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# CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY ACTIVITY OF ESSENTIAL OILS FROM THE LEAVES OF *OCIMUM BASILICUM* L. AND *OCIMUM GRATISSIMUM* L. (LAMIACEAE)

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**ABSTRACT:** *Ocimum basilicum* L. and *Ocimum gratissimum* L. (Lamiaceae) are widely distributed aromatic herbs used in ethnomedicinal management of a range of inflammatory disorders. In the present work we evaluated the topical anti-inflammatory effects of the volatile constituents extracted from the fresh leaves of these plant species. Fresh leaves of the plants were subjected to hydro distillation to obtain the volatile oils OBV and OGV from *O. basilicum* and *O. gratissimum* respectively. The fresh leaves were also extracted with n-hexane to obtain OBHE and OGHE respectively. OBV, OGV, OBHE and OGHE were screened for anti-inflammatory effect using xylene-induced ear edema as a model of inflammation. Their chemical constituents were also analysed using GC/MS apparatus. At 50 µg/ear OBV, OGV, OBHE and OGHE exhibited significant ($P<0.05$) topical anti-inflammatory effect with edema inhibitions of 50.0, 63.3, 62.7 and 80 % respectively. The effects were comparable ($P<0.05$) with that of 100 µg/ear hydrocortisone (% edema inhibition of 54.8). 11 of the compounds from OGV are monoterpenes while 4 are sesquiterpenes. 8 of the compounds identified in OGHE are oxygenated monoterpene derivatives, 5 are sesquiterpenes, and the others are long chain carboxylic acid, eugenol and phthalate derivatives. OGV and OGHE contain linalool, 1-terpinen-4-ol, alpha-caryophylene and trans longipinocarveol in common. Compounds identified in OBV include 2 monoterpenes, 7 oxygenated monoterpene derivatives, 2 sesquiterppenes, a long chain monocarboxylic acid and a triterpene, alpha-amyrin. OBV and OBHE contain eugenol acetate in common. Some of these identified volatile constituents may be contributing to the observed anti-inflammatory effects.

**INTRODUCTION:** *Ocimum basilicum* L. and *Ocimum gratissimum* L. (Lamiaceae) are widely distributed aromatic herbs [1].

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*Ocimum basilicum* (Sweet basil) is an erect herb that grows up to a height of 90 cm.

The stem is purplish and the leaves are lanceolate, glossy, and fragrant. The flowers are tubular, bilabiate, purplish, and packed in whorled racemes.

*Ocimum gratissimum* (Wild basil) is also a perennial herb with erect stem and can grow up to 1-3 m tall. The stem is round glandular, much branched, glabrous or pubescent and woody at the

base. The leaves are opposite, slender and the leave blade elliptical to ovate [2]. The leaves of *Ocimum basilicum* have been used in ethnomedicine for a variety of ailments ranging from respiratory disorders [3], fever [4], as remedy for gonorrhoea, catarrh conditions, cough, constipation, dysentery, ringworm, carminative and hypertension [4, 5].

The whole plant and the essential oil of *Ocimum gratissimum* have many applications in traditional medicine, especially in Africa and India.

Preparations from the whole plant are used as stomachic and in treating sunstroke, headache and influenza. The seeds have laxative properties and are prescribed against gonorrhoea. The essential oil is applied against fever, inflammations of the throat, ears or eyes, stomach pain, diarrhoea and skin diseases [1, 2, 6, 7] and previous studies have confirmed its hypoglycaemic effect [8].

Although there are reported studies on the anti-inflammatory effect of some species of *Ocimum* [9-11] there is to date no known scientific study validating the ethnomedicinal use of *Ocimum basilicum* and *Ocimum gratissimum* in inflammatory conditions.

Inflammatory mycoses, a clinical condition associated with inflammation, fungal and bacterial infection is commonly treated with a combination of anti-inflammatory, antifungal and antibacterial agent in one formulation [12-15]. There are several reported studies on the antimicrobial potentials of *Ocimum* species [16-20].

Our aim in the present study is thus to evaluate the topical anti-inflammatory potentials of the two *Ocimum* spp with the view of ascertaining there suitability for use in topical herbal formulations for inflammatory mycoses. We have also adopted two methods of essential oil extraction namely hydro distillation and solvent extraction.

## MATERIALS AND METHODS:

**Plant Material:** Fresh leaves of *Ocimum basilicum* and *Ocimum gratissimum* were purchased from a local market in Nsukka, Enugu State Nigeria. The leaves were shopped into smaller pieces and used immediately for essential oil extraction

**Extraction of the essential oils:** About 600 g fresh leaves of *Ocimum basilicum* and *Ocimum gratissimum* were coarsely milled and the volatile constituents isolated by hydro-distillation for 4 h using Clevenger apparatus. The volatile oil fractions OBV and OGV respectively were recovered. About 200 g each of coarsely milled fresh leaves of *Ocimum basilicum* and *Ocimum gratissimum* were extracted in 500 mL hexane for 48 h with constant checking at room temperature. The hexane extracts were concentrated *in vacuo* to yield the volatile oil fractions OBHE and OGHE respectively. OBV, OGV, OBHE and OGHE were stored in refrigerator ($0^oC$) before topical anti-inflammatory tests and analysis of the constituents with GC/MS apparatus.

**Gas chromatography-mass spectrometry:** OBV, OGV, OBHE and OGHE were analyzed by GC/MS (GCMS-QP2010 PLUS, Shimadzu Japan). Operating conditions were as follows: carrier gas, helium with a flow rate of 2 ml/min; column temperature, 60-280ºC at 5ºC/min; injector and detector temperatures, $250^oC$; injected volume 2 µl; split ratio, 1:50. The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 1A; ion source temperature, $200^oC$ and resolution of 1000.

**Identification of compounds:** Identification of components in OBV, OGV, OBHE and OGHE were based on comparison of the retention indices and computer matching of MS fragments with the NISTO5.LIB.

**Topical anti-inflammatory tests:** The effect of OBV, OGV, OBHE and OGHE on acute topical edema was evaluated by a modification of previously reported methods [21, 22]. Adult albino mice (20 ± 5 g) of either sex were divided into groups of 5 animals. The treatment groups received the extracts dissolved in xylene at doses of 50, 100, 200 and 400 µg/ear applied on the anterior surface of the right ear. Control animals received either equivalent volume of the phlogistic agent (xylene) or hydrocortisone dissolved in xylene (100 µg/ear). Two hours after application, the mice were sacrificed and both ears removed. Circular sections (5 mm) of both the right (treated) and left (untreated) ears were punched out using a cork borer, and weighed.

Edema was quantified as weight differences between the two earplugs. The anti-inflammatory activity was evaluated as percent edema inhibition in the treated animals relative to the control animals [21, 22] using the relation:

$$\% \ Edema \ Reduction = \left[1 - \frac{Rt - Lt}{Rc - Lc}\right] x\ 100$$

Where Rt = mean weight of right earplug of treated animals; Lt = mean weight of left earplug of treated animals; Rc = mean weight of the right earplug of control (vehicle treated) animals; Lc = mean weight of the left earplug of control (vehicle treated) animals.

**Statistical analysis:** Results of anti-inflammatory effect obtained were analyzed by SPSS version 11 using one way ANOVA and subjected to Fischer LSD post hoc tests and expressed as mean ± SEM. Differences between means were considered significant at P<0.05.

## RESULTS:

**Anti-inflammatory activity:** The result of the topical anti-inflammatory screening of the volatile oils is shown in Tables **1** and **2**. At the dose of 50 µg/ear, all the tested oils exhibited significant (p<0.05) inhibition of edema induced by topical application of xylene. OGHE showed better anti-inflammatory effect than OGV at the tested doses, while OBV showed better anti-inflammatory effect than OBHE. When compared with hydrocortisone, OGHE and OBV exhibited better anti-inflammatory effect. The anti-inflammatory effect exhibited by the oils are however not dose-dependent.

**TABLE 1: EFFECT OF THE VOLATILE OILS FROM *OCIMUM GRATISSIMIUM* LEAVES ON XYLENE-INDUCED EAR EDEMA IN MICE**

| Test material (µg/ear) | Dose | Mean ear edema (Mean ±SEM) at 2 h) | Inhibition at 2 h (%) at 2 h | Inhibition vs hydroc (%) |
|---|---|---|---|---|
| OGV | 50 | 2.68 ± 0.81* | 52.57 | 126.40 |
| | 100 | 3.68 ± 0.56 | 34.87 | 83.84 |
| | 200 | 3.64 ± 1.10* | 35.57 | 85.52 |
| | 400 | 3.66 ± 0.34* | 35.22 | 84.68 |
| OGHE | 50 | 0.80 ± 0.37** | 85.84 | 206.40 |
| | 100 | 2.40 ± 0.37* | 57.52 | 138.30 |
| | 200 | 1.40 ± 0.40* | 75.22 | 180.86 |
| | 400 | 2.60 ± 0.68* | 53.98 | 129.79 |
| Hydroc. | 100 | 3.30 ± 0.00* | 41.59 | 100 |
| Xylene | 5 µL | 5.65 ± 0.91 | - | - |

OGV = Volatile oil from Ocimum gratissimium leaves obtained by hydrodistillation. OGHE = Volatile oil from Ocimum gratissimium leaves obtained by solvent (hexane) extraction. The test materials were applied topically at the stated doses. Treatment animals were compared to control animals which had received vehicle only or prednisolone; **P<0.05, **P<0.01, n = 5.*

**TABLE 2: EFFECT OF THE VOLATILE OILS FROM *OCIMUM BASILICUM* LEAVES ON XYLENE-INDUCED EAR EDEMA IN MICE**

| Test material | Dose (µg/ear) | Mean ear edema (Mean ±SEM) at 2 h) | Inhibition at 2 h (%) at 2 h | Inhibition vs hydroco (%) |
|---|---|---|---|---|
| OBV | 50 | 2.00 ± 0.45* | 64.60 | 155.32 |
| | 100 | 2.4 0 ± 0.75 | 57.52 | 138.3 |
| | 200 | 2.4 0 ± 0.51* | 57.52 | 138.3 |
| | 400 | 1.6 0 ± 0.40** | 71.68 | 172.35 |
| OBHE | 50 | 2.72 ± 0.60** | 51.86 | 124.69 |
| | 100 | 4.72 ± 0.81 | 16.46 | 39.58 |
| | 200 | 4.72 ± 0.81* | 16.46 | 39.58 |
| | 400 | 3.40 ± 0.00* | 39.82 | 95.74 |
| Hydroc. | 100 | 3.30±0.00* | 41.59 | 100 |
| Xylene | 5 µL | 5.65 ± 0.91 | - | - |

OBV = Volatile oil from *Ocimum basilicum* leaves obtained by hydrodistillation. OBHE = Volatile oil from *Ocimum basilicum* leaves obtained by solvent (hexane) extraction. The test materials were applied topically at the stated doses. Treatment animals were compared to control animals which had received vehicle only or prednisolone. **P<0.05, **P<0.01, n = 5.*

**Volatile oil compositions of the leaves:** The results of the chemical composition of the volatile oils extracted from the two species of *Ocimum* by solvent extraction and hydro distillation are shown in **Tables 3** and **4**. A total of 15 compounds where identified in OGV as against 18 compounds identified in OGHE. 11 of the compounds from OGV are monoterpenes while 4 are sesquiterpenes. 8 of the compounds isolated from OGHE are oxygenated monoterpene derivatives, 5 are sesquiterpenes, and the others are long chain carboxylic acid, eugenol and phthalate derivatives. OGV and OGHE contain linalool, 1-terpinen-4-ol, alpha-caryophylene and trans longipinocarveol in common. A total of 14 and 11 compounds where identified in OBV and OBHE respectively. Compounds identified in OBV include 2 monoterpenes, 7 oxygenated monoterpene derivatives, 2 sesquiterppenes, a long chain monocarboxylic acid and a triterpene, alpha-amyrin. OBV and OBHE contain eugenol acetate in common

**TABLE 3: CHEMICAL COMPOSITION OF THE VOLATILE OILS EXTRACTED FROM *OCIMUM GRATISSIMIUM* AND LEAVES**

| S. No. | Compounds identified in OGV | Compounds identified in OGHE |
|---|---|---|
| 1 | Alpha thujene | Linalool |
| 2 | Camphene | Hexyl butanoate |
| 3 | Beta-myrlene | Borneol (1,8 cineole) |
| 4 | Thujanol | 1-Terpinen-4-ol |
| 5 | D-fenchone | Thymoquinon |
| 6 | Linalool | O-Tertbutylphenol |
| 7 | Gamma-terpene | Durenol |
| 8 | Thujone | Verbenone 9 |
| 9 | Beta thujene | Caryophyllene |
| 10 | - | Methyl 9-oxo nonanoate |
| 11 | 1-terpinen-4-ol | Alpha caryophyllene |
| 12 | - | Eudesma-4(14), 11-diene |
| 13 | Thujene-2-one | (-) Alpha panasinsen |
| 14 | Alpha caryophylene | Alpha cardinol |
| 15 | (-) Alpha panasinsen | Trans longipinocarveol |
| 16 | Naphthalene | n-Hexadecanoic acid |
| 17 | Azulene | Dehydrodihydrodiisoeugenol |
| 18 | - | Di-n-octyl phthalate |

OGV = Volatile oil from *Ocimum gratissimium* leaves obtained by hydrodistillation. OGHE = Volatile oil from *Ocimum gratissimium* leaves obtained by solvent (hexane) extraction.

**TABLE 4: CHEMICAL COMPOSITION OF THE ESSENTIAL OIL ISOLATED FROM OCIMUM BASILICUM LEAVES**

| S. No. | Compounds identified in OBV | Compounds identified in OBHE |
|---|---|---|
| 1 | Eucalyptol | 2-Mthyl benzyl alcohol |
| 2 | cis-beta-Ocemene | Thymol |
| 3 | gamma-Terpene | Eugenol acetate |
| 4 | D-Fenchone | Eugenol methyl ether |
| 5 | Linalool | Cubenol |
| 6 | D-2-Bornanone | Hexadecanoic acid |
| 7 | L-Terpinen-4-ol | 13-Hexyloxacyclotridec-10-en-2-one |
| 8 | Eugenol acetate | 8-(2-octylcyclopropyl) octanal |
| 9 | Borneol (1,8 cineole) acetate | Linoleic acid |
| 10 | Alpha Bergamoten | Di-n-octyl phthalate |
| 11 | Germacrene | 2-methyl Benzaldehyde |
| 12 | Dotracotane | - |
| 13 | Hexadecanoic acid | - |
| 14 | Alpha- Amyrin | - |

OBV = Volatile oil from Ocimum basilicum leaves obtained by hydrodistillation. OBHE = Volatile oil from Ocimum basilicum leaves obtained by solvent (hexane) extraction

**DISCUSSION:** The whole leaves and essential oil of various species of *Ocimum* have several applications in traditional medicines especially in Africa and India. Previous studies have validated the hypoglycaemic effect [8], antibacterial [17-20], antinociceptic and anti-inflammatory [10, 11] properties of some *Ocimum* species. Our present studies have shown that the essential oils from *Ocimum gratissimum* and *Ocimum basilicum* possess significant topical anti-inflammatory effect in xylene-induced mouse ear edema model. The anti-inflammatory activity was found to vary based on method of essential oil extraction, which has a direct bearing on the chemical compositions. In general, the activity increases with increase in the proportion of some essential oils like eugenol, linalool, D-fenchone, 1-terpene-4-ol, thymol, alpha-caryophylene and the presence of diterpenes and triterpenes, which have earlier been reported to possess anti-inflammatory activity [23-26].

Essential oils obtained from *Ocimum gatissimum* by solvent (n-hexane) extraction (OGHE) showed better topical anti-inflammatory activity than the oils obtained from the same plant species by hydro-distillation (OGV). Conversely, the essential oils obtained from *Ocimum basilicum* by hydro-distillation (OBV) showed better anti-inflammatory activity than the oils obtained from the same plant species by solvent (n-hexane) extraction (OBHE). These observations can be correlated with the chemical compositions of the essential oils from both plant species isolated by the two reported methods (Tables 3 and 4).

OBV and OBHE both contained eugenol derivatives previously reported to possess significant anti-inflammatory activity [27, 28]. OBV, however, in addition contained several other compounds like eucalyptol, linalool, borneol acetate, alpha-bergamoten, germacrene and a triterpenoid alpha-amyrin (Table 4). These compounds have been reported to exhibit anti-inflammatory activity [29-31] or detected in essential oils reported to exhibit anti-inflammatory activity [32, 33]. This might explain the significantly higher anti-inflammatory activity of OBV compared to OBHE. OGV and OGHE both contained linalool, alpha-caryophyllene, (-) alpha panasinsen and 1-terpnene-4-ol along with other volatile constituents like alpha-thujone, camphene, borneol, verbenone, D-Fenchone, alpha cardinal etc (Table 3). These volatile constituents have been detected in some essential oil isolated from plants with anti-inflammatory activity [32, 33]. The presence of a eugenol derivative (Dehydrodihydrodiisoeugenol), a diterpene (Eudesma-4(14), 11-diene) and Di-n-octyl phthalate in OGHE might be the plausible explanation for the observed better anti-inflammatory activity that OGV. It is also an interesting observation than OGHE and OBV (at 50 µg/ear) exhibited close to 2 times topical anti-inflammatory activity compared to hydrocortisone (100 µg/ear) (Table 1 and 2). This observation thus supports the use of volatile oils obtained by solvent (n-hexane) extraction from *Ocimum gratissimum* and the oils obtained by hydro distillation from *Ocimum basilicum* for topical application in inflammatory conditions.

Previous studies have indicated the chemical variability in the composition of the essential oil extracted from *Ocimum* species. At least six chemotypes namely eugenol, thymol, citral, ethyl cinnamate, geraniol and linalool have been identified, with eugenol being the most important economically [2]. In a recent study, phenylpropanoids were shown to be the major constituents of *Ocimum gratissimum* and *Ocimum basilicum* [34]. Specifically in the study, methyl chavicol and linalool were shown to be the major constituent of *Ocimum basilicum* along with bicyclogermacrane and alpha-terpenol. Also in the study the major constituents in *Ocimum gatissimum* were eugenol, 1, 8-cineole, germacrene D and beta-caryophylene. Our current results show somewhat a slightly different variability. Linalool, germacrene, alpha-terpenol were also shown to be present in *Ocimum basilicum* along with eugenol derivatives, while caryophyllene and 1, 8 cineole were shown to be present in *Ocimum gratissimum* along with linalool and alpha-terpenol.

Our results so far have shown that the volatile oils isolated from *Ocimum basilicum* and *Ocimum gatissimum* show varying degrees of topical anti-inflammatory activity. This supports the ethnomedicinal uses of these plant materials in the management inflammatory disease states. These plant materials have also been reported to possess significant antifungal and antibacterial activities.

Our work thus supports there use as topical agents in the management of inflammatory mycoses.

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