# From Phytochemicals to Molecular Targets: Integrated Antioxidant, Antimicrobial and In Silico Characterization of *Ocimum americanum* L.

Supplementary Information
Phytochemical Profiling, Antioxidant, and Antimicrobial Evaluation of *Ocimum americanum* Leaf Extracts
*Journal of Medico Informatics*

Supplementary Tables

Table S1: DPPH Radical Scavenging Activity

| Concentration (µg/mL) | % Inhibition — Extract (mean ± SD) | % Inhibition — Ascorbic Acid (mean ± SD) |
|---|---|---|
| 10 | 30 ± 1.2 | 75 ± 1.0 |
| 25 | 38 ± 1.5 | 80 ± 1.2 |
| 50 | 47 ± 1.3 | 85 ± 1.1 |
| 75 | 56 ± 1.6 | 90 ± 1.4 |
| 100 | 65 ± 1.8 | 95 ± 1.3 |
| $IC_{50}$ (µg/mL) | 58.3 ± 1.4 (95% CI: 55.6–61.0) | 18.2 ± 0.8 (95% CI: 16.7–19.7) |

Values are mean ± SD (n = 3). ANOVA: F = 32.76, p = 0.0008, $\eta^2$ = 0.929. Error bars in Figure S3 represent SD.

Table S2: ABTS Radical Scavenging Activity

| Concentration (µg/mL) | % Inhibition — Extract (mean ± SD) | % Inhibition — Ascorbic Acid (mean ± SD) |
|---|---|---|
| 10 | 41 ± 1.1 | 70 ± 1.2 |
| 25 | 46 ± 1.3 | 75 ± 1.4 |
| 50 | 52 ± 1.5 | 80 ± 1.3 |
| 75 | 58 ± 1.7 | 85 ± 1.5 |
| 100 | 63 ± 1.9 | 90 ± 1.6 |
| $IC_{50}$ (µg/mL) | 49.7 ± 1.2 (95% CI: 47.4–52.0) | 15.4 ± 0.6 (95% CI: 14.2–16.6) |

Values are mean ± SD (n = 3). ANOVA: F = 28.54, p = 0.0012, $\eta^2$ = 0.919.

Table S3: FRAP and Total Antioxidant Capacity (TAC)

| Extract | FRAP (µmol $Fe^{2+}$ eq/g) | TAC (mg AAE/g) |
|---|---|---|
| Aqueous | 124.6 ± 3.2 | 88.3 ± 2.4 |
| Methanol | 98.4 ± 2.8 | 72.1 ± 1.9 |
| Hexane | 41.2 ± 1.6 | 28.7 ± 1.3 |
| Ascorbic acid (standard) | 245.3 ± 4.1 | 198.5 ± 3.6 |

AAE: ascorbic acid equivalents. Values are mean ± SD (n = 3).

Table S4: ANOVA Summary — All Bioactivity Parameters

| Assay | Source | SS | df | MS | F | p | $\eta^2$ |
|---|---|---|---|---|---|---|---|
| Antimicrobial (ZOI) | Between | 186.3 | 2 | 93.15 | 18.45 | 0.002* | 0.672 |
| | Within | 91.6 | 18 | 5.09 | — | — | — |
| | Total | 277.9 | 20 | — | — | — | — |
| DPPH (% Inhibition) | Between | 4218.4 | 4 | 1054.6 | 32.76 | 0.0008** | 0.929 |
| | Within | 322.6 | 10 | 32.26 | — | — | — |
| | Total | 4541.0 | 14 | — | — | — | — |
| ABTS (% Inhibition) | Between | 1964.4 | 4 | 491.1 | 28.54 | 0.0012* | 0.919 |
| | Within | 172.2 | 10 | 17.22 | — | — | — |
| | Total | 2136.6 | 14 | — | — | — | — |

*p < 0.05; **p < 0.01. Tukey's HSD post hoc: aqueous vs. methanol (ZOI) p = 0.312 (ns); both vs. hexane p < 0.01.

Table S5: ANOVA — Antimicrobial activity (zone of inhibition)

| Parameter | Source | SS | df | MS | F | p |
|---|---|---|---|---|---|---|
| Zone of Inhibition | Between Groups | 186.3 | 2 | 93.15 | 18.45 | 0.002* |
| | Within Groups | 91.6 | 18 | 5.09 | — | — |
| | Total | 277.9 | 20 | — | — | — |

$\eta^2$ = 0.672; Observed Power = 0.94. *p < 0.05. Tukey's HSD: aqueous vs. methanol p = 0.312 (ns); both vs. hexane p < 0.01.

Table S6: ANOVA — DPPH antioxidant activity

| Parameter | Source | SS | df | MS | F | p |
|---|---|---|---|---|---|---|
| % Inhibition (DPPH) | Between Groups | 4218.4 | 4 | 1054.6 | 32.76 | 0.0008** |
| | Within Groups | 322.6 | 10 | 32.26 | — | — |
| | Total | 4541.0 | 14 | — | — | — |

$\eta^2 = 0.929$; Observed Power = 0.99. **p < 0.01. $IC_{50}$ (DPPH) = 58.3 µg/mL (95% CI: 55.6–61.0).

Table S7: ANOVA — ABTS antioxidant activity

| Parameter | Source | SS | df | MS | F | p |
|---|---|---|---|---|---|---|
| % Inhibition (ABTS) | Between Groups | 1964.4 | 4 | 491.1 | 28.54 | 0.0012* |
| | Within Groups | 172.2 | 10 | 17.22 | — | — |
| | Total | 2136.6 | 14 | — | — | — |

$\eta^2 = 0.919$; Observed Power = 0.99. *p < 0.05. $IC_{50}$ (ABTS) = 49.7 µg/mL (95% CI: 47.4–52.0). Error bars represent SD throughout.

Table S8: In silico toxicity prediction parameters for Limonene Dioxide 1 (pkCSM output).

| Model Name | Predicted Value | Unit |
|---|---|---|
| AMES toxicity | Yes | Categorical (Yes/No) |
| Max. tolerated dose (human) | 0.504 | Numeric (log mg/kg/day) |
| hERG I inhibitor | No | Categorical (Yes/No) |
| hERG II inhibitor | No | Categorical (Yes/No) |
| Oral Rat Acute Toxicity (LD50) | 1.985 | Numeric (mol/kg) |
| Oral Rat Chronic Toxicity (LOAEL) | 2.063 | Numeric (log mg/kg_bw/day) |
| Hepatotoxicity | No | Categorical (Yes/No) |
| Skin Sensitisation | Yes | Categorical (Yes/No) |
| *T. pyriformis* toxicity | -0.197 | Numeric (log µg/L) |
| Minnow toxicity | 1.853 | Numeric (log mM) |

Table S9: In silico toxicity prediction for Dehydrodieugenol (pkCSM output).

| Model Name | Predicted Value | Unit |
|---|---|---|
| AMES toxicity | No | Categorical (Yes/No) |
| Max. tolerated dose (human) | 0.652 | Numeric (log mg/kg/day) |
| hERG I inhibitor | No | Categorical (Yes/No) |
| hERG II inhibitor | Yes | Categorical (Yes/No) |
| Oral Rat Acute Toxicity (LD50) | 2.196 | Numeric (mol/kg) |
| Oral Rat Chronic Toxicity (LOAEL) | 1.575 | Numeric (log mg/kg_bw/day) |
| Hepatotoxicity | No | Categorical (Yes/No) |
| Skin Sensitisation | No | Categorical (Yes/No) |
| *T. pyriformis* toxicity | 0.678 | Numeric (log µg/L) |
| Minnow toxicity | -0.041 | Numeric (log mM) |

Table S10: In silico toxicity profiles of Dehydrodieugenol and Limonene Dioxide 1 (pkCSM)

| Parameter | Dehydrodieugenol | Limonene Dioxide 1 | Unit |
|---|---|---|---|
| AMES toxicity | No | Yes | Categorical |
| Max. tolerated dose (human) | 0.652 | 0.504 | log mg/kg/day |
| hERG I inhibitor | No | No | Categorical |
| hERG II inhibitor | Yes | No | Categorical |
| Oral Rat Acute Toxicity (LD50) | 2.196 | 1.985 | mol/kg |
| Oral Rat Chronic Toxicity (LOAEL) | 1.575 | 2.063 | log mg/kg_bw/day |
| Hepatotoxicity | No | No | Categorical |
| Skin Sensitisation | No | Yes | Categorical |
| T. Pyriformis toxicity | 0.678 | −0.197 | log µg/L |
| Minnow toxicity | −0.041 | 1.853 | log mM |

Highlighted cells indicate potential safety concerns requiring experimental validation. Favorable parameters shown without highlight.

Supplementary Figures

Figure S1: In Silico Characterization — Limonene Dioxide 1

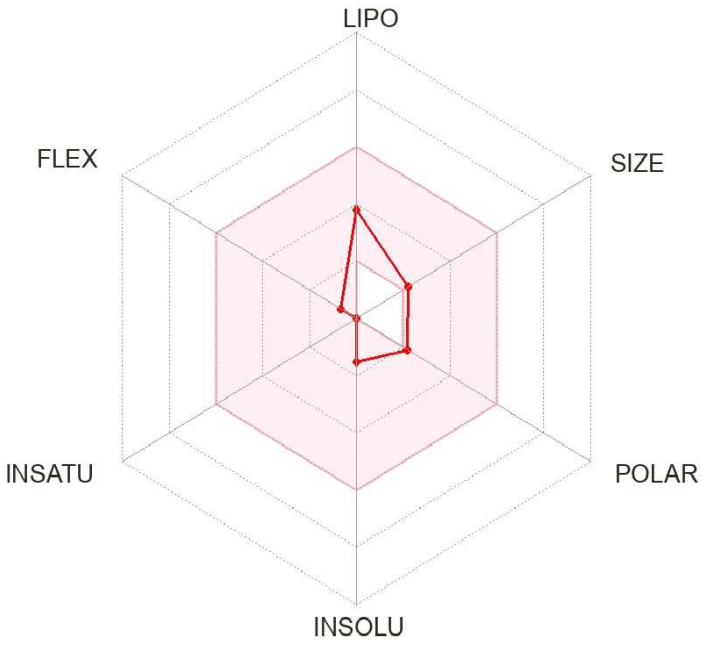
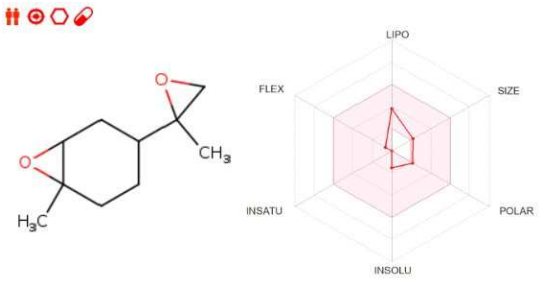



Figure S1 a: Radar chart (drug-likeness physicochemical space) for Limonene Dioxide 1. All six parameters (LIPO, SIZE, INSOLU, INSATU, POLAR, FLEX) fall within the optimal pink drug-space zone, indicating excellent physicochemical compliance.




SMILES CC1(OC1)C1CCC2(C(C1)O2)C

| Physicochemical Properties | |
|---|---|
| Formula | C10H16O2 |
| Molecular weight | 168.23 g/mol |
| Num. heavy atoms | 12 |
| Num. arom. heavy atoms | 0 |
| Fraction Csp3 | 1.00 |
| Num. rotatable bonds | 1 |
| Num. H-bond acceptors | 2 |
| Num. H-bond donors | 0 |
| Molar Refractivity | 46.09 |
| TPSA | 25.06 Å² |
| **Lipophilicity** | |
| Log $P_{o/w}$ (iLOGP) | 2.53 |
| Log $P_{o/w}$ (XLOGP3) | 1.15 |
| Log $P_{o/w}$ (WLOGP) | 1.73 |
| Log $P_{o/w}$ (MLOGP) | 1.52 |
| Log $P_{o/w}$ (SILICOS-IT) | 3.06 |
| Consensus Log $P_{o/w}$ | 2.00 |

| Water Solubility | |
|---|---|
| Log *S* (ESOL) | -1.54 |
| Solubility | 4.83e+00 mg/ml ; 2.87e-02 mol/l |
| Class | Very soluble |
| Log *S* (Ali) | -1.27 |
| Solubility | 9.02e+00 mg/ml ; 5.36e-02 mol/l |
| Class | Very soluble |
| Log *S* (SILICOS-IT) | -1.86 |
| Solubility | 2.34e+00 mg/ml ; 1.39e-02 mol/l |
| Class | Soluble |
| **Pharmacokinetics** | |
| GI absorption | High |
| BBB permeant | Yes |
| P-gp substrate | No |
| CYP1A2 inhibitor | No |
| CYP2C19 inhibitor | No |
| CYP2C9 inhibitor | No |
| CYP2D6 inhibitor | No |
| CYP3A4 inhibitor | No |
| Log $K_p$ (skin permeation) | -6.51 cm/s |
| **Druglikeness** | |
| Lipinski | Yes; 0 violation |
| Ghose | Yes |
| Veber | Yes |
| Egan | Yes |
| Muegge | No; 1 violation: MW<200 |
| Bioavailability Score | 0.55 |
| **Medicinal Chemistry** | |
| PAINS | 0 alert |
| Brenk | 1 alert: Three-membered_heterocycle |
| Leadlikeness | No; 1 violation: MW<250 |
| Synthetic accessibility | 3.61 |

**Limonene Dioxide 1**

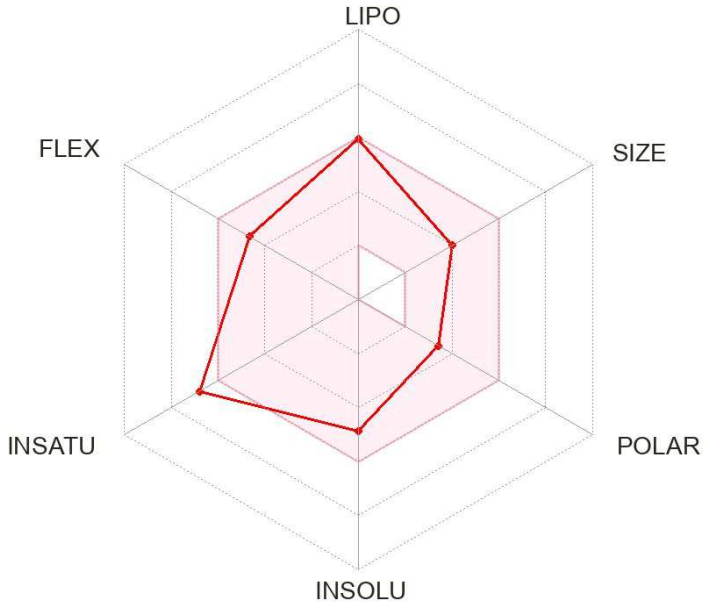
Figure S1b: Complete physicochemical, pharmacokinetic, drug-likeness, and medicinal chemistry profile of Limonene Dioxide 1 (SwissADME). Key findings: Log P = 2.00; TPSA = 25.06 Å²; GI absorption = High; BBB permeant = Yes; Lipinski = 0 violations; Bioavailability score = 0.55.

| Model Name | Predicted Value | Unit |
|---|---|---|
| AMES toxicity | **Yes** | Categorical (Yes/No) |
| Max. tolerated dose (human) | **0.504** | Numeric (log mg/kg/day) |
| hERG I inhibitor | **No** | Categorical (Yes/No) |
| hERG II inhibitor | **No** | Categorical (Yes/No) |
| Oral Rat Acute Toxicity (LD50) | **1.985** | Numeric (mol/kg) |
| Oral Rat Chronic Toxicity (LOAEL) | **2.063** | Numeric (log mg/kg_bw/day) |
| Hepatotoxicity | **No** | Categorical (Yes/No) |
| Skin Sensitisation | **Yes** | Categorical (Yes/No) |
| *T.Pyriformis* toxicity | **-0.197** | Numeric (log ug/L) |
| Minnow toxicity | **1.853** | Numeric (log mM) |

**Limonene Dioxide 1**

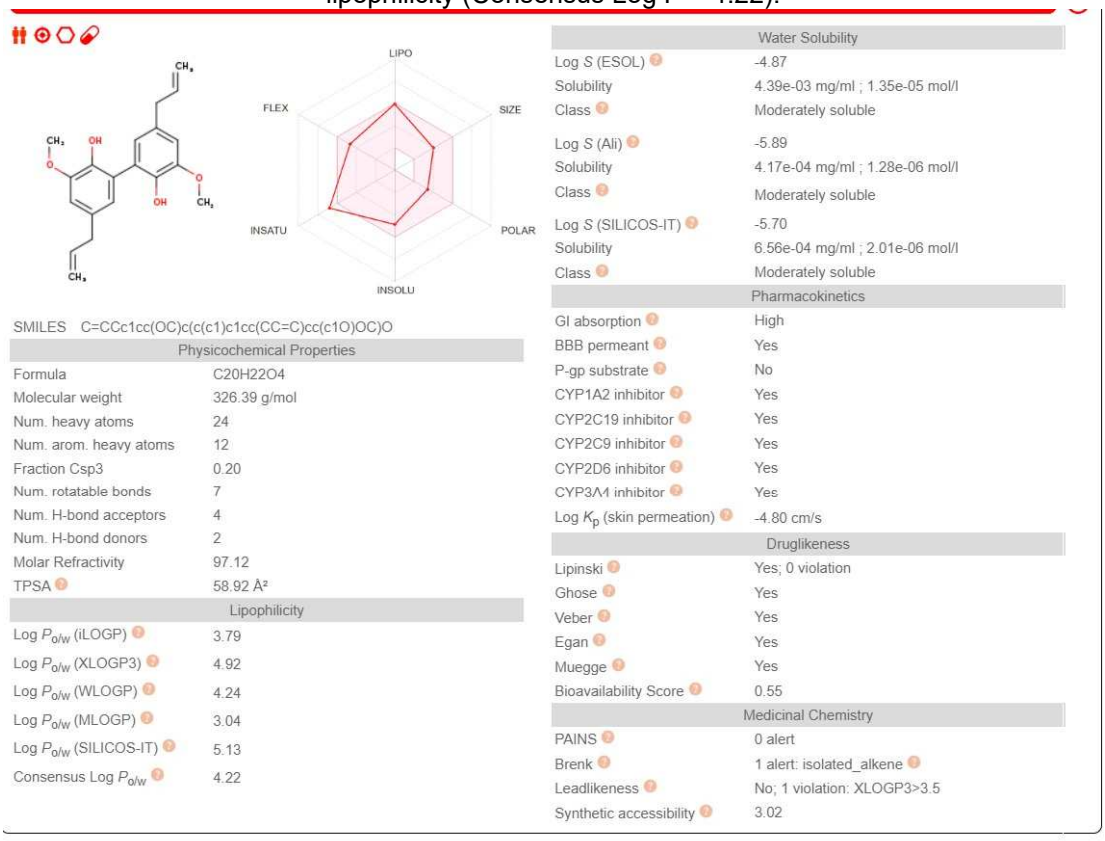
Figure S1c: In silico toxicity profile of Limonene Dioxide 1 (pkCSM). Note: AMES toxicity positive (attributed to reactive epoxide moiety) and skin sensitization positive warrant structural optimization. All other parameters within acceptable range.

Figure S2: In Silico Characterization — Dehydrodieugenol




**Dehydrodieugenol**

Figure S2a: Radar chart (drug-likeness physicochemical space) for Dehydrodieugenol. LIPO and SIZE axes approach outer boundaries of the hexagonal space, consistent with the higher molecular weight (326.39 g/mol) and lipophilicity (Consensus Log P = 4.22).




**Dehydrodieugenol**

Figure S2b: Complete ADMET physicochemical, pharmacokinetic, drug-likeness, and medicinal chemistry profile of Dehydrodieugenol (SwissADME). Key findings: Log P = 4.22; TPSA = 58.92 Å²; GI absorption = High; BBB permeant = Yes; Lipinski = 0 violations; CYP inhibition: 5/5 enzymes; Bioavailability score = 0.55.

| Model Name | Predicted Value | Unit |
|---|---|---|
| AMES toxicity | **No** | Categorical (Yes/No) |
| Max. tolerated dose (human) | **0.652** | Numeric (log mg/kg/day) |
| hERG I inhibitor | **No** | Categorical (Yes/No) |
| hERG II inhibitor | **Yes** | Categorical (Yes/No) |
| Oral Rat Acute Toxicity (LD50) | **2.196** | Numeric (mol/kg) |
| Oral Rat Chronic Toxicity (LOAEL) | **1.575** | Numeric (log mg/kg_bw/day) |
| Hepatotoxicity | **No** | Categorical (Yes/No) |
| Skin Sensitisation | **No** | Categorical (Yes/No) |
| *T.Pyriformis* toxicity | **0.678** | Numeric (log ug/L) |
| Minnow toxicity | **-0.041** | Numeric (log mM) |

# Dehydrodieugenol