

Formulation of an Anti-inflammatory Pharmaceutical Drinkable Emulsion Based on Artemisia Absinthium L Essential Oil

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Abstract

The importance of aromatic plants, their essential oil content and the chemical nature of their constituents, give them great prospects for application. Nowadays, essential oils present a major source of a variety of bioactive molecules, they are also used as an alternative to conventional drugs.

The evaluation of phytotherapeutic properties, such as antioxidant, antimicrobial and anti-inflammatory are gaining great interest in the research field.

The objective of this work is the evaluation of the antibacterial, antifungal and anti-inflammatory effect of the essential oil of wormwood from the region of Blida, Algeria and that of a drinkable emulsion formulated in the presence of this essential oil. Average yield of 0.52% of essential oil extracted by hydrodistillation using a Clevenger from the fresh aerial part of absinthe was obtained. GC/MS analysis demonstrated that wormwood essential oil is dominated by the presence of the compounds β Thujone with 45.646% and 7-ethyl, 1,4 dimethyl Azulene with a percentage of 25.95%. The absinthe essential oil extracted showed an activity against all bacteria and fungi tested. The drinkable emulsion exerted remarkable an anti-inflammatory activity which induced an edema reduction rate of 35.70%, close to the one obtained by a Clofenal reference.

Toxicity tests of the essential oil of *Artemisia absinthium* and the drinkable emulsion which were followed for two weeks, showed neither mortality nor abnormality.

1. Introduction

The plants represent an inexhaustible and renewable source of active ingredients, they have been known since a long time for both traditional and medical use. Algeria possesses a rich and a diverse plant flora. Among them, the *Artemisia absinthium* L commonly known in Algeia as “chiba ou chajrat Maryam” [1, 2] Jor chiba, largely localized in semi-arid regions. In Algeria. Absinthe has been used for a long time in moderate quantities in tea preparations, also used to treat digestive disorders, ulcers and burn [3]. Indeed, some international organizations such as the EMA (European Medicines Agency) [4] the ESCOP (European Scientific Coordination in Phytotherapy) [5] recognize the use of wormwood (*Artemisia absinthium* L.) to treat the loss of appetite, indigestion, gastric emptying and gallbladder problems due to a treatment ranging from 2 to 4 weeks maximum.

Authors report the therapeutic uses of absinthe, like lack of appetite, gastric ulcers, an antiemetic, for digest, fissure, jaundice, cirrhosis, gout, rumathism, improve vision, moxibustion treatments for cancer [6, 7]. Studies conducted by B.Omer et al [8] have demonstrated that in clinical trials on subjects intestinal inflammation (Crohn disease), 65% of the group of people given absinthe were almost free of Crohn disease symptoms compared to none in the placebo group.

The essential oil (EO) of absinthe have also been used since antiquity in traditional medicine. A lot of researches have been done to demonstrate the benefits of essential oils in general, and of the wormwood

particularly [9, 10, 11, 12, 13].

In this study, we are interested in the extraction of essential oil from *Artemisia absinthium* and the determination of its chemical composition by gas chromatography coupled with mass spectroscopy.

A formulation of a Lipophilic / Hydrophilic drinkable emulsion conforming to pharmaceutical use and meeting the requirements of the green chemistry has been prepared. A study of the evaluation of the microbiological and anti-inflammatory activity of oil and emulsion was the subject of this work.

2. The Material and Method

2.1. vegetable matter

The plant material is Absinthe (*Artemisia absinthium* L), which was harvested in the region of Blida (50km north of Algiers) 36°31'06" North, 2°54'19" east. The harvest was carried out on March 2020. The phylogenetic classification is given in Fig. 1.

The aerial parts of the plant were dried at the room temperature, protected from light and in well-ventilated areas (to avoid mould) and kept in bags.

Extraction of the essential oil was carried out by hydrodistillation taking into account the influence of certain parameters on the yield (the mass of the plant material and the extraction time). The essential oil was separated from the water by liquid-liquid extraction then recovered and stored at 4°C away from light for analysis.

2.2. Characterisation of Essential Oil:

The organoleptic (appearance, color, smell) and physico-chemical properties) constitute a means of verification and quality control of the essential oil of *Artemisia absinthium* L extracted by hydrodistillation. Our tests were carried out according to a specific protocol and comply with the standards laid down by AFNOR [14].

The analysis of the essential oil's chemical composition by gas chromatography type Hewlet Packard series Agilent 6890 N chromatograph coupled with mass spectrometer type Hewlet Packard, mass spectrometer, Agilent 5973 series. The operating conditions are : The injector: Temperature: 250°C, Mode of injection: Splitless Volume injected: 0.2 µl. The column: Type: Hp-5ms, Dimensions: long 30m, D int 250 µm, film thickness 0.25 µm Stationary phase: diphenyl (5%), Dimethyl arylene siloxane, Oven temperature: 60°C for 8 min,

2°C/min up to 280°C, 10 min, Carrier gas: 99.9998% pure helium, GV flow rate: 0.5 ml/min. The mass detector: Temperature of the interface: 280°C, Type of ionization: electronic, Intensity of the filament: 70 Ev, Type of the mass analyzer: Quadrupole Temperature of the quadrupole: 150°C.

2.3. Formulation of Drinkable Emulsion:

A formulation of a drinkable emulsion (DE) Lipophiles/Hydrophiles in accordance with pharmaceutical use and respecting the requirements of green chemistry has been prepared; the tests have been predefined by a matrix of experiments which meet the objective of the RSM is a D-optimal mixing design. This matrix generated by a MODDE 6.0 software algorithm.

The products used in the formulation are the oily phase, soy lecithin with an HLB = 8 (emulsifier), Glucose (educolorant), Xanthan (thickening agent), Grapefruit seed (preservative) and demineralized water.

The aqueous phase and the organic phase (EO of *Artemisia Absinthium* L) are mechanically stirred at a temperature of 25–30°C for fifteen minutes and then homogenized using an ULTRA-TURRAX type homogenizer (IKA T10 Basic) in order to ensure a good stability of the formulated emulsion, grapefruit seed extract is introduced as a conservative.

In order to keep the most stable emulsions, the coalescence of the drops or the phase shift were sought by centrifugation using a centrifuge type Hettich zentraifugen-EBA20 at a constant speed of 6000 rpm for 20 minutes and by aging at 50°C for 72 hours.

2.4. Evaluation of the Microbial and Fungal Activity of Essentiel Oil and Drinkable Emulsion:

To evaluate the antimicrobial activity of EO and the drinkable emulsion, we adopted the method of diffusion on agar medium called aromatogram (technique in solid medium). The aromatogram is based on a technique used in the medical bacteriology, called Antibiogram [15]. It has the advantage of being very flexible in the choice of products to be tested and of being applicable to a large number of bacterial species [16].

The strains used in our study are referenced ATCC (American Type Culture Collection). They are summarized in Table 1, they were chosen for their high contamination on food stuffs and their pathogenicity.

Table 1
Microbial Strains Tested and their References.

Strains	Bacterial species used	Gram	ATCC Reference
Bacteria	<i>Escherichia coli</i>	(-)	25922
	<i>Staphylococcus aureus</i>	(+)	25923
	<i>Pseudomonas aeruginosa</i>	(-)	27853
	<i>Bacillus ceureus</i>	(+)	10876
Mushrooms	Sacare	/	4006
	<i>Candida albicans</i>	/	24433
	<i>Aspergillus flavus</i>	/	2035

2.5. Evaluation of the Anti-inflammatory Activity:

The tests of the pharmacological activity (anti-inflammatory test) of both the essential oil and the drinkable emulsion based on the essential oil of Absinthe carried out by the Carrageenan edema method on mice according to the Levy's method [17]. The injection of Carrageenan under the plantar aponeurosis of the mouse paw causes an inflammatory reaction which can be reduced by an anti-inflammatory product. This study makes it possible to compare the reaction of plantar edema after administration of a dose of drinkable emulsion formulate and a corresponding reference product.

The test consists of evaluating the anti-inflammatory effect of the drinkable emulsion formulate in presence of essential oil of Absinthe at a concentration of 0.01 g on the edema of the hind legs caused by the injection of a solution of 1% carrageenan to mice. The albino mice are divided into 3 batches of 5 whose body weight is between 18g and 23g.

At time T_0 : Administer the following suspensions to the three batches:

- Control batch: each mouse receives 0.5 ml of distilled water
- Trial batch 1: each mouse receives 0.5 ml of DE at a dose of 0.01 mg
- Test batch 2: each mouse receives 0.5 ml of the reference product (Clofenal) (Declofenac sodium at 12.5 mg/kg).

At time $T_0 + 30\text{min}$, the 1% Carrageenan solution is injected under the plantar aponeurosis of the left hind paw in a volume of 0.025ml to all the mice tested.

At time $T_0 + 4\text{h}$, the hind legs at joint height are weighed on an analytical balance.

The results are expressed by calculating the arithmetic mean of the weights of the left paw and the right paw for each batch. Then calculate the percentage increase in paw weights (% edema) and (% reduction in edema) by the following formulas [17].

$$\%ofedema = \frac{Averageleftpawweight - Averagerightpawweight}{Averagerightpawweight} \times 100$$

$$Reductionofedema = \frac{\%ofwitnessedema - \%oftrialedema}{\%ofwitnessedeme} \times 100$$

2.6. Toxicity Test:

The ketones present in the EO of absinthe can be aggressive for the nervous tissues and lead to the risk of convulsions. It is therefore, important to know that when talking about the toxicity of ketones, it is necessary to take into account not only the concentration in the EO, but also the nature of the ketone, the doses used, the route of administration and the threshold of tolerance [18].

The toxicity test Drinkable Emulsion was adopted by the method of oral toxicity test. A group of 5 animals from the same sex which receive predetermined doses of 0.5ml of DE to Albino mice weighing between (17g -24 g). Which corresponds to 0.1 mg/g of EO.

The test substance is administered in a single dose using a gastric tube. These mice are fasted for at least 4 hours. Animals should be observed individually at least once during the first 30 minutes and regularly during the first 24 hours. Special attention is required during the first 4 hours and daily for 15 days after administration of the substance.

3. Results and Discussion

3.1. Characterization of Essentiel Oil :

The maximum yield of EO for 75 minutes and 50g is 0.25% is similar to the one found by Weiss et al[19], the extension of the extraction time makes it possible to increase the yield to 0.52 for 105 minutes. The essential oil of the aerial part of Artemisia Absinthium L obtained by hydrodistillation presents the organoleptic characteristics grouped in Fig. 2.

The physico-chemical properties of the Artemisia Absinnthium' essential oil show that the value of the refractive index of the essential oil measured is 1.375 higher than the refractive index of water 20°C (1.333), with a specific gravity of 0.94. The acid index is 1.2 which gives an idea about the level of free acids in the EO. A high value indicates EO degradation (ester hydrolysis) during storage[20].

In our case, the acid index value is low, which proves a good preservation of the essential oil with a very high ester index of 26.6.

The analysis of the essential oil extracted by hydrodistillation of the aerial part of Artemisia Absinthium L by gas chromatography coupled with mass spectroscopy (CG/MS) revealed the presence of 20 compounds, presented in Table 3.

Table 3
The Compounds of the Essential oil of *Atemisia Absinthium* L.

Elution Order	Compound Names	Retention Time (min)	Percent
1	α .-Pinene	11.449	0.530%
2	β .-Myrcene	12.630	0.646%
3	3-Carene	13.430	0.268%
4	1,4-Cyclohexadiene	14.852	0.441%
5	Cyclohexadiene,	17.208	0.343%
6	α Thujone	20.529	1.337%
7	βThujone	21.676	45.646%
8	1-ethyl-3,5-dimethyl- Benzene	25.801	0.958%
9	Caryophyllene	41.788	2.101%
10	3-ethenyl- Cyclopentene	44.973	0.321%
11	(+)-Epi-bicyclosesquiphellandrene	45.658	0.844%
12	Naphthalene	51.692	1.002%
13	Cyclohexene	51.918	0.782%
14	-2,6-Octadien-1-ol	52.988	0.422%
15	Acide benzoique	53.378	0.594%
16	1-Naphthalenol	53.788	2.541%
17	1-Naphthalenemethanol	55.099	0.958%
18	2-Naphthalen methanol	55.769	3.415%
19	7-ethyl 1,4-dimethyl- Azulene	60.265	25. 95%
20	1-ethyl-3,5-dimethyl- Benzen	74.025	1.685%

The concentration of each individual volatile oil varies considerably, depending on a number of parameters, a time of a year [21]and location [22].

The results of the GC/MS analysis show that the most common compound is β -thujone with a rate of 45.646%, a value close to the one found by Militello et al [11]. α -/ β -thujone is mainly known for its contribution to anti-inflammatory activity as demonstrated by Miriam et al [23] However, thujone, in high doses or in repeated consumption, can be neurotoxic and convulsant [24] compared to β -thujone, α -thujone is 2.3 times more toxic [25][26]. α -thujone, has an effect on the central nervous system (CNS). The

effect of α -thujone on CNS takes place by binding γ -aminobutyric acid type A receptors and blocking chloride channels[27]. The quantity of α -thujone is infinitely small 1.337 in the absinthe EO extracted.

It has also been hypothesized that the biochemical process of thujone generation competes with that of chamazulene production [28]. These compounds are potentially used in various fields of medicine, including anti-inflammatories with peptic ulcers, antineoplastics with leukemia, antidiabetics, antiretrovirals with HIV-1, antimicrobials including antimicrobial photodynamic therapy, and antifungals [29]. also, It has been shown by Salamon et al the interesting anti-inflammatory properties of chamazulene [30].

The presence of azulonic derivatives is remarkable 25.95%, which suggests that the oil has interesting anti-inflammatory properties.

3.2. Characterization of the DrinkableEmulsion:

The matrix of tests obtained contains 21 tests, of which 4 represent the center of gravity of the plan to check the reproducibility. The stability tests enabled us to retain four drinkable emulsions with the physico-chemical characteristics presented in Table 4.

Table 4
The Different Stable Drinkable Emulsions and their Physico-chemical Characteristics

Trial N°	Stability	pH (23°C)	Density	Viscosity (Pa. s)
N4	Stable	5 ,74	1 ,028	34.21
N12	Stable	5 ,98	1 ,026	30.15
N18	Stable	5 ,28	1 ,025	29.06
N21	Stable	6,06	1,024	21.29

3.3. Biological Activities :

After incubation, the appearance of inhibition zones of different diameters which surround the discs impregnated with the essential oil and the drinkable emulsion can be noticed. This is explained by the fact that the bacterial strains and fungi, are more or less sensitive to our samples.

The observations made on the effect of the essential oil and the drinkable emulsion on the growth of the bacterial strains represented in the histogram (Fig. 3) show that the essential oil has a good sensitivity towards the bacteria *Escheichia colli*, *staphylococcus aureus* and *bacillus* ; while the susceptibility to *Pseudomonas aeruginosa* bacteria is low. He was found that the drinkable emulsion is not sensitive except for the bacterium *Escheichia colli* and *staphylococcus*. These results agree with those found by Sura baykan et al. [30] and Wendakoon et al [31].

The results of the antifungal effect of the essential oil and the drinkable emulsion of *Artemisia absinthium* L tested on the three fungi namely *Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus flavus* are expressed in the histogram (Fig. 4).

These results are interesting concerning the essential oil for phytosanitary applications, such as biological control methods based on natural substances in order to eradicate infections of fungal origin. The high level of thujone has been shown to be responsible for its antifungal effect [22].

Lopes-Lutz et al [32] have studied the antifungal power on several EO strains of *Artemisia absinthium* L. from Canada, in particular for *Candida albicans* and *Aspergillus flavus*, they have respectively obtained inhibition zones of 13mm and 18mm.

3.4. In Vivo Anti-inflammatory Activity Test :

After administrating the saline solution, the reference product (Clofenal), and the drinkable emulsion to mice in which inflammation was induced by injecting 1% carrageenan into the plantar surface of the hind paws. We measured hind paw weights in (g) in the three groups.

A considerable reduction in edema (54.71%) by Absinthe essential oil demonstrating anti-inflammatory activity linked to the presence of 7-ethyl 1,4-dimethyl-Azulene compound. The drinkable emulsion based on wormwood essential oil induced a reduction rate of edemas of 35.70%. This rate is close to that obtained following treatment with Clofenal. Indeed, the latter caused a decrease in edema of 37.53%, which confirms that the drinkable wormwood emulsion has exerted a rather remarkable anti-inflammatory activity.

Several studies seem to indicate that flavonoids possess anti-inflammatory properties and they are capable of modulating the functioning of the immune system by inhibiting the activity of enzymes which may be responsible of inflammation, they can also modulate the adhesion of monocytes during inflammation by inhibiting expression of inflammatory mediators [33, 34, 35] .

3.5. Toxicity Tests of Artemisia Absinthium L EO and Drinkable Emulsion:

After the administration of the essential oil of *Artemisia absinthium* and the drinkable emulsion to mice in which, we found after 2 weeks, that no mortality or abnormality was observed. Therefore, the two products are consistent with the pharmacological techniques of Saidal [36]. A thirteen-week toxicity study on 2% absinthe extract was performed by Muto et al. He finds that no toxicological effects preclude the human use of this plant [37].

4. Conclusion

The present work aims to enhance a medicinal and aromatic plant widespread in the world and in Algeria, known as Absinthe. The study of certain parameters influencing the yield of the essential oil allowed us

to obtain a maximum yield of around 0.52%. CG/MS analysis of the essential oil of *Artemisia absinthium* L shows the presence of the compound Azulene with a percentage of 25.95%. The antimicrobial activity tests carried out on the absinth essential oil demonstrated the inhibiting actions against all the bacteria and the champions bet in study. Prepared stable drinkable emulsions exhibit less sensitivity to these bacteria and champions.

The study of the anti-inflammatory activity in vivo on mice, led us to deduce that the drinkable emulsion of *Artemisia absinthium* is effective to administer. The oral emulsion exerted anti-inflammatory activity, which induced an edema reduction rate of 35.70%, close to that obtained by a Clofenal reference. The results obtained during this study are very interesting, but further studies are needed to understand the molecular and cellular mechanisms of these effects. After administrating the essential oil of *Artemisia absinthium* L, and the drinkable emulsion to mice in which we have noticed no mortality or abnormality.

Declarations

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Conflicts of Interest: The authors declare no conflict of interest.

Author Contributions

Djedri formulated the pharmaceutical product and carried out the other tests.

Chemat Zoubida is occupied by the extraction and characterization part of the essential oil.

Belhadji Linda is occupied by the biological effect of the product. All authors have read and agreed to the published version of the manuscript.

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Figures



Figure 1

Phylogenetic Classification of Absinthe

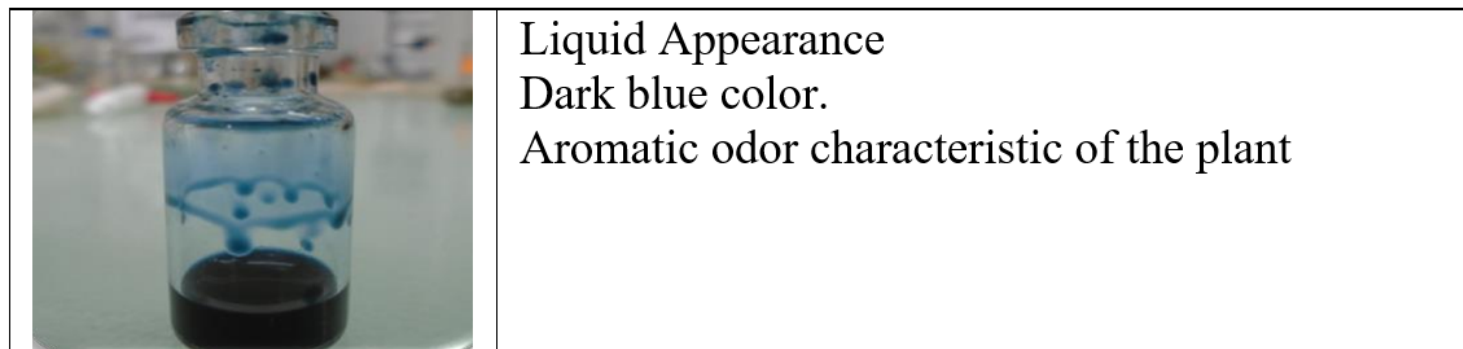


Figure 2

Organoleptic Characteristics of the Essential Oil

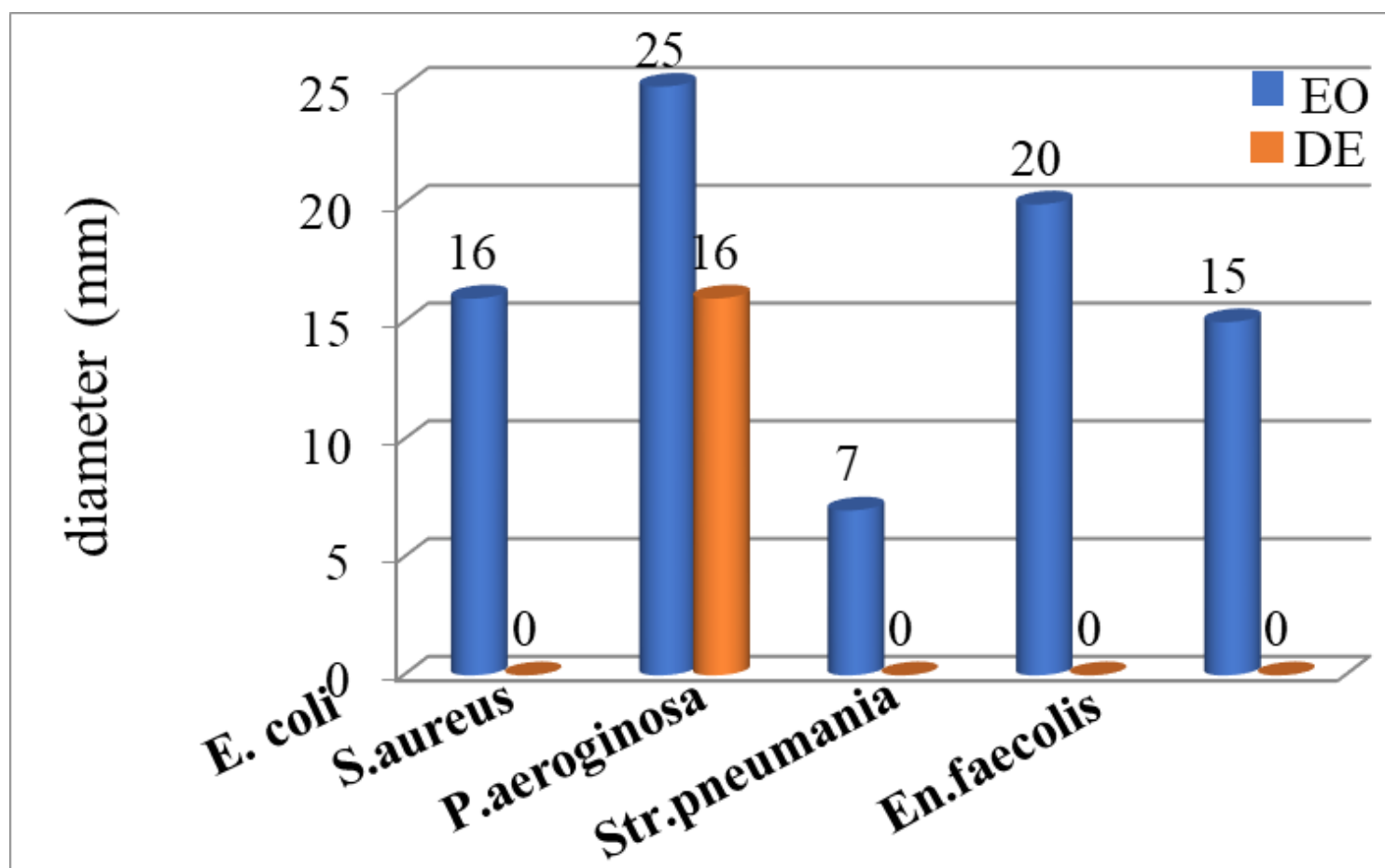


Figure 3

Histogram of Bacteria Inhibition Zone Diameters

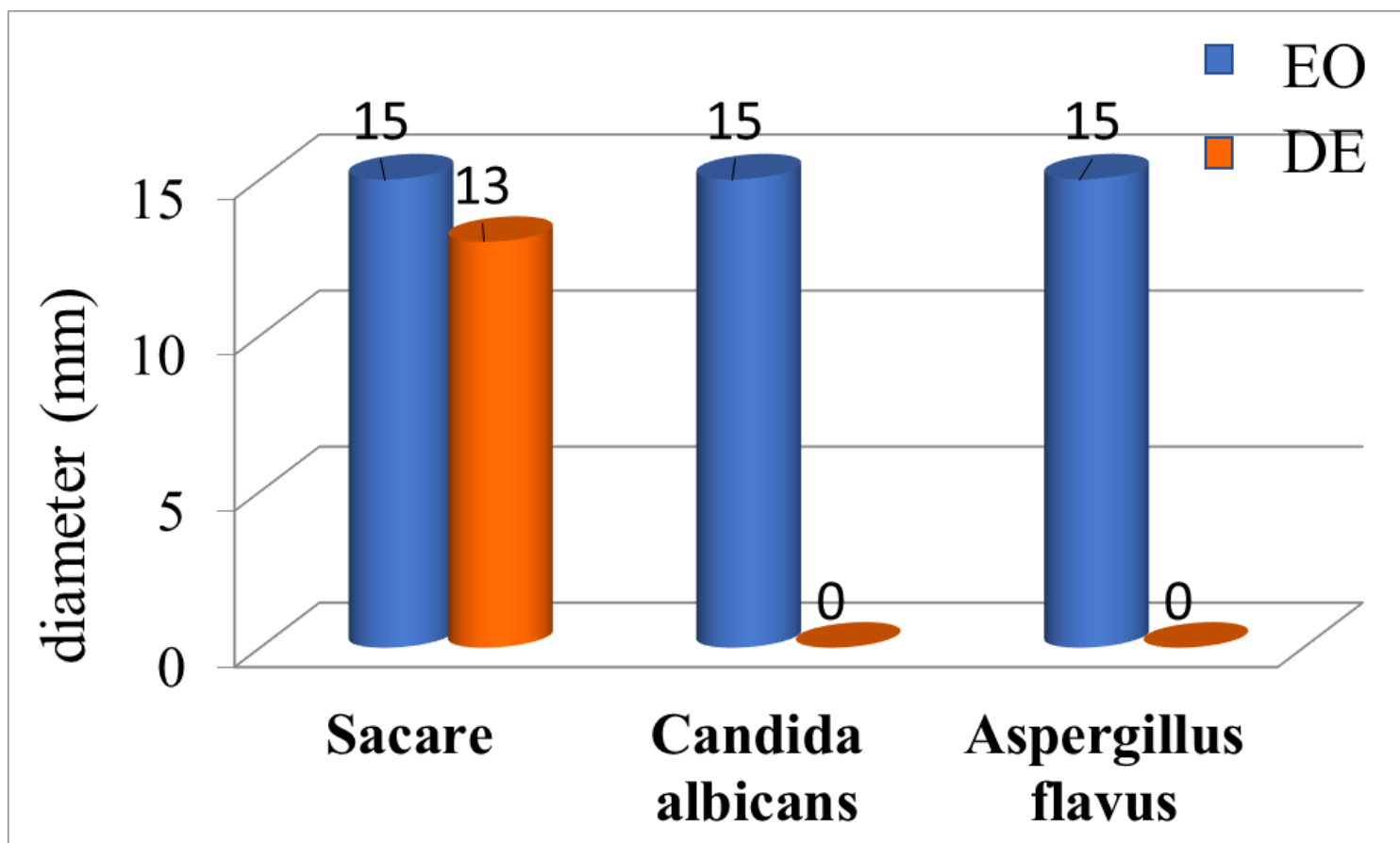


Figure 4

Histogram of Fungal Inhibition Zone Diameters

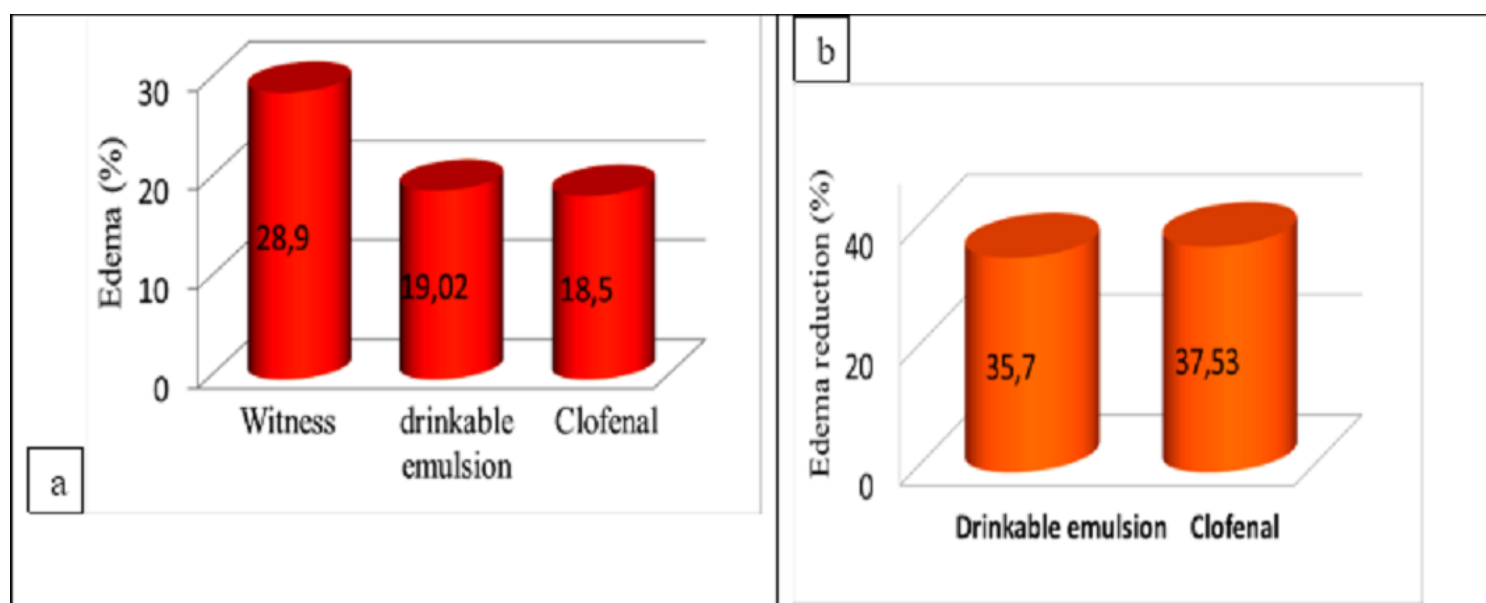


Figure 5

Percentage of edema (a) and reduction of edema (b) of oral emulsion and Clofenal