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Original Research Article

Identification of Unique Bioactive Compounds in *Dissotis rotundifolia* Ethnomedicine for Theragnosis of Plague-like Disease and Inflammatory Ailment in Rural Settings of Abakaliki, Nigeria

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Abstract: In rural settings of Abakaliki, *D. rotundifolia* direct leaf extract is commonly used for diagnosis of suspected Plague-like diseases as well as management of redness associated with eyes injuries. However, there is limited information on bioactive compositions of this ethnomedicine. This study evaluated the homeopathic theragnostic effects, nutritional and bioactive compositions of the *D. rotundifolia* ethnomedicine. The diagnostic and anti-inflammatory effects were evaluated by interviewing herbalists and observing along with them, the biological effects on patients after being administered with the ethnomedicine. Blood samples of the patients were tested for *Y. pestis* F1 antigens. The bioactive composition of the extract was determined by GCMS and nutritional composition by proximate analysis. Administration of *D. rotundifolia* direct leaf extract on patients suspected of plague-like disease through the eyes, ears, nose or mouth, which showed exacerbated swelling and inflammation of the head regions within 30 minutes, was interpreted to indicate positive for plague-like diseases by herbalists. Laboratory investigation confirmed 97.1% (68/70) of patients diagnosed positive for plague-like disease using the ethnomedicine, were confirmed positive for *Y. pestis* F1-antigen. Application of the direct extract on eye injuries with redness, relieved pains and cleared the redness/inflammation within 2 days. GCMS analysis revealed the presence of seven (7) bioactive compounds including; pyrrolidine,2-butyl-1-methyl; Heptane,2,2-dimethyl; 2,4-dihydroxybenzaldehyde, 2TMS derivatives; Octane,3,5-dimethyl. 3,7,11,15-tetramethylhexadecane-1,2,3-triolsilylated, 1,8-nonadien-3-ol and 2,6-Dimethyldecane. Out of the seven compounds, two novel compounds were identified as 1,8-nonadien-3-ol and 2,6-Dimethyldecane. The study also showed that direct extract of *D. rotundifolia* contained high amounts of protein, carbohydrate and lipid bioactive compounds. The presence of these compounds is supportive of the roles in diagnosis and management of diseases such as plaque-like diseases, infections, oxidative stress and inflammatory injuries as reported by herbalists in rural area.

Keywords: *Drosera rotundifolia*, Ethnomedicine, Theragnostic, Yersinia pestis, Plague-like diseases, Herbalists, Anti-inflammatory.

INTRODUCTION

The eye is a paired organ located in the orbital cavity and its function is to capture images that are sent to the cortical vision centre for processing and recognition of objects as appropriate (Sangeetha and Asokan, 2018). Due to the constant exposure to the external medium, the eyes along with associated structures are subjected to intense microbial contamination/infection (Trindaderita *et al*., 2000; Sangeetha and Asokan, 2018). The eye is a unique organ which could be infected by bacteria, fungi, parasites or viruses. The external ocular surface acquires a microbial flora at birth and some of the commensals may become resident flora in the conjunctival and lids and have the potential to become pathogenic (Sharma *et al*., 1988; Sangeetha and Asokan, 2018). Apart from resident flora, any microorganisms, from the environment can form a transient flora in the eye and as well cause diseases (Sharma *et al*., 1988; Sangeetha and Asokan, 2018).

The eye infection is caused by various bacterial species such as *Steptococcus pneumonia, Haemophilus infiuenza, Staphylococcus aureus, Eschericha coli, Pseudomonas aeruginosa, Staphylococcus epidermidis, Bacillus cereus*, *Chlamydia trachomatis* and *Neisseria gonorrhoae* (Hirotoshi *et al*., 2006; Sandhu *et al*., 2011). The common causative agents for external ocular

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bacterial infections are *S. aureus* and *S. epidermidis* (Starr *et al*., 2000; Sandhu *et al*., 2011). Symptoms of bacterial eye infectious diseases include burning sensation, irritations, tearing and usually mucopurulent or purulent discharge. Eye lids may be stuck together, particularly in the mornings. Although, there are usually considered to be self-limiting (Papa *et al*., 2002; Sandhu *et al*., 2011). Various fungal species associated with eye infections are *Fusarium solani, Fusarium oxysporum, Aspergilus niger, Aspergilus flavus, Candida albicans* and *Penicilum notatum* (Hedayati *et al*., 2007; Sandhu *et al*., 2011). These infections are difficult to treat and can result in eye degeneration such as corneal ulcer and possibly blindness (Fabiana *et al*., 2004; Sandhu *et al*., 2011). Symptoms include redness, blurring vision and photophobia (Fabiana *et al*., 2004; Sandhu *et al*., 2011).

In many parts of the world, medicinal plants have been used as traditional methods for treatment of numerous human diseases including ocular infections for thousands of years, especially in rural areas of developing countries where herbal material has continued to serve as the primary source of medicines (Biswas *et al*., 2001; Sangeetha and Asokan, 2018). The world health organization (WHO) has revealed the importance of herbal cures and it has been active in creating guidelines and standards of botanical medicine (Sangeetha *et al*., 2018). Ocular medication such as eye drops or ointment are mainly used to treat and prevent eye diseases. Herbal eye drops can be polyherbal formulation used as anti-inflammatory, antihistaminic effect and anti-degenerative ophthalmic disorder (Klauss *et al*., 1994; Sangeetha and Asokan, 2018).

Different plant species including *Dissotis rotundifolia* have been associated with ocular disease management especially in developing countries such Nigeria (Sandhu *et al*., 2011). *Dissotis rotundifolia* is native of Africa occurring naturally in central and western Africa including Ebonyi State, Nigeria. It has been introduced as a ground cover and ornamental plants to other tropical areas such as Puerto rico, Malaysia and Australia. The grow in a variety of ways, from straight up and erect to lying flat and prostrate to the branches lie flat on the ground but turn up the ends (Wikipedia, 2020; Umberto, 2012). The plant is also known to be used as spices for sauce, vegetable, diuretic and very importantly extract for treatment of ocular diseases (Wikipedia, 2020; Johannes, 2005).

The growth pattern of the *D. rotundifolia* which most common lie flat on the ground; increases the chances of its microbial contamination with both indigenous and pathogenic microbes. Contamination of the leaves with pathogenic organisms would pave way for human infection when used raw especially in ocular applications. Among the Izzi speaking ethnic group of Ebonyi State Nigeria, including Ndidenyi Ishieke, where the plant used in this study were collected; the *D. rotundifolia* plant non-solvent extract is used by herbalists directly without any form of sterilization prior to application on patients' eye. Application of the non-sterile extract on ocular diseases increases the chances of eye infections which arise from environmental contamination/contamination during extraction process. The extract can also be used to treat eye diseases such as cataract and corneal ulcer associated with infectious agents like *S. aureus, S. epidermidis, Aerococcus* spp, *E. coli, Chlamydia* spp etc. This study is therefore focused on evaluating the diagnostic effects, nutritional and bioactive compounds in the direct extract of *D. rotundifolia* leaves. This became necessary as previous studies have focused solvent based extraction of the plant with limited information on the non-solvent extract.

This study focused on determining the microbiological quality, antimicrobial effects and bioactive compounds of *D. rotundifolia* direct extract.

MATERIAL AND METHODS

The Study Area

This study was carried out in Ebonyi Local Government of Ebonyi State, South Eastern part of Nigeria. It is bounded by Enugu state in West, Cross River in the East, in the South by Abia and Benue by the North and located between longitude 70 30IN and 60 45IE. Abakaliki which is the state capital has a tropical climate with average relative humidity of 74% and may reach 80% during the rainy season. The vegetation characteristics are predominantly rainforest with average atmospheric temperature of about 30degree. The state enjoys two distinct seasons, rainy (between April and October) and dry season (November and March) respectively. The state Capital and the largest town is Abakaliki. Other towns in the state are Afikpo, Ikwo, Izzi, Onicha, Edda, Onueke, Ezzamgbo, Nkalagu, Uburu, Ishiagu Amasiri and Okposi. Ebonyi local government residents speak Izzi language. The people are predominately of Igbo stock. The areas are traversed by streams and rivers which constitute the major source of water supply to some communities in the areas. Their major occupations are farming and trading as well as emerging civil servant class.

Plant Collection and Preparation

The leaf was collected from Ndidenyi Ishieke in Ebonyi Local Government of Ebonyi State, Nigeria. The leaf was identified by a botanist, Nwankwo O.E. from the Department of Applied Biology, Ebonyi State University Abakaliki, Nigeria. The fresh leaf was transported to the laboratory and ground to homogenous paste.

Extraction of Plant Materials

The freshly ground leaf paste was placed on a 2mm pore size mesh and pressed directly to extract the content, placed on flat surface and allowed to air dry.

Evaluation of Theragnostic Effects of *D. Rotundifolia* Laboratory Detection of *Y. Pestis* F1-Antigens

Proximate Analysis

The lipid, carbohydrate and protein were determined using standard methods.

Carbohydrate Determination

Carbohydrate was determined by preparing 80% phenol in distilled water. Fifty microlitres (50µl) of 80% phenol was added to 800µl of sample suspension in test-tube. The mixture was added 2ml of concentrated H_2SO_4 and heated in a water bath for 30minutes. Then, the absorbance was read at 480nm.

Protein Determination

One mililitre of the sample and standard solution was added to 0.5ml of Coomasie Brilliant Blue G250 (0.1g/l) and incubated for 5minutes at room temperature. The absorbance was read at 595nm.

Lipid Determination

Total lipid was determined by adding 1ml of diethyl ether to 0.1g of the sample and mixed to extract fats. Empty container was weighed (W_1) and container containing lipid extract was also weighed (W_2) and allowed to air dry which was reweighed as the weight of the sample (Ws). Therefore, percentage of the lipid was calculated using the formula; Total lipid W_1-W_2/WS*100.

Gas Chromatography/Mass Spectrometry (GCMS)

The bioactive compounds in extract were determined using Gas Chromatography Mass Spectrometry (Model 5975, Japan).

Statistical Analysis

The data generated were analyzed using simple percentages and two-way ANOVA. The ANOVA was performed using GenStat version 4 statistical software.

RESULTS

Nutritional and Bioactive Constituents of *D. Rotundifolia* Extract

The determination of total carbohydrate, protein and lipid content of the extract showed that protein percentage was the highest (3.79 mg/10 mg of the extract representing 37.9%), followed by carbohydrate (1.12mg/10mg of the extract representing 11.2%), whereas lipid was the least (0.99mg/10mg of the extract representing 9.9%). Other elements which were not determined in this study constituted 41% of the extract (Table 1).

Gas chromatography/mass spectrometry (GCMS) showed that there were seven (7) active compounds in the extract with varying molecular weights (Table 2).

Table 1: Proximate Analysis of *D. rotundifolia* Extract

Nutritional Contents	Quantity in 10mg/ml of extract	Percentage composition
Total Carbohydrates	1.12 mg	11.2%
Total Protein	3.79 mg	37.9%
Total Lipid	0.99 mg	9.9%
Others (N_2, Ca^{2+} etc)	4.1 mg	41%

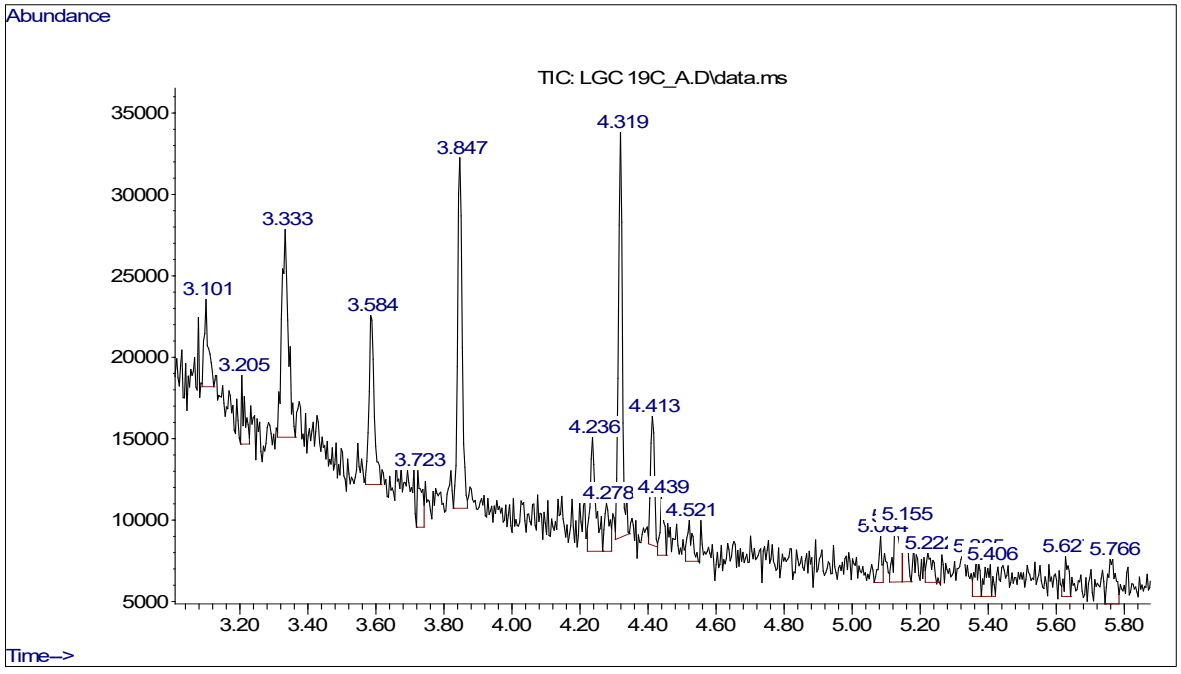

Figure 1

Table 2: Bioactive Compounds Identified in *D. rotundifolia* Direct Extract

MF-RMF	Probability %	Name of Compound	Molecular Weight	Molecular Formula
624-756	31.0%	Pyrrolidine,2-butyl-1-methyl	141	$C_9H_{19}N$
635-635	21.6%	1,8-Nonadien-ol**	140	$C_9H_{16}O$
625-673	41.9%	2,4-Dihydroxybenaldehyde,2TMS derivatives	282	$C_{13}H_{22}O_3Si_2$
708-830	7.8%	2,6-Dimethyldecane**	170	$C_{12}H_{26}$
594-629	21.1%	Heptane,2,2-dimethyl	128	C_9H_{20}
763-867	12.5%	Octane 3,5-dimethyl	142	$C_{10}H_{22}$
797-879	**25.7%**	3,7,11,15-tetramethylhexadecan-1,2,3-triolsilylated	341	$C_{15}H_{24}$

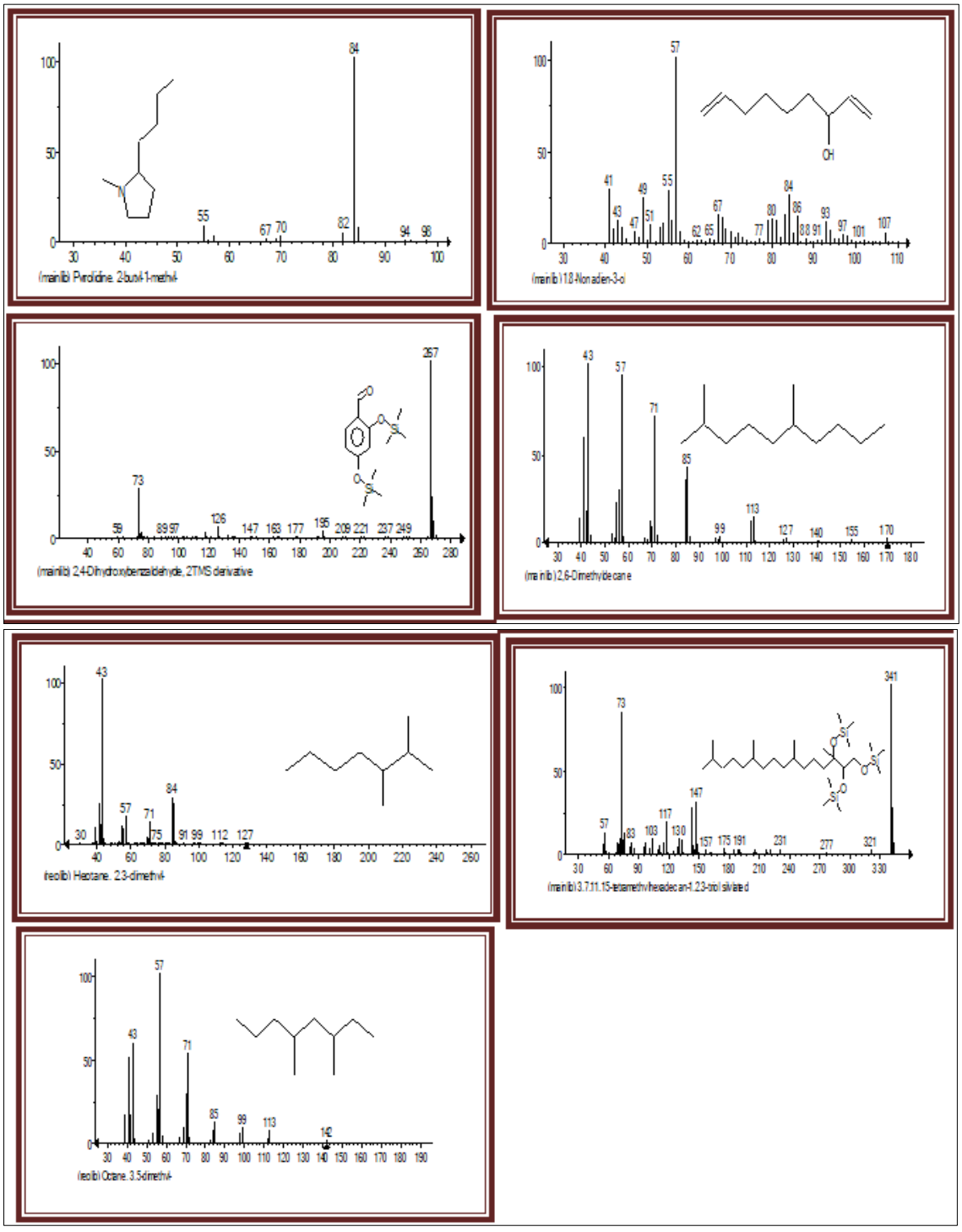

Figure 2

Table 3: Microbial contamination of Dissotis rotundifolia (Nsachi) Extract

Sample	No Examined	Days of Storage for Contamination Evaluation						Identified Organisms
		0	1	2	3	4	5	
Fresh Extract	25	**0**	**0**	**0**	0	M	H	*A. flavus*
Dry Extract	25	**0**	**0**	**0**	0	M	M	*A. niger*
Total	**50**							

DISCUSSION

Herbal medicine has been in use for several purposes including eye treatment and current research still show that it is still highly valuable (Sandhu *et al.,* 2011). However, studies have shown that problem of processing leads to the contamination of the extract, as well the aging (storage) can cause herbal medicines to impact negatively on human health (Sousa Lima *et al* 2020). In this study, *D. rotundifolia* 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 extract was contaminated with *Aspergillus niger* and *Aspergillus flavus,* with *A. niger* being the dominant contaminant (Table 1 and Table 2). This finding is in line with the report of Ronald and Jaap (2001), which showed that wide range of enzymes produced by *Aspergillus* spp are useful for the degradation of plant cell wall polysaccharides. It was also observed in this study that as the time of storage increased, the population of the contaminants also increased. The study also showed that no bacterial pathogen was isolated for the period of five days of storage of the extract ((Table 1). The absence of bacterial pathogens contaminating this extract is possibly due to antimicrobial compounds found in the extract such as pyrrolidine,2-butyl-1-methyl (Table 3).

The presence of inhibition zones with the application of extract after certain period of incubation reveals the level of antimicrobial activity of such 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. pneumonia*e among the bacterial species tested whereas *Candida* spp was highly susceptible to the extract (Figure 1). These different susceptibilities of microorganism to the *D. rotundifolia* extract maybe attributable to the chemical composition of the extract, certain specific internal factors of each test microorganisms and the environment (Desire *et al* 2016). However, *S. pneumoniae* and *P. aeruginosa* which appeared less sensitive may have some kind of additional resistant mechanisms such as enzymatic inactivation, target site modification (Desire *et al*., 2016). It was noticed that *D. rotundifolia* extract inhibited Gram positive and Gram negative bacterial strains with MIC ranging between 5mm (*S. pneumonia*) to 15.5mm (*Candida* spp) (Figure 2). The MIC and MBC ratio showed that direct extract of *D. rotundifolia* exhibited bacteriostatic and bactericidal effect on all the organisms tested. This finding is indicative of strong antimicrobial effect of the constituent of the extract and is consistent with the reports of Gill *et al* (1992), Abere *et al* (2010) and Olufemi *et al* (2014), which showed that *D. rotundifolia* extract is used in the treatment of various ailments caused by various bacterial pathogens, protozoa, and fungi.

Studies have shown that plant extracts contain several bioactive compounds with varied biological effects such as antimicrobial agents, antioxidants and anti-inflammatory agents (ref). In this study, GCMS analysis of the *D. rotundifolia* direct extract revealed the presence of seven bioactive compounds including pyrrolidine,2-butyl-1-methyl; 1,8-nonadien-3-ol; 2,4-dihydroxybenzaldehyde,2TMS derivative; 2,6-dimethyldecane; Heptane,2,2-dimethyl; Octane,3,5-dimethyl and 3,7,11,15-tetramethyhexadecan-1,2,3-triolsilylated (Table 3). Of these compounds identified, some studies have reported that pyrrolidine derivatives and 2,4-dihydroxybenzaldehyde,2TMS derivative have antimicrobial effects on microbes such as *S. aureus, E. coli* and *Candida albican* (Phaechamud *et al* 2012; Daniel *et al* 2020). Previous studies also showed that pyrrolidine derivatives can be used as antimycobacterial and antiviral agent as well for treatment of nervous and immune system diseases (Phaechamud *et al* 2012, Anna *et al* 2021). The antimicrobial activities as observed in this study is attributable to the presence of the compounds; 2,4-dihydroxybenzaldehyde,2TMS derivative and pyrrolidine,2-butyl-1-methyl in direct extract of *D. rotundifolia*.

The interview of the rural dwellers and herbalists revealed that the *D. rotundifolia* direct extract is commonly used for treatment of ocular diseases such as injuries and inflammations. Therefore, the presence of 2,4-dihydroxybenzaldehyde,2TMS derivative in direct extract of *D. rotundifolia* supports the information from rural settings that extract could be important in treating eye injury and inflammation, as previous study has reported the antioxidant and anti-inflammatory effects of the 2,4-dihydroxybenzaldehyde,2TMS derivative (Daniel *et al.,* 2020).

The presence of 2,6-dimethyldecane in plant extract has been shown to have toxic effects on animals; which is consistent with the report of WHO, 2016 that chronic exposure of animals to dioxins which is one of 2,6-dimethydecane derivatives has resulted in several types of cancer. A study conducted by Kessenikh *et al.,* (2021) has shown that heptane derivatives have genotoxic effects on bacterial cells leading to DNA damage and cell death. The findings of this study

revealed the presence of heptane,2,2-dimethyl in direct extract of *D. rotundifolia*. Hence, the presence of heptane,2,2-dimethyl is attributable to the antimicrobial effects of the extract and is in line with the findings of the study conducted by Kessenikh *et al.,* (2021) in which heptane,2,2-dimethyl derivatives was shown to have genotoxic effects causing bacterial death.

Conventionally, anti-inflammatory drugs are mostly steroid and lipid related compounds such as Betamethasone, Dexamethasone. In this study, the proximate analysis of *D. rotundifolia* direct extract revealed high presence of proteins and lipid compounds. It revealed 9.9% lipid related compounds and steroids being lipids indicate the potentials anti-inflammatory roles of the extract.

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