University of Fort Hare
Together in Excellence

Bioactive Constituents, Antibacterial, Antioxidant and Cytotoxic Properties of Flower and Leaf Essential Oils of *Plectranthus madagascariensis* (Pers.) Benth Grown in South Africa

Sunday O. Okoh[1, 2], Omobola O. Okoh [1] , Anthony I. Okoh[1]

[1]University of Fort Hare, Department of Biochemistry &Microbiology, South Africa
[2]Federal Institute of Industrial of Research Oshodi, Department of CFET, Nigeria
[1]University of Fort Hare, Department of Pure & Applied Chemistry, South Africa

SUNDAY 5TH – THURSDAY 9TH AUGUST 2018

Introduction

Antimicrobial resistance has been listed as one of the top 3 threats to global public health (Matias *et al*., 2016).

- ROS and infectious diseases (IDs), particularly those due to MDR bacterial strains almost are impossible to combat globally.
- Besides challenges posed by microbial resistance to synthetic antibiotics, these drugs are known to exhibit some side effects (Mahmoud and Hanan, 2012).
- The worth of plant-based products on the global market is approximately 62 billion USD & will be up to 5 trillion USD by 2025 (Bhattacharya *et al*., 2017).
- There have been increasing demands for EOs rich in SM in food, cosmetics, nutra/pharmaceutical industries (Morten *et al*., 2012). And in the mgt of IDs & OSD - cholera, malaria, cancers, cardiovascular and diabetes (Bakkali *et al*., 2008).
- Paucity of information of most indigenous plants such ***Plectranthus madagascariensis*** (**Spur flower/Candle plant**) used in folk medicine for mgt of OSR &IDs in Africa.

CP

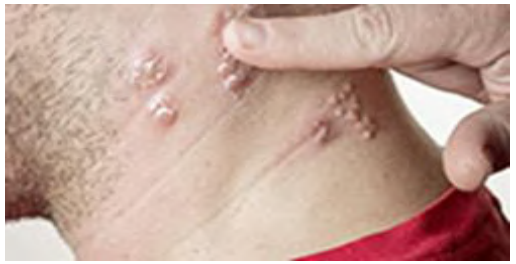
Objectives

We aimed to characterize the constituents and investigate the antibacterial, antioxidant and cytotoxic properties of leaves & stem EOs of endemic in ECP, South Africa

Specific objectives:

- To collect, process the leaves, stem and extract the EOs
- Characterize the SMs from the leaf & stem EOs
- Evaluate anti-pathogen effects on 6 bacterial strains linked to some IDs.
- Examine their radical scavenging effects & evaluate the antioxidant capacity on 4 different radicals including those associated with OSD.
- To determine the toxic effects of the EOs.

Carbuncle



Shingles

Flow Chart of Methodology

- Ethnobotanic Survey of Plants used against IOSD
  - Collection, Identification, & Processing Plant Material

❑ Extraction plant EOs from flower &leaf

➢Characterization of SMs in EOs

❖Quantitative of RS properties of the EOs

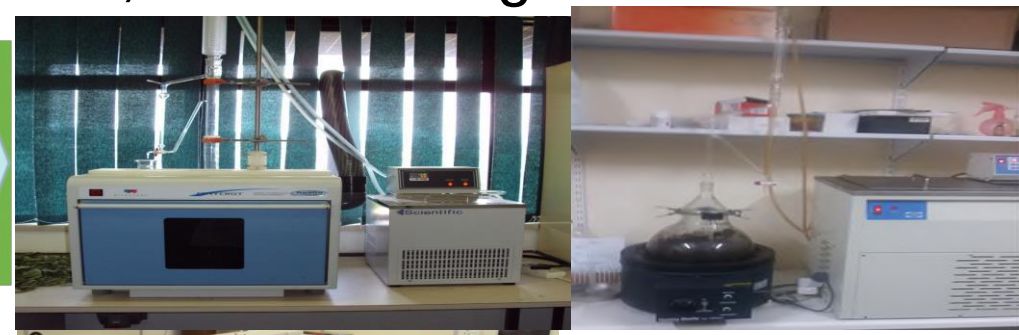
DPPH, ABTS, LP NO radicals protocols (Watanptan et al. 2010)

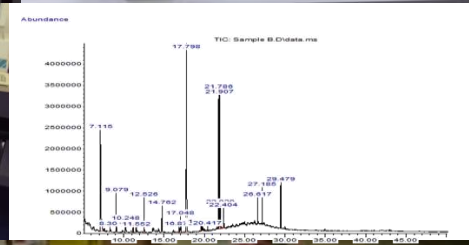
- Determination of the IC50 of each EO & RCs

○MIC & MBC evaluation of the EO (CLSI 2014)

✓Toxicity effects EOs haemolytic technique

✓





Experimental Setting

❖ Antioxidant assays

(1) 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Assay: EOs & RCs at 0.025-0.5 mg/mL prepared in DMSO & 20 ml of 0.135 Mm of DPPH Spectrophotometrically at 517 nm (Watanptan et al. 2010).
% inhibition = {(Abs control – Abs sample)}/ (Abs control)100, - Abs control = absorbance of the DPPH radical + DMSO, - Abs sample = absorbance of DPPH radical + EO or RCs.

(2) 2,2-azino-bis (3-ethylbenzothiazolin-6- sulfonic acid) diammonium salt (ABTS) assay: The working solution - 7mM ABTS + 2.4 mM K2S2O8 (1:1) & left for 12hr at room temperature in the dark. The absorbance, after 7 min is read at 734 nm.

(3) Lipid Peroxidation(LP) assay: Thiobarbituric acid (TBA) assay using egg-yolk as LP radical rich medium (Badmus et al., 2010). Absorbance read at 532 nm.

(4) Nitric Oxide (NO) assay: The NO radical was generated from sodium nitroprusside in 0.5 ml Griess reagents (Raju et al.,2006). The absorbance of the colour developed was read at 546 nm.

○ Antibacterial assays

Six reference bacterial strains [*S. aureus* (NCINB 50080), *L. ivanovii* (ATCC 19119) *M. smegmatis* (ATCC 19420), *E. cloacae* (ATCC 13047), *E. coli* 180, and *V. paraheamolyticues*] were used to examine the antibacterial activities of the EOs. They were grown in MHB and incubated at 37 oC for 24 h. The micro-dilution and pour plate (CLSI 2014) guidelines as described Iweriebor et al., 2015 were used to perform MIC, and MBC of the EOs. Ciprofloxacin and DMSO were used as positive and negative control respectively.

➢ Cytotoxic assay

The haemolytic technique (Helander et al.1998) was used to assess the toxicity of the EOs. RBC from one of our projects involving human blood was used in preparing BAP. Wells were bored on the BAP at 0.10 – 0.90 mg / mL in DMSO. Into each well 25 µL of the EO was poured while BAP and well with only DMSO was used negative control. Thereafter, incubated at 37 oC for 24 h. The presence of hemolytic activity was examined on wells and the assay was carried out in triplicate.

Results and Discussion

Physiochemical & Bioactive constituents of CP EO

The flower EO (FEO) & leaf EO (LEO) colour was slightly milky & colourless. Yield was 0.34 & 0.45 % respectively

- FEO slightly pungent,while LEO was odourless.GC/MS revealed 42 constituents FEO, LEO contained 34 representing 95.32 and 94.06% of the total oil content respectively.

Candle Plant

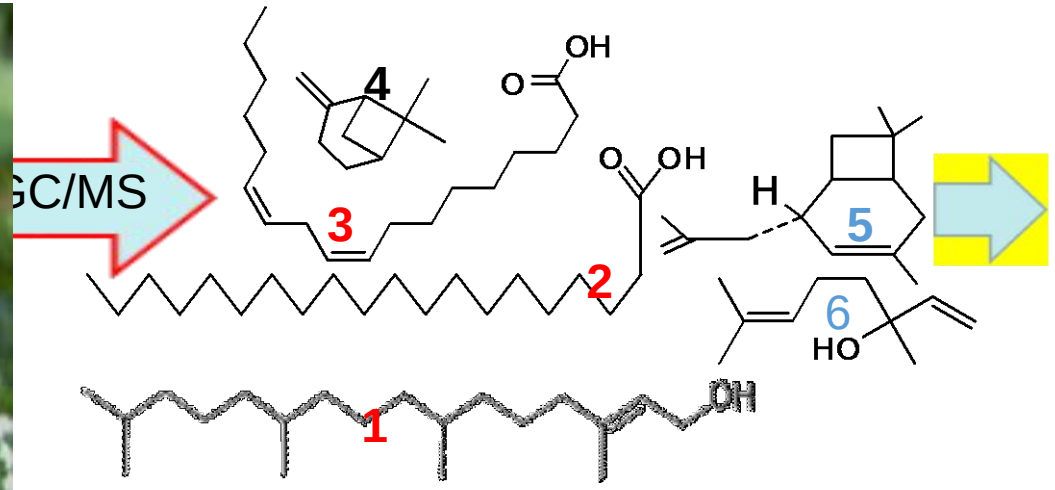


Fig. 1: Dominant Constituents in FEO and LEO of *P. madagascariensis*

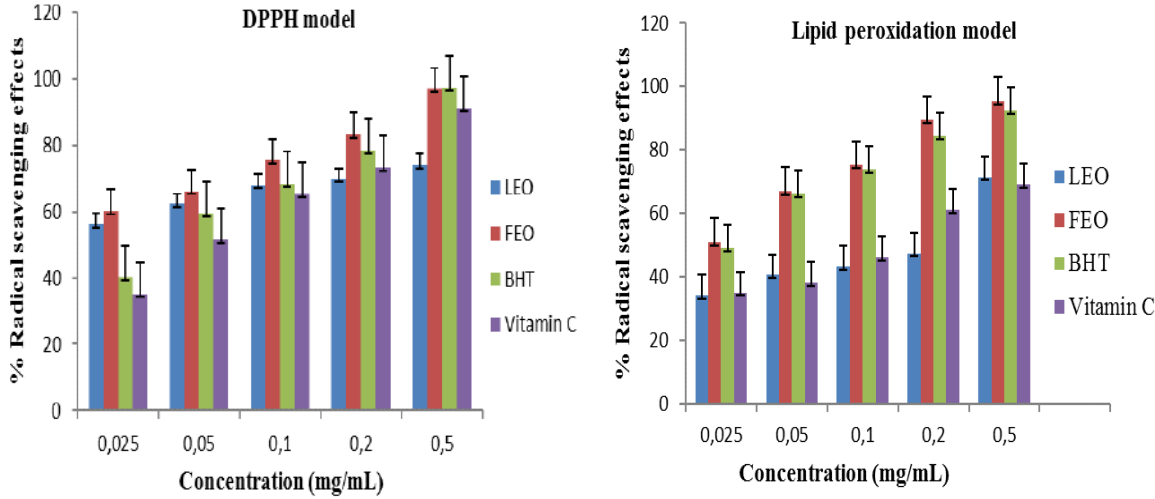


Fig 2. Antioxidant effects of *P. madagascariensis* EOs & RCs on DPPH & lipid peroxyl radicals

FEO Terpenoids & PUFA phytol **1** (25.60%), eicosanoic acid **2** (16.90 %),linoleic acid **3** (12.84%) &pinene **4** (9.12%

LEO caryophyllene **5** (20.30%), linalool **6** (15.21%) and pinene **4** (8.46%) were dominant constituents in the **LEO**.

FEO IC_{50} for (0. 52 mg/mL) indicated a higher antioxidant property than **LEO** (3.30 mg/mL) and RCs (1.20-3.34 mg/mL) in scavenging DPPH radical. The EOs efficiently reduced ABTS, LP and NO radicals in concen. dependent fashion . None of the EO was toxic to human RBC at below 0.85mg/ml.

Results and Discussion

- The MICs value for FEO & LEO against *E. coli*, *E. cloacae*, *M. smegmatis*, *L. ivanovii*, *S. aureus*, & *V. paraheamolyticus* range from 0.05 - 0.15 and 0.10 - 0. 30 mg/mL respectively.

➢ Both EOs were more sensitive to G+ve than on G-ve bacteria. The FEO was bactericidal against *S. aureus* and *L. ivanovii* at 0.20 & 0.15 mg/mL, while the LEO was bactericidal on *L. ivanovii* & *V. paraheamolyticus* at 0.35 and 0.45mg/mL, respectively.

❑ The results from the current study are in agreement with other reports that have implicated aliphatic terpenes & terpenoids with bioactive properties, while the effect of cyclic monoterpenes and sesquiterpenes with double bonds were similar to the properties of phenolic compounds such as α –tocopherol (Edris, 2007; Bakkali *et al*., 2008).

❑ The ability of the FEO & LEO from **candle plant** to scavenge four varieties of free radicals and to exhibit strong activity against 6 RBS including confirmed MDR bacterial strain (*V. paraheamolyticus*) is noteworthy.

Conclusions & Recommendations

- The high phytol & PUFA content in the FEO in this present study is remarkable & might have enhanced the bioactivity of the oil.

- Phytol, a precursor for synthesis of vitamin E, was reported by Camilla [42] to demonstrate good antioxidant effect in vivo and has high capacity to quench OH and NO radicals & prevent the formation of lipid peroxide (Probst *et al*., 2011),

- These observations may suggests that the EOs of **CP** could possibly be new active candidate in the search for lead constituents for the mgt of infectious and OSRD disorders such as diarrheal, impetigo, cancers, arteriosclerosis, and dementia on further investigation.
  - ✓ This present study indicates that apart from the traditional uses of spur flower, the FEO and LEO contained strong bioactive constituents; thus, might be good candidates as novel antimicrobial agents in this present era of increasing MDRBS, also an option to synthetic antioxidant and may be used as food preservative.

References

Badmus JA, Odunola OA, Obuotor E. Phytochemical and in vitro antioxidant potentials of Holarrhena floribunda leaf. Afr J Biotechnol. 2010;9:340–346.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of Essential oil- A review. J Food and Chem Toxo 2008; 46: 446 – 475

Camila CS, Mirian SS, Vanine GM, Luciana MC, Antonia AC et al. Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. Neurosci J. 2013

Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytoth Res. 2007;21:308–23. doi: 10.1002/ptr.2072.

Probst IS, Sforcin JM, Rall VL, Fernandes AA, Fernandes A. Antimicrobial activity of propolis and essential oils and synergism between these natural products. J Vernom Anim Toxins Incl Trop Dis 2011;17:157-69.

Helander IM, Akakomi H, Latva-Kala K, Mattila-Sandholm T, Pol I, Eddy J. Characterization of the action of selected essential oil components on gram-negative bacteria. J Agric Food Chem 1998;46:3590-5.

Mahmoud A, Hanan H. The prevalence of antimicrobial resistance in clinical isolates from Gulf corporation council countries. Antimicrobiol Resist &Infect Cont 2012; 1: 26

Matias EF, Alves MK, Silva CR, Carvalho FA, Medeiros VC, Santos CE, Bitu FG, Souza AA, Figueredo ML, Boligon JG, Athayde HD et al (2016) Potentiation of antibiotic activity of amino glycosides by natural products from *Cordia verbenacea* DC. Micro. Pathog. 95: 111-116

Morten H, Mygind TL, Rikke M. Essential Oils in Food Preservation: mode of action, synergies & interactions with food matrix components. Front. Microbiol. 2012;3:12

Iweriebor BC, Iwu CJ, Obi LC, Nwodo UU, Okoh AI. Multiple antibiotic resistances among Shiga toxin producing Escherichia coli O157 in feces of dairy cattle farmsin Eastern Cape of South Africa. BMC Microbiol. 2015;15:213. doi: 10.1186/s12866-015-0553-y.

Valko M, Leibfritz D, Moncol J, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44–84. doi: 10.1016/j.biocel.2006.07.001.

Witayapan N, Sombat C, Siriporn O. Antioxidant and antimicrobial activities of *Hyptis suaveolens* essential oil. Sci Pharm. 2007;75:35–46. doi: 10.3797/scipharm.2007.75.35

Acknowledgements

ALMIGHTY GOD

✓ AEMREG

❖ GMRDC

Acknowledgements

Thank You all

for your attention