LT-291).[10,6]

$$\%\ \text{of inhibition} = \frac{[Abs\ (Control) - Abs\ (Test)}{Abs\ (Control)}\ x\ 100$$

Whereas Abs- absorbance.

The IC_{50} value denotes the concentration of the test sample (gm/mL) needed to inhibit an enzyme by 50%.

In-vitro antioxidant activity

Hydrogen peroxide was utilized for the scavenging activity of the *in-vitro* antioxidant study. 0.2, 0.4, 0.6, 0.8, and 1-mg/mL test and the standard solution was prepared with hydrogen peroxide and phosphate buffer (pH 7.4). Absorbance was taken by UV spectroscopy at 230 nm. The standard utilized as ascorbic acid.[6,10,23]

$$\%\ \text{Scavenging Activity} = \frac{[Abs\ (Control) - Abs\ (Standard)}{Abs\ (Control)}\ x\ 100$$

Ex-vivo permeation study

We utilized Franz diffusion cells to measure how much of the medication filtered through the skin during the *ex-vivo* research. The donor and receptor chambers were clamped together using chicken skin, and the donor chamber was filled with the appropriate formulations (F1, F2, and marketed formulation). Before each experiment, the whole skin was adjusted for one hour in PBS (pH = 7.2). Skin was 0.8 mm thick, plus or minus 0.05 mm, carefully removing the remaining cream from the skin. The medicated film was applied on the donor side as the skin was placed on a vertical Franz-type diffusion cell so that the receptor compartment's dermis was facing it. Water heated to a thermostatic 37°C was circulated throughout the jacketed cells. pH 7.4 buffer was used as the control sample for the investigation. Periodically, for up to 24 hours, receptor fluid samples (1ml) were taken out and replaced with a new buffer solution. After that, a UV spectrophotometer (LABTRONICS MODEL LT-291) was used to evaluate the sample. Based on the permeation results, we saw that F5 had the maximum amount of penetration in comparison to the other preparations.[24]

In-vitro anthelmintic activity

The experiment was carried out on mature earthworms for morphological and physiological similarities to the human intestinal parasite. Earthworms have been utilized often for the initial *in-vitro* assessment of anthelmintic chemicals due to their accessibility. Six worms of roughly identical size were put in 50 mL four different concentrations of solution such as 5, 10, 15, 20 & 25 mg/mL by distilled water in a petri dish. Up to five hours into the test period, observations were conducted on how long it took for individual worms to become paralyzed or die. After determining that worms did not move when shaken vigorously or when submerged in slightly hot water (50°C), along with the fading of their body colors, the time of death of the worms was noted. To generate three separate sets of data for the study, the experiment was independently repeated. The reports are presented as a mean standard error of the mean. As a reference standard albendazole (5, 10, 15, 20, and 25 mg/mL) and saline water were utilized as a control.[3,25]

RESULT AND CONCLUSION

The appearance of the cream was judged by its pearlescence, color, roughness, and grade (Figure 1). The spreadability test revealed that the cream's formulation had good spreadability. The ploy herbal cream's pH was evaluated at 6.1 and 6.0, respectively, which is a suitable pH for the skin. The herbal formulation had a pH that was closer to what was needed for skin, or a pH of 6.7. The emulsion is mixed with the scarlet crimson color. A drop of the emulsion should be

placed on a microscope surface, then the open slid cover under a microscope and covered with a cover slip. The globules, or "w/o type cream," seem colorless in the crimson background. Table 1 provides a summary of the results.[19] Formulations F1 and F2 were prepared with the same ingredients but with different concentrations of plant extract. Both the cream formulations were observed to have similarities in consistency. There was no apparent change in the phase separation after centrifugation.

Centrifugation Test

There was no apparent change in the phase separation after centrifugation. No phase separation occurred after the centrifugation test.

Table 1: Physical and other different parameters evaluation

S No.	*Parameters*	*F1*	*F2*
1	Color	Creamy mint green	Creamy mint green
2	Odor	Pleasant	Pleasant
3	Consistency	Smooth	Smooth
4	State	Semisolid	Semisolid
5	pH value	6.1	6.0
6	Spreadability gm.cm/sec	33	34
7	Non-irritancy test	Non-Irritant	Non-Irritant
8	Acid value	1.55	1.39
9	Saponification value	38	40

A. F1, B. F2, C. F(STANDARD)

Figure 1: Color of various batches of formulation

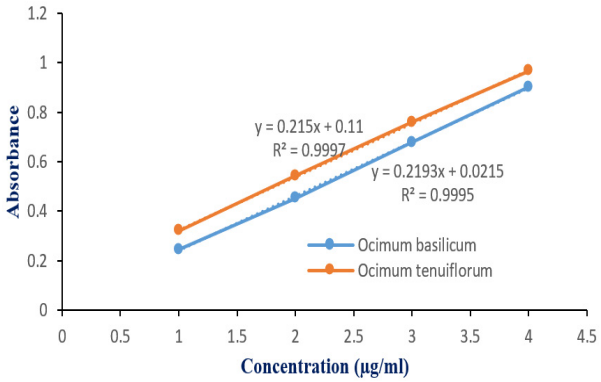
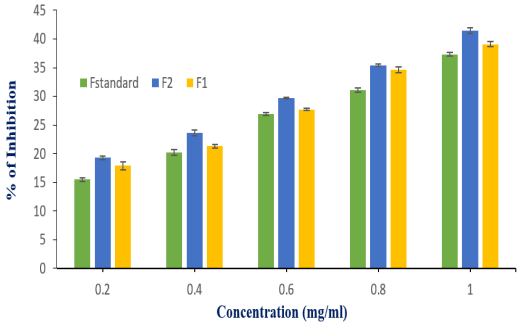


Figure 2: Spectrometric method



(Results are presented as mean ± standard deviation)

Figure 3: Comparison of *in-vitro* anti-inflammatory activity of test & standard samples

Spectrometric Methods

Correlation coefficient (R2) was found to be <1 (0.9997 for *O. tenuiflorum* and 0.9995 for *O. basilicum*) measured by 660 nm λ_{max}.[23,26] Results were summarized in Figure 2**.**

In-vitro Anti-inflammatory Activity

The protein denaturation technique was used to measure the anti-inflammatory effect *in-vitro*. One well-known factor contributing to inflammation is the denaturation of proteins. As part of the analysis of the mechanism behind the anti-inflammatory impact, the potential of a formulation derived from plant extract to retard protein denaturation was examined. It proved effective in stopping heat-induced denaturation of albumin. The reports of the current study showed that diclofenac sodium inhibited protein denaturation by 12 to 44% and that cream formulation was 14 to 54% across the concentration range of 100 to 1000 g/mL (Figure 3). The current study's findings led to the conclusion that the cream formulation has anti-inflammatory properties.[10]

In-vitro Antioxidant Activity

The *O. basilicum* extract demonstrated the greatest antioxidant activity. The extracts added into the *O. basilicum* emulsion exhibited strong anti-free radical action (Figure 4). The plant's potential as a source of natural antioxidants with potential use to lower oxidative stress and reap subsequent health benefits is shown by the formulation's wide spectrum of antioxidant activity.[10]

In-vitro Anthelmintic Activity

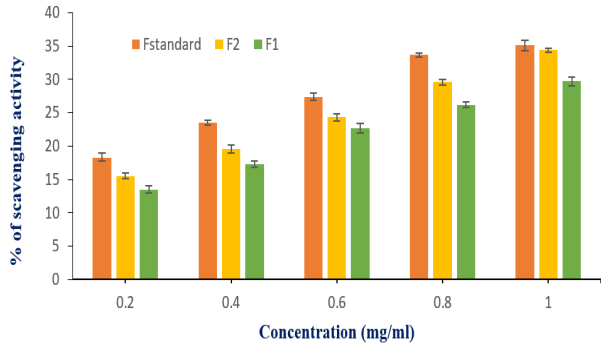
Earthworms were used to test the extracts' anthelmintic capabilities. Table 2 demonstrates that two formulations, which were tested for their anthelmintic efficacy, showed varying death and paralysis times at various dosages (1–5 mg/mL). Compared to the activity of the medicine albendazole, it is very modest. The high anthelmintic efficacy of the ethanol extract may be attributed to the presence of phytochemicals such as tannins, alkaloids, and terpenoids.[3] The paralysis/death time increases with decreasing the amount or concentration of formulation. F2 has less time in paralysis and death time of earthworms compared to F1 and standard also.

Permeation Study (*Ex-vivo*)

This study is illustrated in Figure 5. Formulation F2 is the best formulation with 87% drug permeated through the membrane at the end of 24 hours. No permeation enhancers were used.[24] Both these plants *O. tenuiflorum* and *O. basilicum* are well known for their anti-inflammatory activities. Therefore, the cream was tested against other standard formulations to validate the anti-inflammatory activity of the formulation. The results showed that F2 has a much higher anti-inflammatory impact than standard and other test samples. The present formulation shows the effective therapeutical value against the parasitic worms for the evaluation of anthelmintic activity. Furthermore, the targeting profile of both the formulation must be investigated and *in-vivo* and *ex-vivo* evaluations carried out.

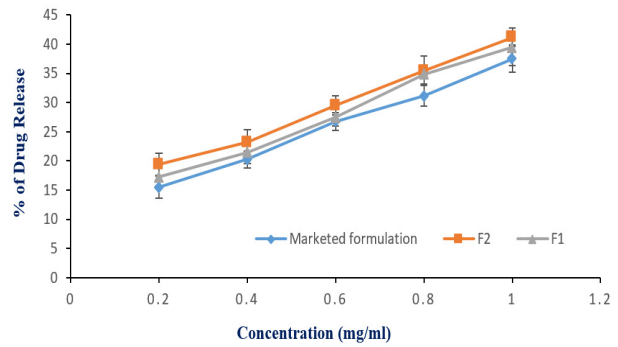
Table 2: In-vitro anthelmintic activity

Concentration (mg/ml)	*Paralysis of Earthworms (mean values) Time (min)*			*Death of Earthworms (mean values) Time (min)*		
	F1	*F2*	*Standard*	*F1*	*F2*	*Standard*
5	31.43 ± 0.90	23.67 ± 0.78	97.90 ± 1.15	48.96 ± 0.69	36.75 ± 0.87	488.23 ± 1.67
4	47.76 ± 0.76	38.28 ± 0.75	110.88 ± 1.12	75.91 ± 0.80	65.89 ± 0.70	503.78 ± 1.95
3	74.20 ± 0.69	63.41 ± 0.56	200.10 ± 0.97	115.35 ± 0.76	97.90 ± 1.17	563.78 ± 2.32
2	98.02 ± 1.02	88.60 ± 0.76	120.54 ± 0.89	133.65 ± 1.21	128.24 ± 1.43	590.70 ± 2.12
1	130.32 ± 1.07	113.67 ± 0.84	235.42 ± 1.12	178.37 ± 1.26	165.76 ± 1.20	601.65 ± 1.87



(Values are represented as mean ± standard deviation)

Figure 4: Comparison of *in-vitro* antioxidant evaluation of test sample and standard samples



(Values are represented as mean ± standard deviation)

Figure 5: Comparison of permeation of test and standard samples

CONCLUSION

Despite significant progress in the field of synthetic medications, plants continue to hold a special place since they have negligible side effects. To employ plants as natural anti-inflammatory drugs with minimum toxicity and increased pharmacological value, a technique for determining the effectiveness of plants against inflammation should be developed. The current findings show that traditional therapeutic practices are supported by science and point to a bright future for plant-based anti-inflammatory drug development. Formulations F1 and F2 were prepared with the same ingredients but with different concentrations of plant extract. Both the cream formulations were observed to have similarities in consistency. There was no apparent change in the phase separation after centrifugation. Both these plants *O. tenuiflorum* and *O. basilicum* are well known for their anti-inflammatory activities. Therefore, the cream was tested against other standard formulations to validate the anti-inflammatory activity of the formulation. The results showed that F2 has a much higher anti-inflammatory impact than standard and other test samples. The present formulation shows the effective therapeutical value against the parasitic worms for the evaluation of anthelmintic activity. Furthermore, the targeting profile of both the formulation must be investigated and *in-vivo* evaluations carried out.

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