JOPAT Vol. 18(2) 304 - 313, July – December 2019 Edition.
ISSN 2636 – 5448

Chemical Composition and Antibacterial Activity of the Essential Oil of *Dysphania ambrosioides* (L.) Mosyakin & Clemants from North Central Nigeria

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ABSTRACT

The chemical composition of the essential oil of *Dysphania ambrosioides* and the antibacterial properties of the oil against two clinical isolates and two typed isolates (one each of Gram positive and Gram negative bacteria) of *Bacillus subtilis*, *Staphylococcus aureus* ATCC 25923; *Escherichia coli*, *Pseudomonas aeruginosa* ATCC 27853 was studied. The essential oil was extracted from the fresh leaves of *D. ambrosioides* with the aid of a Clevenger apparatus by hydrodistillation after which the chemical composition of the oil was analyzed using the Shimadzu GCMS QP2010 plus. The minimum inhibitory concentration (MIC) of the oil against *E. coli*, *S. aureus*, *B. subtilis* and *P. aureginosa* as well as and the zones of inhibition compared with standard antibiotics was determined. The GC-MS analysis revealed a total of twenty (20) compounds in the oil, with alpha-terpinene (48.68%), o-Cymene (21.71%) and trans-beta-terpinyl butanoate (17.15%), ascaridole (5.67%), gamma-terpinene (2.16%) and D-limonene (1.83%) being the major compounds identified. The oil was active against *E. coli*, *S. aureus*, *P. aureginosa* and *B. subtilis* at MIC ranging from 10 to 20 µL/mL. The zones of inhibition revealed that the oil has a good microbial efficacy at 200µL/mL (ranging from 27.5 to 30mm) against *E. coli*, *S. aureus*, *B. subtilis* which is comparable to ciprofloxacin, better than vancomycin, ampicillin and amoxicillin/clavulanic acid, and a relatively lower activity against *P. aeruginosa* (11mm). The oil was rich in compounds that possessed antimicrobial activity.

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INTRODUCTION

Dysphania ambrosioides (Chenopodioideae-Amaranthaceae) is an annual or short lived perennial plant known as Indian worm seed, sweet pig weed, Mexican or Jesuit's tea (English) leaf of excreter- "ewe imi" or arunpale in Yoruba and "Ebigben-suigben in Edo state. It is an aromatic, more or less pubescent leafy plant with a prostrated branched stem with glandular trichomes secreting essential oils.

The leaves vary from narrowly elliptical to elliptical and are green, oblong-lanceolate and serrated with flowers that are small and green occurring in dense terminal panicles of glomerules, each with five sepals [13]. Sepals are usually 3-5 and might be partially or totally united. The fruits and seeds are black and horizontal and enclosed in persistent calyx which is usually less than 0.8mm long. Sepals bear stamens (3-5) which might be free or have adnate filaments [3, 14].

The use of *D. ambroisoides* in traditional medicine spans the continents of South America, North America, Asia and Africa where its uses include: treatment of digestive problems, hangovers, colds, coughs, contusions, digestive sluggishness, diabetes, diarrhea, dyspepsia, falls, flu, fractures, gastric disorders, hemorrhoids, hemorrhages, increasing perspiration, indigestion, intestinal gas, intestinal parasites [23, 2].

Dysphania ambrosioides is rich in flavonoids and terpenoid compounds particularly the toxic and pungent ascaridole, monoterpene and monoterpene derivatives. *D. ambrosioides* essential oil, popularly known for its anthelminthic properties hence its name *Chenopodium ambrosioides* var. *anthelminticum* is now accepted as *Dysphania anthelmintica* attributable to ascaridole [17]. Pollack [22] observed that ascaridole inhibited the growth of *Plasmodium falciparum* in vitro, and that cineol, which is a very similar compound lacking the internal 1,4 peroxide was inactive. Artemisinin, on the other hand, which like ascaridole contains a 1,4endoperoxide, was active against *Plasmodium falciparum*. These observations prompted to conclude that the endoperoxide in ascaridole was essential for its anti-malarial activity. Chekem [7] reported the *in vitro* and *in vivo* anti-fungal activities of *D. ambrosioides* essential oil against *Candida* species. Other activities include sedative and pain-relieving properties due to the presence of ascaridole [4], anti-Helicobacter pylori activity [15] comparable to the combination of lansoprasole, clarithromycin and metronidazole in the elimination of *Helicobacter pylori*. Da Silva [11] reported the acute and sub-chronic toxicity of *D. ambrosioides* leaf aqueous extract by gavage on rats. *D. ambrosioides* has also found usefulness as a natural pesticide. The aim of the study is to determine the antibacterial activity of the essential oil of *D. ambrosioides* and identify new chemotypes if available from the essential oil of *D. ambrosioides* collected from Niger state, north central Nigeria.

MATERIALS AND METHODS

Collection and Extraction

D. ambrosioides leaves were collected from Chaza Niger state in January, 2018 and authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD) herbarium with voucher number NIPRD/H/6976. 200g of fresh leaf of *D. ambrosioides* was chopped into smaller pieces and transferred into a round bottom flask; thereafter the essential oil was isolated by hydro-distillation using a Clevenger type apparatus. 0.5mL of a golden yellow essential oil was obtained, dried over anhydrous sodium sulphate and stored in sealed vials at 4ºC until analysis [10].

Gas Chromatography- Mass Spectrometry Analysis

The essential oil was analyzed by using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (scan range= 45-400 amuelectron energy = 70 eV), and scan rate = 3.99 scans/sec], and Shimadzu GC-MS solution data system. The GC column was Optima-5 ms fused silica capillary with a (5% phenyl)-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 mL/min. The program used for GC oven temperature was 60 - 180°C at a rate of 10°C/min, then held at 180°C for 2 minute, followed by 180 -280°C at a rate of 15°C/min, then again held at 280°C for 4 minutes. The injection port temperature was 250°C while detector temperature was 280°C. Diluted sample (1/100 in hexane, v/v) of 1.0 µL was injected using autosampler and in the split mode with ratio of 10:90. The analysis was performed in triplicates. Individual constituents were identified by referring to compounds known in the literature, and also by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 08). The percentages of each component are reported as raw percentages based on the total ion current without standardization [19].

Antimicrobial Susceptibility Testing

Microorganisms were obtained from the Department of Microbiology and Biotechnology at the National Institute for Pharmaceutical Research and Development (NIPRD). Organisms tested include one typed isolate and one clinical strain each: Gram-positive microorganisms such as *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*, and Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* ATCC 27853.

The antibacterial activity of essential oil of *Dysphania* was determined using the agar diffusion method. A suspension of an overnight culture of each test microorganism in normal saline was standardized to 0.5 McFarland turbidity (10^5cfu/mL). About 1 mL of the standardized inoculum was added to 24 mL of molten Mueller Hinton agar, transferred into sterile petri dishes and allowed to set. Wells were made using a sterile cork borer (diameter= 6mm) into the seeded Mueller Hinton agar plates. The base of each well was sealed with two drops of Mueller Hinton agar and allowed to set. Each well was filled with 100 µL of different concentrations of essential oil and the negative control (20 % dimethyl-sulfoxide). The plates were allowed to stand for 1 hour. A freshly prepared Mueller-Hinton agar was also incubated to assess the sterility of the media before inoculation (Medium sterility control), while the organisms were also inoculated on Mueller-Hinton agar without addition of test agent to confirm the viability of the organisms (Organism viability control) [12].The results were interpreted using the interpretation criteria published by Clinical and Laboratory Standards Institute [1].

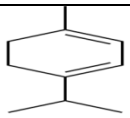
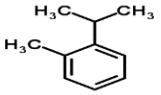
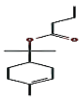
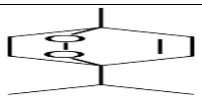
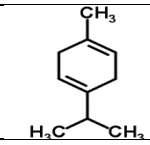
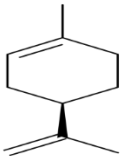
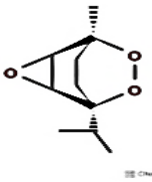
Determination of Minimum Inhibitory Concentration

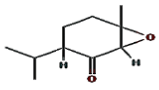
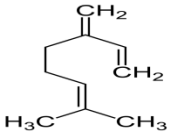
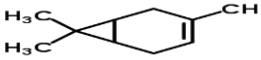
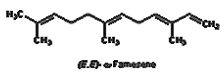
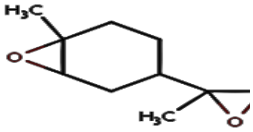
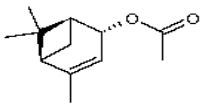
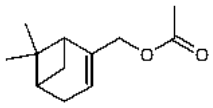
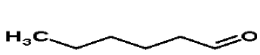
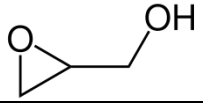
Antimicrobial activity of the essential oil against bacteria was determined using the Microbroth dilution technique in 96 multi-well plates, in triplicates. Mueller-Hinton broth was used. The plant essential oil was diluted in dimethyl sulfoxide (DMSO) in a concentration of (1 in 10) and with DMSO as negative control and then further diluted in Mueller-Hinton broth to reach a final concentration of 20µL/mL. The assay plates used were in a 8 x 12 format. One hundred microliters of the diluted

essential oil was added to the first well, 50µL of broth medium was added to wells 2-12. Two-fold dilution was made by transferring 50µL from the first to the 10th well. Fifty microliters of DMSO was introduced into the 11th well. Fifty microliter of inoculum was transferred from the 1st to the 12th well. Plates were incubated for 24 h at 37 °C. As an indicator of bacterial growth, a 50µL of a solution of three phenyltetrazoliumchloride (TTC, 5 mg/mL-1) was added to the wells and incubated at 37 °C for 30 minutes. The lowest concentration of the essential oil showing no growth was taken as its minimal inhibitory concentration (MIC). The yellow color tetrazolium salt acts as an electron acceptor and was reduced to a red-colored formazan product by biologically active organisms.

RESULTS

Table 1: GC-MS analysis of *Dysphania ambrosioides* Essential oil

Compound	Chemical structure	Molecular weight (g/mol)	Area (%)	Retention time
Alpha-Terpinene		**136.238**	**48.68**	**4.555**
o-Cymene		**134.222**	**21.71**	**4.648**
Trans-beta-Terpinylbutanoate		**224.344**	**17.15**	**7.778**
Ascaridole		**168.23**	**5.67**	**8.725**
Gamma-Terpinene		**136.238**	**2.16**	**5.105**
D-Limonene		**136.24**	**1.83**	**4.702**
Ascaridole epoxide		**184.232**	**0.83**	**8.024**

Piperitone oxide		**168.236**	**0.37**	**7.981**
Nonanal		**142.238**	**0.32**	**5.705**
Terpinolene		**136.23**	**0.24**	**5.539**
Beta-Myrcene		**136.23**	**0.20**	**4.147**
3-Carene		**136.24**	**0.18**	**4.457**
Alpha-Farnesene		**204.357**	**0.14**	**9.087**
Alpha-limonene diepoxide		**152.237**	**0.13**	**7.545**
(-)-trans pinocarvyl acetate		**194.274**	**0.09**	**5.995**
Myrtenyl acetate		**194.274**	**0.07**	**6.244**
Hexanal		**100.16**	**0.06**	**4.297**
Glycidol		**74.08**	**0.06**	**3.535**

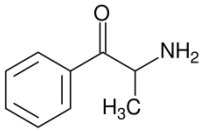
Cathione		**149.19**	**0.03**	**3.813**

Table 2: Determination of Minimum Inhibitory Concentration (MIC) of *Dysphania ambrosioides* essential oil

Test Organism	Minimum Inhibitory Concentration (µL/mL)
Escherichia coli	**10.0**
Staphylococcus aureus ATCC 25923	**10.0**
Pseudomonas aeruginosa ATCC 27853	**20.0**
Bacillus subtilis	**20.0**

Table 3: Antibacterial Activity of *Dysphania ambrosioides* Essential oil

Tested Organisms	Zones of Inhibition (mm)			
	200µL/mL	100µL/mL	10µL/mL	DMSO
Escherichia coli	**27.5**	**14**	**7.5**	**–**
Staphylococcus aureus ATCC 25923	**30**	**9**	**–**	**–**
Pseudomonas aeruginosa ATCC 27853	**11**	**8**	**–**	**–**
Bacillus subtilis	**27.5**	**13**	**7**	**–**

Table 4: Zones of Inhibition Produced by Standard Antibiotics against Test Organisms

Tested Organisms	Zones of Inhibition (mm)						
	VA	AMP	AMC	CIP	CTX	C	GN
Escherichia coli	**0 (R)**	**0 (R)**	**0 (R)**	**31 (S)**	**0 (R)**	**29 (S)**	**25 (S)**
Staphylococcus aureus ATCC 25923	**15 (R)**	**0 (R)**	**0 (R)**	**21 (S)**	**0 (R)**	**24 (S)**	**22 (S)**
Pseudomonas aeruginosa ATCC 27853	**0 (R)**	**0 (R)**	**0 (R)**	**25 (S)**	**0 (R)**	**10 (S)**	**17.5 (S)**
Bacillus subtilis	**12 (R)**	**0 (R)**	**0 (R)**	**20 (S)**	**0 (R)**	**18 (S)**	**23 (S)**

KEY; VA- Vancomycin (30 µg), AMP-Ampicillin (10 µg), CIP- Ciprofloxacin (5 µg), CTX- Co-trimoxazole (25 µg), C- Chloramphenicol (30 µg), GN- Gentamicin (30 µg), R- Resistant, S-Susceptible

DISCUSSION

The essential oil from fresh leaf of *D. ambrosioides* collected from Niger state has a golden yellow appearance with an aromatic odour. The GC-MS analysis revealed a total of 20 compounds in the essential oil of *D. ambrosioides*. The main constituents of the oil were alpha-terpinene (48.68%), o-cymene (21.71%) and trans-beta-terpinylbutanoate (17.15%). Ascaridole, a monocyclic monoterpene, popular for its anthelmintic, antirheumatic and antimicrobial properties, has a concentration of 5.67%.Besides it, the others compound having concentrations above 1% were gamma-Terpinene (2.16%) and D-limonene (1.83%). Owolabi [21] reported the following constituent's alpha-terpinene (63.1%), p-cymene (26.4%) and ascaridole (3.9%) as major; while Onocha [20] reported alpha-terpinene (56%), alpha-terpinylacetate (15.7%), p-cymene (15.5%). However the concentration of ascaridole (5.67%) reported in this study is greater than previous studies by Owolabi [21] (3.9%). The concentrations of ascaridole in the essential oil in this study was significantly lower than those of *D. ambrosioides* oil from Madagascar with ascaridole (41.8%), Cavalli [6], Brazil with ascaridole (61.4%) Jardim [16], China with ascaridole (29.7%) Chu [8], and Cuba with ascaridole (30.5-47.1%) Monzote [18]. Seasonal variations, geographical variation, time of collection, collection from a wild or cultivated source are factors known to affect the concentration of constituents of essential oils. Ascaridole is also known to be a heat sensitive compound as it undergoes partial thermal isomerization to isoascaridole in GC [5].

The antimicrobial assay revealed that *D. ambrosioides* essential oil from Northern Nigeria has potent antimicrobial activity when compared to Standard antibiotics. Ascaridole, cymene, among other oxygenated terpenoids which are mainly esters and oxides such as ascaridole epoxide, limonene diepoxide are constituents believed to be responsible for the observed antimicrobial activities. Oxygenated terpenoids such as alcoholic and phenolic terpenes have been reported to have more antimicrobial activity than the other constituents [16, 26, and 27].The minimum inhibitory concentrations (MICs) of *D. ambrosioides* essential oil that inhibited the growth of the bacteria in this study are listed in Table 3. The essential oil exhibited antibacterial activity at the 100-200 µL/mL, inhibiting the growth of Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*P. aeruginosa* and *E. coli*) bacteria with lower concentrations (10 µL/mL) against *E. coli* and *B. subtilis*. Some literatures state that Gram negative bacteria are less sensitive than Gram positive bacteria, due to its rich polysaccharide cell wall content which reduces the degree to which antimicrobial agents are absorbed (24). From this study however, both gram negative and positive bacteria exhibited similar sensitivities i.e. 10 and 20 µL/mL. The results in this study differ from results from Owolabi [18] who reported no activity against *S. aureus* and *E. coli*. Santiago [24] reported the antibacterial properties of *D. ambrosioides* essential oil against *S. aureus* and *E. coli* although with higher MICs than reported in this study. Differences in the concentrations of the major constituents present in the essential oil could result in the differences in antibacterial activities observed. Factors that affect antibacterial properties of essential oils according to Santiago [24] include isomerism, synergistic properties of constituents and lipophilicity of compounds.

CONCLUSION

The essential oil of fresh leaf of *D. ambrosioides* collected from Northern Nigeria contained alpha-terpinene (48.68%), o-cymene (21.71%), trans-beta-terpinylbutanoate (17.15%) and ascaridole (5.67%); and exhibited antimicrobial activity against both gram-positive and gram-negative bacteria specifically *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

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