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PHYTOCHEMICAL AND INVITRO ANTIBACTERIAL ACTIVITIES OF THE CRUDE AND FRACTIONATED LEAF EXTRACTS OF *DISSOTIS THEIFOLIA* G. Don. (*MELASTOMATACEAE)*

James Eddy Simeon Idoko[1*], Deji Agboola M. A.[2], Femi-Oyewo M. N.[3], Oshiomah Kudirat Olabanjo[4] & Jolaosho Adeyemi Olufunmilayo[2]

[1.] Department of Clinical Pharmacy/Bio-Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Sagamu, Nigeria
[2.] Department of Medical Microbiology and Parasitology, Olabisi Onabanjo University, Sagamu Campus, Sagamu, Nigeria
[3.]Department of Pharmaceutics & Pharmaceutical Technology, Olabisi Onabanjo University, Sagamu, Nigeria
[4.]Department of Pharmacognosy, Olabisi Onabanjo University, Sagamu Campus, Sagamu, Nigeria
[5.]Department of Medical Microbiology and Parasitology, Olabisi Onabanjo University, Sagamu Campus, Sagamu, Nigeria
Email: christheroesj@yahoo.com

Abstract: The increasing prevalence of resistant microorganisms to orthodox antimicrobial agents is a public health problem worldwide. Also, the slow rate of development of new antimicrobial agents has resulted in intensive search for natural products with potent antimicrobial properties. Medicinal plants, extracts, powder and decoctions are used for the treatment of different human diseases to combat the resistant microorganisms with potentials to reduce adverse effects and less toxicities on host cells. Therefore, this study was carried out to determine the in-vitro antimicrobial activities of *Dissotis theifolia G.don.* (Melastomataceae) on clinical isolates. The leaves of *Dissotis theifolia* (M.) were extracted with 50% diethyl-ether, ethanol and methanol solvents, respectively. The photochemical screening of the leaf was carried out using standard procedures and the antimicrobial activities was evaluated using agar well diffusion method on pure clinical isolates and Ciprofloxacin (500mg) was used as the standard antibacterial agent. The photochemical screening revealed that the plant contains alkaloids, anthraquinones, combined anthraquinones, tannins, flavonoids, and cardiac glycosides. The antibacterial screening revealed that the diethyl-ether extract was more potent on the tested isolates than the ethanolic and methanolic extracts. All the tested organisms were sensitive to the diethyl-ether crude extracts. The most sensitive organism was E. *coli* wit 26.0mm zone of inhibition, followed by S.*aureus* (16.0mm). However, the ethanolic extract was resisted by the tested strains of E *coli* and that of methanol by E. *coli,* S.*aureus* and P.*vulgaris*. Furthermore, the chromatographic fractionation of the diethyl-ether extracts showed that the leaf extract has seven compounds. All the fractions exhibited antibacterial activity against all tested isolates with the most outstanding activity of 58.0 mm and 51.0 mm zones of inhibition on band one of the fraction against and S *aureus* respectively. In conclusion, the extracts of D. *theifolia* leaf possess antibacterial activities against both Gram positive and Gram negative bacteria. The plant could, therefore, be a potential source of antimicrobial agent.

Keywords: Antibacteria Activities, *Dissotis theifolia,* Chromatography, Fractionation, Organisms.

INTRODUCTION

Man is endowed by nature with primary sources for curing ailments (Oke and Hamburger, 2002) and with medicinal plants which have been utilized by our

fore- fathers to treat various diseases and has been with us since the beginning of mankind in preventing and curing illnesses (Claudia *et al.*, 2007). Natural products including plants, animals and minerals have been the basis of treatments of ailments of human diseases, as a result, the traditional uses of plants in the treatment of illnesses has a deep root in human history (Grabley and Thiercke, 1999).Alternative form of medical therapy using various plant parts has been flourishing for thousands of years. These forms are *Ayurveda*, *unani*, *kampo* and traditional Chinese medicine (Jamil *et. al.*, 2007). However, in Nigeria medicinal plants are not grown in farms but are rather sourced by people around bushes, road sides, regular wet areas, surface dumping sites, forests and mountains. The other sources where plant parts are obtained for medicinal and research purposes are markets and traditional herbal homes.

Dissotis theifolia is a tropical medicinal herb. Although, folkloric claims about the plant abound on its curative capacities in the treatment of infectious diseases, much work *has not* been carried out on it for scientific proofs. However, earlier works done on D. *theifolia,* by Odumegwu *et al* (2008) reported that the methanolic crude extract of the stem has activity against tested clinical isolates of *staphylococcus aureus* and three strains of *Psuedomonas aeriginosa* obtained from wounds and Ofokansi *et al (2007)* also reported that the incorporation of the crude leaf extract with some disk antibiotics has been found to have enhanced antibacterial activity of the preparation against clinical isolates from wounds.

PLANT COLLECTION, IDENTIFICATION AND EXTRACTION

The leaves was collected at dawn at Orokam farm, Ogbadibo LGA, Benue State, Nigeria and identified the Federal Research Institute OF Nigeria,(FRIN) where a herbarium sample was deposited. The dried leaves were grounded into powder using a blender (KAP T M32, 2000) to allow for solvent percolation in line with Sofowora*et.al.*, (*2001).*250g of the powdered leaf sample was soaked separately in 1.0 liters each of 50% diethyl ether [DE], 50% methanol [ME] and 50% ethanol [EE], respectively for 72 hours at room temperature. The bottles containing the extracts were shaken from time to time for even distribution of the extracting solvents through the powder for 72 hours. After 72 hours, the extracts were filtered according to Aiyegoro *et al. (2008)* with a slight modification. The filtrates were evaporated using rotary evaporator (NYC *R205D*, 2000). The extracts were transferred unto sterile petri dishes, covered and sealed with foil papers and kept in a refrigerator.

ORGANISMS

The test organisms were clinical isolates collected from the Medical Microbiology and Parasitology Department of Olabisi Onabanjo University Teaching Hospital (OOUTH) and Emabel Research Laboratory, Sagamu, Ogun State, Nigeria.

The isolates were identified using Gram stain reactions and biochemically using analytical Profile index (API) and reconfirmed to be Escherichia *coli*, *Pseudomonas aeruginosa, Staphyloccus aureus*, proteus *vulgaris* and *Klebsiella pneumonaea.* They were collected on sterile Nutrient agar slant and incubated at 37^0C for 24 hours ad were kept as stock on Nutrient agar slant in the refrigerator at 4^0C.

PHYTOCHEMICAL ANALYSIS

The ethanolic, methanolic and diethyl ether extracts were tested for the presence of tannins, alkaloids, flavonoids, saponins, anthraquinones, saponins, and cardiac glycosides using standard procedures in line with Adedayo *et al* (2001).

CHROMATOGRAPHY

Thin layer chromatography (TLC) pre coated papers were caught into 20cm by 2cm sizes made of flexible plates of TLC Whattman, Germany. The Diethyl Ether crude extracts of the leaf extract was dissolved in the extraction solvent into paste and spotted on the TLC papers and allowed to dry. The spotted plates were kept in a developing tank made up of methanol, ethanol and diethyl ether in the ratio of 2:2:1 while different analysts ascended the plates. The TLC result was used as a guide into the fractionation. The leaf extract was dissolved in excess mixture of methanol, ethanol and diethyl ether solvent in the ratio of 2:2:1 and introduced into the column loaded with fine silica gel and allowed to elute, then collected immediately after each separation into flexible cork vial bottles and kept. The different fractions were evaporated on the rotary evaporator at controlled temperature of 40^0C.

ANTIBACTERIAL ACTIVITIES

Mueller Hinton Agar plates (diameter 9cm) were inoculated by flooding with 1ml of the standardized test inoculums, swirled and excess was carefully decanted into discard jar. A sterile core borer was used to bore wells (6mm in diameter) on each agar plate. The plates were left on the bench for 1hour to allow for proper diffusion (*NCCLS*, 1990). All incubations were done at 37^0C for 24 hours. The antibacterial activity of both extracts and the standard drug were determined by measuring the zones of inhibitions round each well excluding the core borer diameter.

MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC)

A loopful of the organisms previously diluted to 0.5 McFarland turbidity standard was introduced into a tube containing 0.5ml of varying concentrations of the extracts prepared by serial dilution and 2ml of Mueller Hinton Broth was added in duplicate form. This procedure was repeated on the test inoculums using the standard antibiotic (*Ciprofloxacin*) as the positive control and a tube containing Mueller Hinton Broth only was seeded with test organisms as above as negative control. All the broth was then incubated at 37^0C for 24 hours. After incubation, the tubes were examined for turbidity. The smallest concentration that inhibits the growth was taken as the minimum inhibitory concentration [MIC].For the determination of the MBC, the tubes from the MIC experiment above which did not show any visible growth was inoculated on sterile Mueller Hilton agar by streaking. The plates were incubated at 37^0C for 24 hours. The concentration at which no visible growth was noticed was taken as the Minimum Bactericidal Concentration (MBC].

RESULTS

PHYTOCHEMICAL SCREENING

The phytochemical screening of the three extracts showed the presence of alkaloids, tannins, flavonoids, cardiac glycosides and anthraqinones.

ANTIBACTERIAL ACTIVITIES

The antibacterial sensitivity test results indicate that both the crude extracts and fractions of D. ethyl ether has antibacterial activities on the tested organisms while the strains of E. coli and S. *aureus* screened were resistant to the methanolic and ethanolic extracts. The results are presented in tables' 1 – 6 respctively.

DISCUSSION

The result of the phytochemical screening of *Dissotis theifolia* indicates the presence of alkaloids, cardiac glycosides, tannins, anthraquinones, combined anthaqinones and flavonoids. Earlier works done on secondary metabolites of other medicinal plants have shown that secondary metabolites obtained from plants possess several biological activities including antimicrobial activities (Soforowa, 1993). The flavonoids are recognized for their anti-oxidant property as well as its role in modifying the body's reaction to allergies, viruses and carcinogens has also been reported (Ekam and Ebong, 2007). Also, the result of the antibacteriaal activities of D. *theifolia* is in consonance with some earlier works done on the plant. Ofokansi *et al.*; (2007) reported that the incorporation of the crude leaf extracts of D. *theifolia* into antibiotics disk showed enhanced activity against tested bacteria infiltrating wounds. Also, Odumegwu *et al.*; (2008)

reported on the antibacterial activity of the methanolic crude extract of the D. *theifolia* stem against clinical isolates of *Staphylococcus aureues* and three strains of *Pseudomonas auriginosa* obtained from wounds. Furthermore, both Gram positive and Gram negative bacteria were inhibited by *Dissotis theifolia.* This attribute could suggest that the plant possesses broad spectrum activities. The enhanced antimicrobial activity of the different fractions on the tested isolates revealed the impact of the chromatographic separation the crude extracts.

The findings of this study show that the plant based therapy may just be the lasting panacea to the problem of multidrug resistance. The phytochemical screening revealed quite a number of secondary metabolites. Extraction solvents used in the extraction also showed the role solvents play in extraction of active compounds in a natural product and by extension, their activity on microbes. The leaf extracts of *Dissotis theifolia* in this research work exhibited a broad spectrum activity against the tested organisms indicating that the plant contain potential compounds needed for standardization, purification, modification and development into a drug to fight micro-organisms. Also, it could be concluded that the findings of this study uphold the folkloric claims that D. *theifolia* has antimicrobial activity.

Further works need to be carried on the plant toxicologically at animal level to ascertain its safety in a biologic system and structurally to understand the compound(s) exhibiting the antibacterial activities.

LIST OF TABLES

Table 1: Percentage Weight Yield (%)

Solvent	
Diethylether	12.5
Methanol	15.0
Ethanol	15.0

Table 2: Phytochemical Screening of D. *Theifolia*

Phytochemical Constituents	Status
Alkaloids	+
Anthraquinones	+
Combined Anthraquinones	+
Cardiac	+
Glycosides	+
Flavonoids Taninns	+

Key: + = Present, - = Absent

Table 3: Antibacterial Activities of the Crude Extracts (zones of inhibitions in mm/mg, mean ± SD, Pvalue = 0.10)

SE	EC	SA	PV	PA	KP
DE	26.0 ± 0.00	16.0 ± 2.00	12.0 ± 1.01	12.0 ± 1.01	14.0 ± 0.70
EE	0.00 ± 3.00	13.0 ± 0.00	14.0 ± 0.00	12.0 ± 1.00	05.0 ± 6.00
MT	0.00 ± 0.00	0.00 ± 0.00	00.0 ± 0.00	20.0 ± 00.00	22.0 ± 0.00
A	53.0 ± 0.00	50.0 ± 0.00	48.0 ± 0.00	36.0 ± 0.01	15.0 ± 2.00
B	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 4: Antibacterial Activities of the Fractions

Bands	EC	SA	PV	PA	KP
1	28.0	58.0	27.0	56.0	38.0
2	26.2	26.4	26.4	27.1	33.0
3	26.0	31.0	20.2	21.8	38.0
4	26.2	27.6	27.1	27.0	38.9
5	27.2	27.6	27.1	27.1	28.0
6	42.0	28.0	27.0	27.1	28.0
7	30.3	26.3	19.4``	19.4	31.0
FSC	0.00	0.00	0.00	0.00	0.00

Table 5: Minimum Inhibitory Concentration of the Fractions in mg/ml

Organisms	B_1	B_2	B3	B4	B_5	B_6	B_7
E. *coli*	155	310	155	310	310	9.6	77.5
P.*vulgaris*	38.8	310	77.5	77.5	310	38.8	155
K. *pneumonae*	155	155	155	38.8	155	77.5	155
Staph. Aureus	77.5	310	310	310	310	38.8	310
p.aeuriginosa	77.5	155	38.5	155	310	310	310

Key: B_1-B_7 Stands for Fractions 1 to 7

Table 6: Minimum Bactericidal Concentration (MBC) in mg/ml

Organism	B_1	B_2	B_3	B_4	B_5	B_6	B_7
E. *coli*	310	>310	310	310	>310	38.6	310
P.*vulgaris*	155	>310	155	155	>310	155	>310
K.*pneumonae*	310	>310	>310	77.5	>310	155	>310
Staph. Aureus	310	>310	>310	>310	>310	77.5	310
p. aeuriginosa	155	310	77.5	310	>310	>310	310

Key: SE = Extraction solvent, DE = diethyl ether, EE = ethanol, MT = methanol, B = band of fraction, EC = *Escherichia coli,* SA = *Staphylococcus aureus,* PV = *proteus vulgaris*, PA = *Pseudomonas auriginosa,* KP = *Klebseila puenmonae,Ciprofloxacin/Fluconazole,* B= Negative control (50% diethyl ether, ethanol and methanol respectively, FSC = Solvent combination (50% ethanol, methanol and di ethyl ether in ratio 2:2:1)

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Several aspects of life to man as observed in human medicine, veterinary medicine, agriculture, Pharmacognosy, ethno botany, etc. Ethno pharmacologic use of plants are gaining popularity among Nigerian native people and Scientists conscientious activities is doing everything possible within their powers to establish the antimicrobial activity are yielding positive results (Olukoya *et al.*, 1986; Perumal and Ignacitnuthu, 2001; Adedayo *et al.*, 2001; Ndukwe *et al.*, 2005). There is need for the discovery of new antimicrobial agents with different spectral of activities , mechanisms of actions, chemical structures, little toxicity to host cells, to oppose the rapid wide spread antimicrobial resistance to clinically use antimicrobial and newly introduced antimicrobial agents (Coates *et al.*, 2002; Bajpal *et al.*, 2005; Singh *et al.*, 2010). As a result of the above, Pharmaceutical companies are in high demand for medicinal plants and as such researches, developmental programmes and strategies are in place to encourage the striving for the novel drugs (Ogunnusi and Dosumu, 2008, Funari and Ferro, 2005).

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