Beyond Vibranium: Exploring Antimicrobial Activity in a Traditional African Healing Herb

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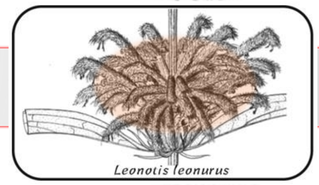


Introduction

The United Nations called **antibiotic resistance** the most urgent global risk causing the World Health Organization to propose a five-step action plan[1]. This suggests **finding alternatives to current antibiotic treatment is of paramount importance**. Africa contains a wealth of promising herbs given their history of medicinal use such as ***Leonotis,*** which is known as imvovo or imyuwane by the Zulu (fig. 1)[2]. According to **traditional healers**, *Leonotis leonorus* and *L. neptifolia* have been used against colds, influenza, stomach ailments, diarrhea, skin maladies, fungal infections, and inflammation. However, **scientific research to back these claims is sometimes equivocal**[3].

In instances where positive results were reported[4,5,6] the **zone of inhibition** (ZOI) against select microbial strains was **dependent on the preparation methodology**. Consistency in reproducibility is necessary in experiments. We wondered if the ZOI effect was reproducible by adhering to the **hot water extraction methods** that traditional African healers employ. If so, **is the effect chemotaxonomically present in additional *Leonotis* taxa** as well? We ran assays using ***L. leonorus, L. nepetifolia*** and a third taxon, ***L. menthifolia***, to test against different classes of non-pathogenic microbes**.** For comparison's sake, extracts were prepared from **foliage** versus **flowers** and **hot water** (dH_2O) versus **methanol** (MeOH).

Figure 1. Medicinal herbs for sale by an inyanga (healer) at an open air market[2].

Materials and Methods

I. Extract preparation.

Foliage and flower samples of ***L. leonurus, L. menthifolia*** and ***L. nepetifolia*** were air dried, ground, and hand-sieved through a #20 mesh screen, and transferred to beakers covered with perforated foil[7]. Simple dH_20 extraction ran for 12 h on a hot plate at low boil. Extractions with cold 80% MeOH followed a 1:20 ratio w/v for flowers and 1:40 ratio w/v for foliage and ran for 36 h[7]. Constant agitation was provided using magnetic stir bars. Final extracts were spun, squeezed through cheese cloth twice, with supernatant allowed to evaporate under a sterile hood[9]. Dried samples were scraped from beakers and resuspended in PBS solution (pH 7.4) to a final conc. of 500mg/ml[10] and stored at 1.6ºC until use.

II. Disc preparation.

25 µl of resuspended extract was pipetted onto blank discs and air dried inside a hood. Antibiotic discs (Ampicillin, Chloramp, Gentamycin, Streptomycin, Tetracycline) were commercially sourced[11]; antifungal discs were hand-prepared (10% w/v Undecylenic acid, 1% Clotrimazole, tea tree oil solution, neem seed tincture). A 2nd *L. leonurus* sample from commercially-sourced resuspended extract was used as a back-up measure.

III. Antimicrobial assays.

Streaked agar plates in triplicate were prepared from LB-cultured[11] non-pathogenic (BH level 1) bacteria and SDA-cultured[11] fungi. A single control plate per trial was left unstreaked. Treated plates used the disc diffusion method[7] using four extracts, four (+) antimicrobials, and a blank on two bacteria: gram (-) *Escherichia coli* and gram (+) *Staphylococcus epidermidis*, and two fungi: *Candida albicans* and *Aspergillus niger*. Bacteria were trialed on MH agar (Mueller-Hinton); an additional trial for *Staph. epidermidis* used MSA agar (Mannitol Salt). Fungi were trialed on SDA agar (Sabaroud Dextrose)*;* an additional trial for *Candida* used YM agar (Yeast Malt). Plates were incubated at 37°C (bacteria) or 30°C (fungi) for 16-24 hours[9], and then stored at 1.6°C until ZOI was recorded.

Results

Bars in figure 2A&B show plates that returned a **(+) reaction** (blue) in relation to total (orange) in each microbial trial per category.

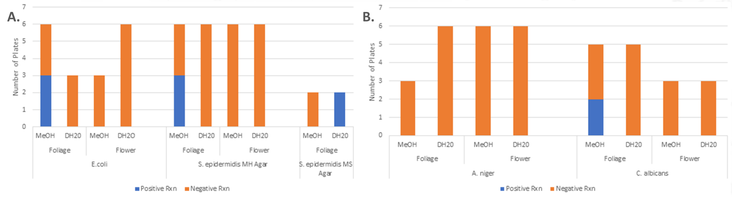
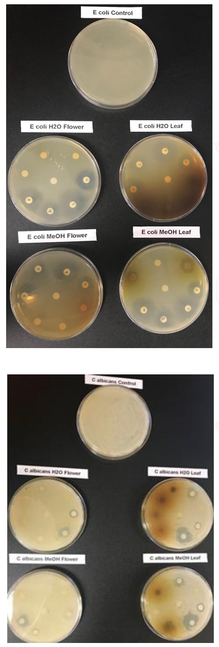


Figure 2. 90 total plates were used for antibiotic trials (A) and antifungal trials (B).

Overall, **floral extracts** showed **no reaction** (Table 1 + images). **Antimicrobial activity** (fig. A-D) was seen in **MeOH foliage extracts** of ***L. leonurus*** and ***L. nepetifolia*** on **MH agar** (fig. 3B-C) but not for *L. menthifolia*. Retrialed foliage extracts (both **MeOH** and **dH2O**) on *Staph. epi.* using **MSA agar** returned **positive results for all three taxa** (fig. 3D). **Antifungal activity** only occurred on ***L. nepetifolia* MeOH extracts** for ***Candida*** on **YM agar**. **Box plots** show comparison in figure 4 (A-C).

Table 1. Average ZOI in mm with **red font** indicating inhibition. Base-line disc-size = 6 mm, no effect. MH = Mueller Hinton, MSA = Mannitol Salt, SDA = Sabourand Dextrose, YM = Yeast Malt.

Plant	*Pathogen (+ agar)*		
Extract	*E. coli* (MH)	*Staph. epi.* (MH)	*Staph. epi.* (MSA)
L. leonurus			
-MeOH (leaf)	**12.7**	**9.7**	6.0
-MeOH (flower)	6.0	6.0	N/A
-dH_2O (leaf)	6.0	6.0	**15.0**
-dH_2O (flower)	6.0	6.0	N/A
L. menthifolia			
-MeOH (leaf)	6.0	6.0	6.0
-MeOH (flower)	6.0	6.0	N/A
-dH_2O (leaf)	6.0	6.0	**12.5**
-dH_2O (flower)	6.0	6.0	N/A
L. nepetifolia			
-MeOH (leaf)	**13.0**	**9.0**	6.0
-MeOH (flower)	6.0	6.0	N/A
-dH_2O (leaf)	6.0	6.0	**18.0**
-dH_2O (flower)	6.0	6.0	N/A
Extract	*C. albicans* (SDA)	*C. albicans* (YM)	*A. niger* (SDA)
L. leonurus			
-MeOH (leaf)	6.0	6.0	6.0
-MeOH (flower)	6.0	6.0	6.0
-dH_2O (leaf)	6.0	6.0	6.0
-dH_2O (flower)	6.0	6.0	6.0
L. menthifolia			
-MeOH (leaf)	6.0	6.0	6.0
-MeOH (flower)	6.0	6.0	6.0
-dH_2O (leaf)	6.0	6.0	6.0
-dH_2O (flower)	6.0	6.0	6.0
L. nepetifolia			
-MeOH (leaf)	6.0	**9.3**	6.0
-MeOH (flower)	6.0	6.0	6.0
-dH_2O (leaf)	6.0	6.0	6.0
-dH_2O (flower)	6.0	6.0	6.0



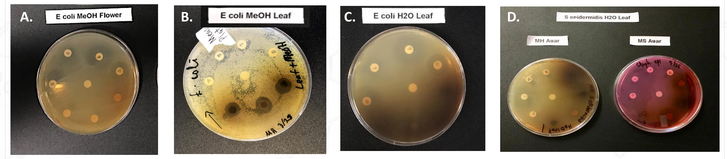


Figure 3. Examples of trialed plates. **A**. (-) result floral extracts; **B**. (+) result MeOH leaf extracts; **C**. (-) result dH_2O leaf extract; **D**. (+) result dH_2O leaf extract on MSA.

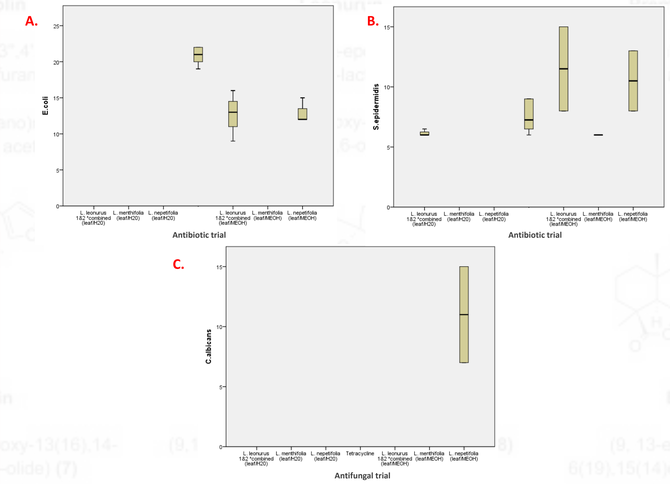


Figure 4. Box plots[12] indicate effect of MeOH leaf extracts against three microbes and show only recorded effect using *L. menthifolia* (B) and only effect using dH_2O leaf extract (B). *Leonotis leonurus* was most efficacious antibacterial; *L. nepetifolia* as antifungal (C).

Discussion

Although modern research sometimes over-emphasizes positive findings in an attempt to have publishable results, reproducibility of any experimental protocol is essential, especially when equivocal, which is why we attempted to replicate earlier work[4,5,6]. Most of our **(+) results** resulted from **foliage extracts using cold MeOH** (see fig.4 A&B), an extraction method that would never be employed by traditional African herbalists. **dH_2O leaf extractions** did work in a retrial with ***Stap. epi.*** cultured on **MSA agar**. To our knowledge, we also report the first antimicrobial activity using ***L. menthifolia***. **Antifungal** activity was exclusive to *L. nepetifolia* extracts against *Candida* on **YM agar**. **Floral extracts produced no effect** despite references suggesting otherwise[4,5,6]. Additional research should test minimum inhibitory control for foliage extracts. The use of pathogenic strains of microbes[13] would also be more informative but would require stringent monitoring in a biohazard-equipped laboratory setting, which is beyond our capabilities.

In conclusion, ***Leonotis* extracts show some antimicrobial activity,** largely against **bacteria** compared to fungi, and when using **MeOH extraction** procedures with **foliage** versus flowers. **Efficacy** of **hot water leaf extracts** is dependent on **culture media** and the pathogen tested.

References

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Contributions: Experimental design and implication was a group effort oversaw by Ordonez; Kalima and Thomas took photos, Thomas wrote Intro, Ordonez wrote M&M, Kalima and Tabi wrote Results and Discussion and did analyses.

Acknowledgements: Sincere thanks to Adam McCabe, Christina West, and Biology Department for technical support.