

## ANTI INFLAMMATORY AND BACTERICIDAL OINTMENT USING OLIVE OIL AND IBUPROFEN

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Abstract:

*The aim of this work had consisted of the formulation of an anti-inflammatory ointment based on virgin olive oil, the determination of skin tolerance and anti-inflammatory activity, the identification of different chemical groups of the olive leaves, the determination of antibacterial activity, and the formulation of an antiseptic ointments based on the extracts obtained.*

*The determination of the organoleptic characteristics, physical and chemical indices, and UV and IR spectrum, gave results in conformity with the standards of the European Pharmacopoeia.*

*The determination the skin tolerability of the formulated ointment classifies it in the category of non-irritating product (IP= 0.16), thus non toxic for use on the human skin. The anti inflammatory activity of the ointment has been proved by the croton oil method with a percentage of reduction of edema (% =33.78).*

Topical routes of administration of drugs are often desirable, because other problems can be avoided, for example oral administration is often associated with variable removals and gastrointestinal irritation, especially gastrointestinal irritation associated with oral administration of the anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, and naproxen. The administration of these drugs and other non-steroidal anti-inflammatory can cause significant irritation of the gastrointestinal tract intestinal, including ulceration and bleeding bowel disease, nausea, dyspepsia [1-4].

Oral administration of NSAIDs can also lead to other systemic side effects such as headaches of head, dizziness and hypertension, in addition, a significant amount of the drug may be lost following administration by oral route because of the partial metabolism of the product by action of the liver before reaching the bloodstream.

In an effort to avoid the disadvantages of oral administration of NSAIDs, especially ibuprofen, it has carried to the introduction of the latter in a formulation semi solid that can be applied directly on the skin above the source of inflammation, although such a formulation can avoid some traditional issues related to the administration of NSAIDs, there are other problems associated with topical drugs (the stratum corneum limit the diffusion of molecules through the) (skin) [6-8].

Olive oil has a nutritional balanced fatty acids, moderately rich in oleic acid and Palmitic acid

Nevertheless, it is the presence of specific phenolics which gives it high stability against oxidation with a color and a unique flavor the distinguishing other oils [9-13].

The extraction process of oil from olive releases six carbon atoms responsible for its characteristic aroma volatile alcohols and aldehydes. *Olea europaea* is one of the rare plant species to synthesize both (mannitol) polyols as oligosaccharides (stachyose and raffinose), metabolites of photosynthesis at the level of its leaves.

These carbohydrates are sent, with sucrose, of the leaf to the fruit, the olive, to participate as precursors in the synthesis of oil [14].

Developing olives contain chloroplasts capable of fixing CO<sub>2</sub> thus contributing to the carbon of the fruit economy. The overall quality of olive oil depends directly on this stage of maturation of the olive.

The ripening is the result of a combination of physiological and biochemical changes under the influence of cultural and environmental conditions, even

if most of the biochemical reactions are under strict genetic control [15-18].

Olive oil contains many antioxidant agents: of tocopherols (vitamin E), carotenoid as the betacarotène, simple phenolic compounds as the hydroxytyrosol and the tyrosol, SECO-iridoïdes as the oleuropein, the déméthyleuropéine and the ligstroside, flavonoids and finally of the Lignans like the acétoxy-pinorésinol and the pinorésinol.

Note that the hydroxytyrosol is well absorbed by the intestine, but its bioavailability is low, because after sulfation and glucuronidation, plasma free form becomes almost undetectable, making it difficult to understanding of its activity in vivo antioxidant and health benefits derived from its use; unless the hydroxytyrosol is also a metabolite of dopamine and endogenous and exogenous sources combine to express an effect [19-21].

Daily consumption of 50 g of extra olive oil Virgin by postmenopausal women for eight weeks has resulted in a significant decrease in urinary markers of oxidative damage of DNA. In a Spanish study, the phenolic compounds of olive oil increased antioxidant defenses of human hepatocytes in culture under induced oxidant stress. Daily consumption of 50 g of extra olive oil Virgin by postmenopausal women for eight weeks has resulted in a significant decrease in urinary markers of oxidative damage of DNA. In a Spanish study, the phenolic compounds of olive oil increased antioxidant defenses of human hepatocytes in culture under induced oxidant stress.

Normolipémiant and antiperoxy lipidique activity [22]

A study on healthy volunteers receiving a daily dose of 40 ml has shown that most ingested oil contains phenolic compounds, contain more LDL of participants and the level of their LDL oxidation becomes low in postprandial [22]

Biological markers of inflammation (IL6 and CRP) could be significantly lowered in stable coronary patients after the daily intake of 50 ml of olive oil refined during two periods of three weeks preceded by two weeks of wash-out

The phenolic compounds of olive oil have shown their ability to modulate the expression of certain metalloproteases (MMP-9) by action on the NF-kappa B pathway, and to reduce some mediators of inflammation (IL, TNFalpha, IL1-beta, PgE2). These mechanisms updated explain reduction with olive oil for inflammation associated with pathological processes such as atherosclerosis.

The hydroxytyrosol, isolated from olive oil, expressed also anti-inflammatory properties on human monocytes via inhibition of the expression of COX-2 and oxide nitric Synthase (iNOS) A rich in phenolic compounds Virgin olive oil can change the profile of hemostasis in hypercholesterolemic patients postprandial period, allowing the acquisition of status less thrombogenic [23].

Olive oil decreased platelet sensitivity to 'aggregation and thromboxane A2 and Willebrand factor plasma levels. Olive oil decreased platelet sensitivity to 'aggregation and thromboxane A2 and Willebrand factor plasma levels. In addition, chronic consumption of olive oil is accompanied by plasma PAI-1 decreased, thus increasing the plasma fibrinolytic activity. The MUFA identified subjects young and healthy, as moderators of platelet aggregation and factor VI

The hydroxytyrosol and the erythrodiol, they demonstrated in vitro inhibitory activity against human cells of cancer of the colon (actions antiproliferative and proapoptotic). Phenolic compounds of the Virgin olive oil (hydroxytyrosol, tyrosol, pinorésinol and caffeic acid) showed in vitro a capacity to inhibit the settlement of cultures by cells of cancer of the colon, it is '-i.e. their ability to metastasize. Unlike a' corn, oil extra olive oil Virgin would protect the breast cancer by promoting the proliferation/apoptosis balance and reducing the levels of DNA damage [24]

## Experimental part

### Plant material:

Botany: botanical family: Oleaceae taxonomic synonyms: Olea hispanica Mill. Nomenclatural synonyms: Olea gallica Mill., (Tela Botanica data) nomenclatural Code (nn): BDNFFnn44593

taxonomic Code (nt): BDNFFnt3956

Olive oil was obtained by pressure cold in Kabylia (Algeria) in the region of Vadhis (M' Chédallah)

The leaves were collected at the same time

Drying was carried out in the shade at room temperature.

After drying, the leaves were mashed traditional mortar

Parameters	Values	Standard Values
Acidity factor mg de KOH /g oil	1.06	2.0
Peroxyde factor	14	≤20
Extinction à 270nm	0.2087	0.3
Density	0.913	0.910-0.916
Refraction value	1.4681	1.4677-1.4705
Water rate	0.05	≤0.1%
Iodide factor	86	75-94

to obtain a coarse, then reduced powder into a fine powder using a propeller type Mill Retsch DM 2000.

It have been obtained a clear fine greenish powder, that was used for the further phytochemical and pharmacological investigations.

### quality control of the raw material:

oil d'olive Virgin (olivae oleum virgale) (pharmacopée European 01/2008:0518)

Definition: the Virgin olive oil is the oil obtained from the drupes mures of Olea europaea L., pressure cold or other appropriate mechanical means.

Characters: liquid clear, transparent, yellow or yellow-green, of characteristic, virtually insoluble in alcohol, miscible odor to petroleum ether (50-70 ° C). Cooled, the

Virgin olive oil becomes blurred at 10 ° C and solidifies into buttery mass to 0 ° C. The density of the Virgin olive oil is close to 0.913.

Identification:

organoleptic characteristics (smell and taste) of olive oil quality criteria:-colour, odour, flavour:

### Identification:

Test: 2.1.

Acid value:

Principle: (see annex) determine on Virgin olive oil 5. 0 g, acid index is not greater than 2.0. Result: Sample *standard acid value (mg KOH/g of oil)*

1 ≤ 2.0 1.06

2.2. Peroxide: principle (see annex) standard: maximum of 20.0.

Result: Sample standards peroxide index

1 ≤20.0 14.06

### Appareillage :

UV-170PHARMASPECUV-VISIBLE  
SPECTROPHOTOMETER SHIMADZU

Dissolve 1. 00 g Virgin olive oil in cyclohexane and complete 100. 0 ml with the same solvent. Determine à270nm, the absorbance is not greater than 0.20. the relationship between absorbance at 270nm and 232nm is greater than 8.

Résultat :

Echantillon Norme à 270nm Absorbance à 270nm

1 Au max 0.20 0.2087

Fatty acid Composition: the mixture of the constituent fatty acids according to the pharmacopoeia Virgin olive oil is composed of:-fatty acids saturated (see chromatogram and above reported table)

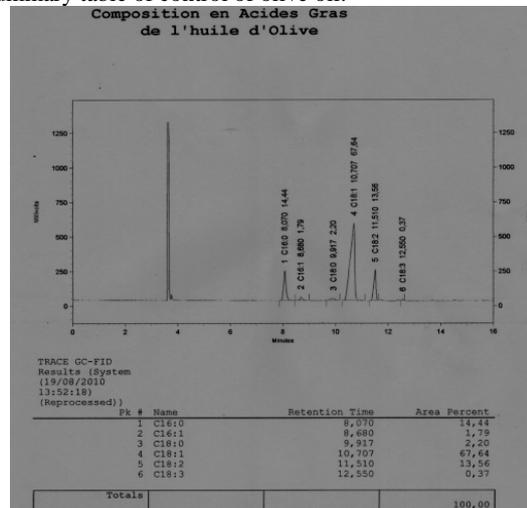
Methylation with a hot methanolic solution of sodium methoxide followed by esterification into acid

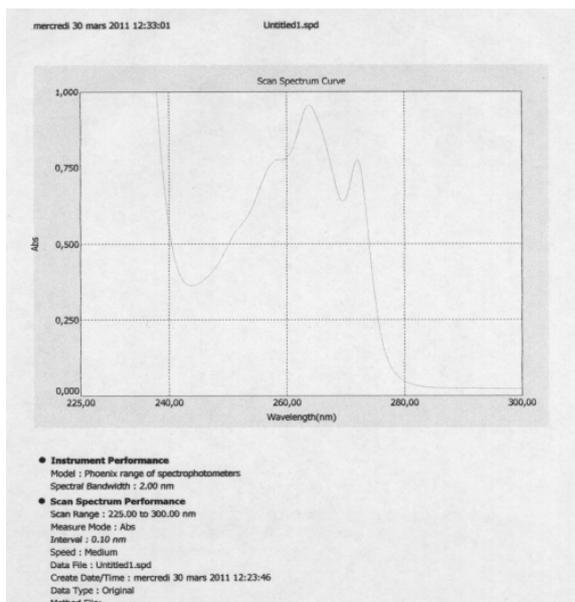
### Operating conditions

Operating conditions: GC: column the column length stainless steel: 2 m internal diameter: 2 mm carrier gas: nitrogen flow: 25 ml/min detector: GC - FI (flame ionization detector). Column temperature: 180 ° C temperature of the injection Chamber and that of detector: 200 ° C

Température de la chambre à injection et celle de détecteur : 200°C

Summary table of control of olive oil:





### Sesame oil:

to a tube of glass with ground stopper, shake 10 ml of 1 min approximately ave Virgin olive oil a mixture of 0. 5 ml of a solution of furfural to 0.35% V/v in acetic anhydride and 4. 5 ml of acetic anhydride. To the filtrate add 0. 2 ml of sulphuric acid.

He doesn't develop staining blue-green.

### .Formulation of the ointment:

The composition of the proposed anti inflammatory ointment formulation is reported in the next table:

Component	Rate (%)
Ibuprofen	X
Olive Oil (Oleocanthal)	X
Vaseline	16,0
Glycerol	0.5
Bee Wax	1.0
Propylene glycol	X
stearylic Alcohol	4.0
glycerol Stéarate	3.0
Menthol	1.0
Lauric Acid	X

### Extraction of the oleocanthal:

2 ethyl (4-hydroxyphenyl) (3 S, 4) - 4 formyl - 3-(2-oxoethyl) hex-4-enoate (C<sub>17</sub>H<sub>20</sub>O<sub>5</sub>) the Oleocanthal can be obtained from any appropriate source. The formulation was prepared by mixing olive oil, of ibuprofen, beeswax, stéarylique alcohol, and petroleum jelly and heating to 30-35 ° C.

The heating has been interrupted and the rest of the components have been added by. The mixture is left to cool to room temperature, how much menthol has been added.

Quality control parameters:

macroscopic, organoleptic qualities and pH:

homogeneity: we have verified homogeneity of ointment by spreading the thin layer on a flat surface with a spatula. Regular distribution of the excipients extracts were observed.

Measurement of pH : pH is determined by measuring the dilution to the tenth of ointment in hot distilled water.

(2) Skin irritation test:

the skin tolerance test is designed to predict from knowledge of the toxicity on a living organism, the toxicological risk in humans. The method is performed on 6 New Zealand strain rabbits, each animal's back is divided into two areas, after removal of hair, one of the areas the product is applied directly, the other area it is applied after scarification. The product is held in contact by a dressing for 24 hours. Readings are made 24 hours and 72 hours after the application. The rating of the phenomena observed system to identify a skin primary irritation index (PI) calculations to classify the product in 4 categories. The test is performed in a quiet place or no disturbance is likely to excite the animals.

### Determination of anti-inflammatory activity:

it was carried out in the animal House of the Centre National Support (Algiers) to the fight against the disease (C.N.A a.m.). We used the method of edema of the leg of mouse (Winter et al., 1962).

Apparatus and instruments

Plétysmomètre UGO BASIL 71.40 is a unit of measurement of the volume of the paw of the mouse either in absolute or in relative value compared with the untreated paw. It consists of a measuring in perspex cell containing water which plunges the leg of the mouse and the difference in the level of the water after immersion of the leg is measured by a transducer design, it is displayed on a digital device

Method: Test of inhibition of edema of the leg of the mouse to the carrageenan

Evaluation of anti-inflammatory activity:

$$\%Inb = Po - Pt / Po.100\%$$

Determination of anti inflammatory activity by the method of croton oil:

Erythème	Œdème	
Pas d'érythème	Pas d'œdème	0
Léger érythème (à peine visible)	Très léger œdème (à peine visible)	1
Erythème bien visible	Léger œdème (conditions bien définis, gonflement).	2
Erythème important	Œdème moyen (épaisseur environ 1mm)	3
Erythème grave (rouge pourpre)	Œdème grave (épaisseur supérieure à 1mm)	4

### Antimicrobial activity:

micro-organisms and the culture conditions: the bacterial strains used were Bacillus cereus CECT 148, b. subtilis CECT 498, Staphylococcus aureus ESA 7, Escherichia coli CECT 101, Pseudomonas aeruginosa CECT 108 and Klebsiella pneumoniae ESA 8. Strains of fungi are Candida albicans CECT 1394 and Cryptococcus neoformans ESA 3.

The passage proved very active on several species including Staphylococcus aureus (S-research and research-R), S.epidermidis, and Pseudomonas aeruginosa. The results are very encouraging and suggest that the leaves of the olive tree have compounds with significant antimicrobial properties, which leaves provide their application in pharmaceutical and/or agri-food industry.

## Conclusion

It had been proposed a new anti-inflammatory ointment formulation in the composition of which Ibuprofen and Olive Oil from Kabylia (Algeria) can be used together, providing an synergic effect.

The leaves were also used to extract the Oleoconthal, a specific high biological antibactericidal activity molecule.

Also the parameters pharmacological were determined successfully

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