

## Microbial Contamination, Antimicrobial Activities of *Dissotis rotundifolia* Leaf: A Common Ethnomedicine for Ocular Diseases

V.U. USANGA<sup>1AEF</sup>, N.L. AGBO<sup>2B</sup>, M.E. KALU<sup>1\*BC</sup>, U.A. UDE<sup>1D</sup>, S.O. AZI<sup>1EF</sup>

<sup>1</sup>Department of Medical Laboratory Science, Ebonyi State University, Abakaliki, Nigeria

<sup>2</sup>Department of Laboratory Services, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State, Nigeria.

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

### Abstract

**Background:** Direct leaf extracts of *Dissotis rotundifolia* are commonly used in rural settings including Abakaliki, Ebonyi State, Nigeria as traditional medicine for the treatment of eye injury and related diseases with limited information on the scientific basis of the microbiological quality/safety of the extract.

**Objective:** This study investigated the microbial quality, antimicrobial effects of *D. rotundifolia* direct leaf extract.

**Materials and Methods:** The extracts were evaluated for microbial contamination and stored for 5 days with daily screening for microbial quality. Antimicrobial test was assessed by agar diffusion at various concentrations against five clinical isolates namely; *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter freundii* and *Candida* species. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined.

**Results:** The findings of the study showed that *D. rotundifolia* fresh direct extract was not contaminated by any pathogenic bacteria or fungi until after 2 days of storage. The fresh extract was significantly ( $p < 0.05$ ) more contaminated with *Aspergillus flavus* (20%) and *Aspergillus niger* (80%) whereas *Aspergillus niger* was the only contaminant in the dry extract up to 5 days of storage. The extract significantly ( $< 0.05$ ) inhibited the growth of all test organisms with zone of inhibition varying between 4mm (*S. Pneumoniae*) to 15mm (*Candida species*). MIC for *Candida* species was observed at 500mg/ml and 200mg/ml for other test organisms while MBC was 500mg/ml for other test organisms

**Conclusions:** The findings of this study are supportive of the use of *D. rotundifolia* extract in the treatment of bacterial-related ocular diseases and inflammation.

**Keywords:** *Dissotis rotundifolia*, Ethnomedicine, Ocular disease, Antimicrobial activity, Microbial contamination

### INTRODUCTION

One of the nature's gifts to mankind is the availability of medicinal plants which have been greatly exploited for the treatment and prevention of ailments and diseases. Research and utilization of medicinal plants in the management of diseases increases as the day goes by and the internet is awash with research confirming the efficacy of medicinal plants in treating diseases. Mankind has leveraged on

the potency of medicinal plants to alleviate and eradicate infections (Aberé *et al.*, 2010) culminating in their uses in the manufacturing of drugs such as codeine, emetine, aspirin, morphine cocaine, and ephedrine most of which mimics the mechanism of action of medicinal plants (Aja *et al.*, 2015). The reason for this would not be unconnected to their cultural acceptability, availability and adaptability

with the human body as they possess fewer side effects (Oladeji, 2016). In different parts of the world, traditional methods of managing ailments with the use of medicinal plants have continued to grow (Sangeetha and Asokan, 2018), hence arousing interest in the screening of plant parts for their biological and pharmacological properties by researchers. These efforts are aimed at evaluating the merit of trado-medicine in the light of modern science with the purpose of adopting effective beneficial medical practices (Abere et al., 2010).

*Dissotis rotundifolia* also referred to as Spanish Shawl, Trailing Tibouchina and Pink Lady, is a perennial plant in the family of Melastomataceae. It has an inconsistent pattern of growth, from straight up and erects to lying flat and prostrate on the ground with stems that are woody at the lower part of the plant and root extending from the nodes. It has beautiful pink to purplish red flowers, the petals of the flowers are ovate and 3 to 4cm in diameter (Umberto, 2012). In the tropics, the plant is cultivated as an ornamental and ground-covering plant. *Dossotis rotundifolia* has been reported to be effective in the treatment of rheumatism, oedema, diarrhoea, dysentery and to relieve stomach upset (Abere et al., 2010), gastric ulcerations (Adnorley et al., 2020), and malaria (Djehoue et al., 2020). In Nigeria specifically in Ebonyi State, *Dissotis rotundifolia* is used as an ocular medication for the management of ocular disease (Sandhu et al., 2011). The efficacy of *Dissotis rotundifolia* could be attributed to its phytochemical components which include flavonoids, saponins, tannins, anthocyanin,

cyanogenic glycoside and anthroquinone (Aja et al 2015; Adnorley et al., 2020). A study by Balogun and Owoseni (2013) revealed that methanolic extract of *Dissotis rotundifolia* is rich in isoorientin, orientin, vitexin and isovitexin. These bioactive compounds which also have been extracted from other medicinal plants form a key step in the production of plant-derived drugs (Lam et al., 2016). The antioxidative, antiaging, antimicrobial, anti-inflammatory as well as cardio-protective has been well established (Lam et al., 2016). In different parts of Africa, *Dissotis rotundifolia* is used for different purposes, such as antihelmintic and anti-inflammatory in tropical Africa and antidiarrheal in Liberia (Abere et al., 2010).

The chances of microbial contamination of *Dissotis rotundifolia* could be higher due to its growth pattern of lying flat on the ground. This may pose a risk of human infection when used without any form of sterilization prior to utilization for ocular disease management. Among the Izzi speaking ethnic group of Ebonyi State, Nigeria, including Ndidenyi Ishieke, where the plant used in this study was collected, the *Dissotis rotundifolia* non-solvent extract is commonly used by herbalists without any form of sterilization prior to application on patients' eyes. This study sought to evaluate the microbial contamination, antimicrobial activities of direct extract of *Dissotis rotundifolia* leaves. This became necessary as previous studies have focused on solvent-based extraction of the plant with limited information on the non-solvent extract.

## METHODOLOGY

### Plant collection and preparation

Fresh leaves of the plant were gathered from 50 different sites within the environs of Ndidenyi Ishieke Local Government Area of Ebonyi State, Nigeria and identified by a botanist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. The fresh leaves were carefully washed with clean tap water and ground to a homogenous paste. The paste was placed on a 2mm pore-size mesh and pressed directly to extract the content, part (25) of which was shade-dried to a powder and reconstituted prior to usage and the other part (25) was stored fresh.

### Test organisms

The organisms used in this study including *Staphylococcus aureus*, *Streptococcus pneumoniae*,

*Pseudomonas aeruginosa*, *Citrobacter freundii* and *Candida* species were clinical isolates obtained from a Federal University Teaching Hospital in the State. All the organisms were confirmed using standard cultural, morphological and biochemical methods and maintained at 4°C in a nutrient agar slant.

### Preparation of Macfarland standard solution

One percent sulphuric acid solution was prepared by adding 1 ml of concentrated sulphuric acid in 99 ml of distilled water in a conical flask. Barium chloride (1.175%) was also prepared by adding 1.175g of barium chloride salt to 100 ml of distilled water. Macfarland standard solution was prepared by adding slowly with agitation, 0.5 ml of 1% barium chloride solution to 99.5 ml of 1% sulphuric acid to make 0.5 Macfarland standard solution (Lonsway, 2021).

### Preparation of inoculum

The test organisms were inoculated in Brain Heart Infusion broth and incubated for 24 hours at 37°C. Suspension of the inoculum was made in a test tube containing normal saline and the turbidity of the test organism was determined by comparing it with the turbidity of 0.5 Macfarland standard solution. The prepared inoculum yielded approximately  $1 \times 10^8$  cells/ml.

### Microbial contamination screening

Ten microlitre of the fresh extract was inoculated on Mackonkey agar, Cysteine Lactose Electrolyte Deficient (CLED), Xylose Lysine Deoxycholate (XLD) and Sabouraud dextrose agar (SDA) with chloramphenicol in duplicate. Mackonkey, CLED and XLD were incubated at 37°C for 5 days, but SDA supplemented with chloramphenicol was incubated for 7 days and repeated daily for 5 days.

### Antimicrobial screening of the extract

Antimicrobial screening of the extract was done by agar well diffusion method (Bhattacharaya *et al.*, 2014). Briefly, Nutrient agar was prepared and 20 ml was poured into a petri dish and allowed to solidify, 10 microlitres of the test organism suspension containing  $1 \times 10^8$  cells/ml were inoculated each on the agar plates using a sterile pipette and spread over the surface using a glass spreader. Wells of 5mm in diameter were made using sterile cork borer. The wells were filled with 50 µl of different concentrations of the extract i.e. 500mg/ml, 400mg/ml, 300mg/ml, 200mg/ml and 100mg/ml and

## RESULTS

### Evaluation of microbial contamination of *Dissotis rotundifolia* leaf extract

The fifty (50) samples made up of 25 freshly extracted and 25 concentrated to dryness prior to storage were evaluated for microbial contamination over 5 days of storage. All the extracts were either contaminated with *Aspergillus niger* or *Aspergillus flavus* after 3 days of storage with an increasing

### Antimicrobial susceptibility pattern of *Dissotis rotundifolia* leaf extract

The result showed that at a low concentration (< 200mg/ml), *D. rotundifolia* leaf extract had no inhibitory effect against all test organisms. At 500mg/ml, the extract showed a significant effect on all the test organisms except for *Candida* species which was significantly inhibited at 1000mg/ml. The inhibitory effect of the extract was directly proportional to the concentration (figure 1).

incubated at 37°C for 48 hours. The inhibition zone was measured using a transparent meter rule. The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method (Andrews, 2001). The lowest concentration of the extract that showed inhibition (clear zone) for each organism was serially diluted in test tubes containing Mueller Hinton Broth and incubated at 37°C for 24 hours. After the incubation, the tubes were examined for turbidity. The lowest concentration without visible turbidity was considered to be the MIC. The minimum bactericidal concentration (MBC) was determined by taking a loopful of the MIC and streaked on a freshly prepared nutrient agar and SDA and incubated for 48 hours and 5 days respectively. The plates were examined for the presence or absence of growth.

### Identification of contaminant organism

Fungal contaminants were identified based on their morphological characteristics using cultural (direct examination of plates) and microscopic (wet mount using lacto-phenol cotton blue solution and Gram stains) methods.

### Statistical analysis

The difference in the zones of inhibition was analysed using statistical package for social science (SPSS) version 22.0 (IBM SPSS Statistics 22, Chicago, II, USA). Descriptive and inferential statistics were used in the analysis of the data. Values were presented in tables of percentages.

microbial population. However, those of dry extract were contaminated with *Aspergillus niger* only with a likewise increase in fungal load after the 4<sup>th</sup> day of storage (Table 1). Out of the 25 fresh extract samples, 5 were contaminated with *Aspergillus flavus* representing 20% while the remaining 20 (80%) were contaminated with *Aspergillus niger*. The dry extracts were all contaminated with *Aspergillus niger* (Table 2).

The minimum inhibitory concentration (MIC) for *Candida* species was observed at 500mg/ml and 200mg/ml for other test organisms (Figure 2) while minimum fungicidal concentration (MFC) was 1000mg/ml for *Candida* species and minimum bactericidal concentration (MBC) was 500mg/ml for other test organisms (Figure 3). The zones of inhibition for the MIC and MBC varied between the

bacteria species; 8mm and 13mm for *S. aureus*, 4mm and 11mm for *S. pneumoniae*, 6mm and 12mm for *P.*

*aeruginosa* and 8mm and 14mm for *C. freundii* respectively.

**Table 1: Microbial contamination of *Dissotis rotundifolia* leaf extract**

Samples	No. of samples examined	Days of storage for contamination evaluation					Organism identified
		1	2	3	4	5	
Fresh extract	25	0	0	M	H	TNC	<i>A. Flavus</i> and <i>A. niger</i>
Dry extract	25	0	0	0	M	H	<i>A. niger</i>

M = Moderate (5-50 colonies), H – high (50-100 colonies), TNC – Too numerous to count (> 100 colonies).

**Table 2: Occurrence of organisms contaminating *Dissotis rotundifolia* leaf extracts**

Type of sample	Number examined	Number contaminated with <i>A. flavus</i> (%)	Number contaminated with <i>A. niger</i> (%)
Fresh extract	25	5 (20%)	20 (80%)
Dry extract	25	0 (0%)	25 (100%)
Total	50	5 (10%)	45 (90%)

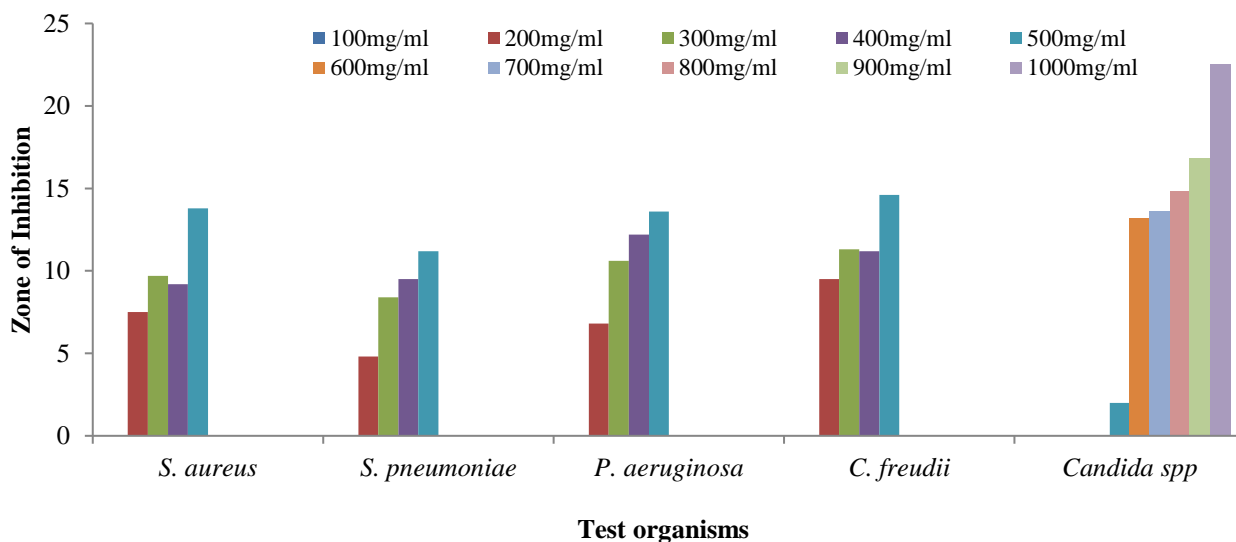


Figure 1: Zone of growth inhibition of microbial pathogens by *D. rotundifolia* leaf extract

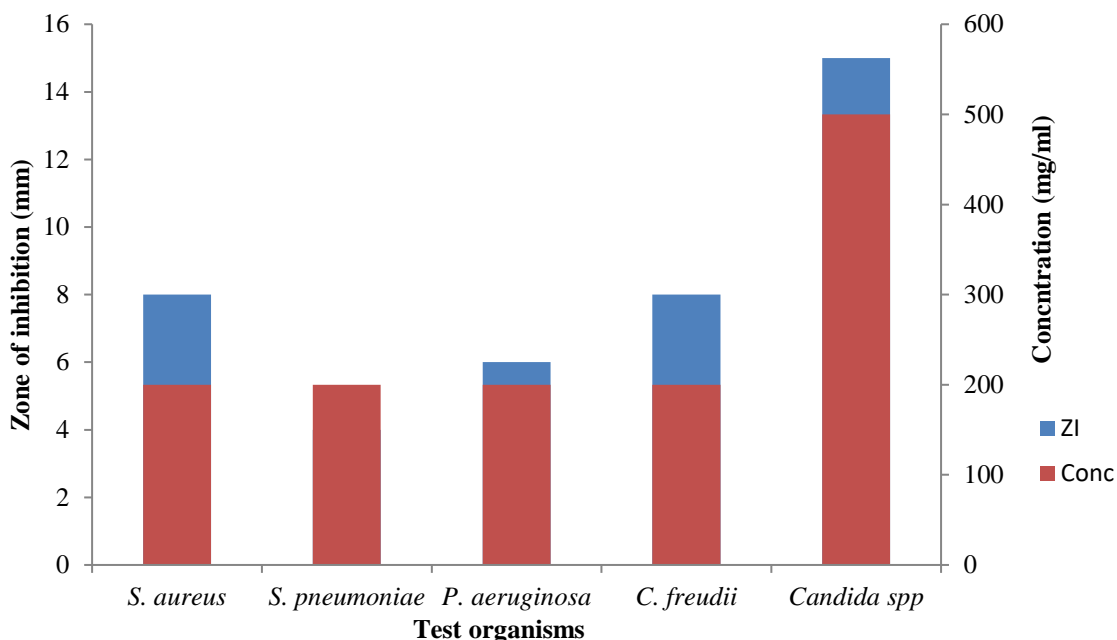


Figure 2: Minimum inhibitory concentration of *D. rotundifolia* leaf extract

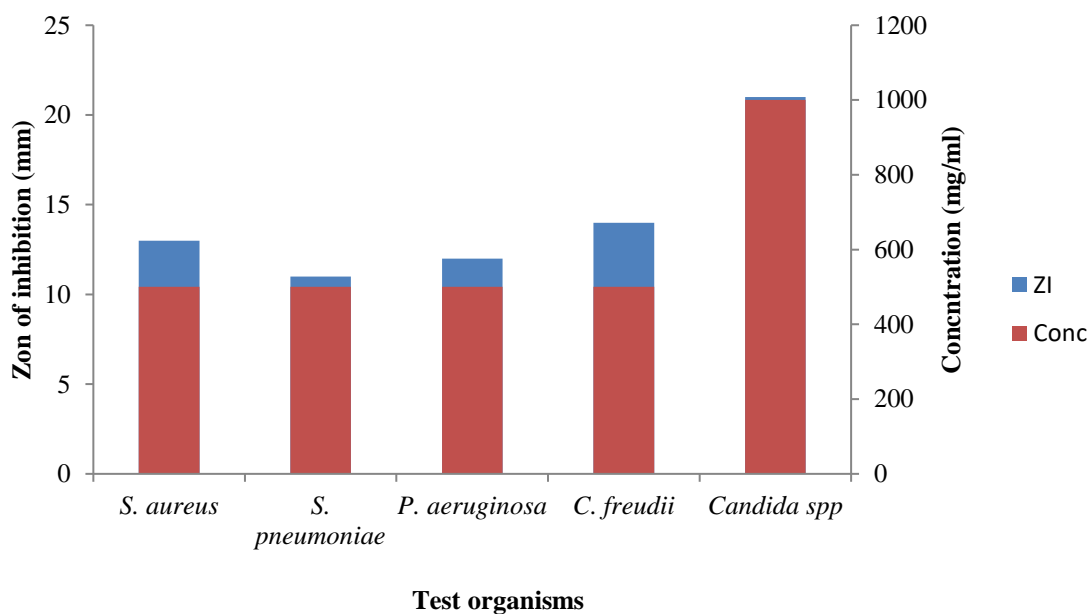


Figure 3: Minimum bactericidal/fungicidal concentration of *D. rotundifolia* leaf extract

### DISCUSSION

Herbs have long been in use for several purposes including eye treatment and current researchers still show that it is still highly valued (Sandhu *et al.*, 2011). However, studies have shown that problem of

processing leads to the contamination of the extract, as well as the storage condition can cause herbal medicines to impact negatively on human health (Shi, *et al.*, 2004). In this study, *Dissotis rotundifolia* leaf

extract which is commonly used for treatment, especially eye-related injury or inflammation in rural settings of Ebonyi State was evaluated. The findings showed that fresh extract was contaminated after two days of storage whereas dry extract was contaminated after three days of storage. Both extracts were contaminated with *Aspergillus niger* and *Aspergillus flavus*, with *Aspergillus niger* being the dominant contaminant (Table 1 and 2). It was also observed in this study that as the time of storage increased, the population of the contaminants also increased and no bacterial pathogen was isolated for five days of storage of the extract.

The presence of growth inhibition zones with the application of extract after a certain period of incubation reveals the level of antimicrobial activity of the extract against the organisms tested. In this study, it was observed that the extract was most active against *C. freundii*, followed by *S. aureus*, *P. aeruginosa* and the least was *S. pneumoniae* among the bacterial species tested whereas *Candida* species was fairly susceptible to the extract. The differences in the susceptibility of microorganisms to *D. rotundifolia* leaf extract could be attributed to the chemical composition of the extract, certain specific internal factors of each test microorganism and the

## CONCLUSION

The findings of this study provide more evidence to support the use of *D. rotundifolia* extract for the treatment of bacterial-related ocular diseases and

environment (Desire *et al.*, 2016). The MIC and MBC ratio showed that at a concentration above 500mg/ml, direct extract of *D. rotundifolia* exhibits bacteriostatic and bactericidal effects on all the organisms tested. This finding confirmed the antimicrobial effect of the constituents of the extract and their potency in the treatment of various ailments caused by various bacteria pathogens, protozoa and fungi as reported by earlier studies (Gill *et al.*, 1992; Abere *et al.*, 2010; Olufemi *et al.*, 2014).

Oral interview of the rural dweller and herbalists within the study area revealed that *D. rotundifolia* direct leaf extract is commonly used for the treatment of ocular diseases such as injuries and inflammation. The presence of 2,4-dihydroxybenzaldehyde, 2TMS derivative in direct extract of *D. rotundifolia* supports the information from rural dwellers that extract could be important in treating eye injury and inflammation due to its antioxidative and anti-inflammatory effect (Daniel *et al.*, 2020). The antimicrobial efficacy of *D. rotundifolia* could also be linked to the presence of heptane,2,2-dimethyl which has been strongly associated with genotoxic effects on bacterial cells, thus culminating in DNA damage and cell death (Kessenikh *et al.*, 2021).

inflammations. However, caution should be applied in processing the extract to minimize contamination during preparation and prolonged storage.

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\*Address for correspondence: Michael E. Kalu  
Department of Medical Laboratory Science, Ebonyi  
State University, Abakaliki, Nigeria  
Telephone: +2348064028592  
E-mails: [kalu.ere@ebsu.edu.ng](mailto:kalu.ere@ebsu.edu.ng)

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