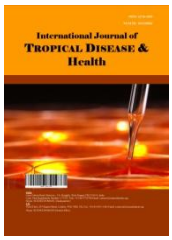

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Phytochemicals and Comparative antimicrobial activities of *D. velutinum*, *S. nodiflora*, and Honey against Micro-Organism of Public Health Importance

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Antimicrobial resistance is a global public health challenge with numerous implications. A growing number of infections are becoming harder to treat due to resistance to the antibiotics used to treat infectious diseases leading to multidrug resistant strains as typically seen in staphylococcus aureus. Microbial resistance to antibiotics has been on the increase leading to increased infection therefore this study evaluated the antimicrobial profile of *D. velutinum*, *S. nodiflora* and honey against the multidrug resistant *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella spp*, *Salmonella typhi*, *Staphylococcus aureus* and a fungus; *Candida albicans* which causes different diseases leading to public health implications using standard laboratory procedures.
The result showed the presence of alkaloids, glycosides, flavonoids, tannins, phlobatanins, saponins, proteins, carbohydrates and anthocyanins across the three samples (*D. velutinum*, *S.*

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nodiflora and honey). The result from the findings showed a dose dependent activity against the selected organisms. It showed that *D. velutinum* had the best activity against *P. aeruginosa, Shigella* spp and *C. albicans* which implied that *D. velutinum* could be harnessed for the treatment of urinary and joint infections caused by P. aeruginosa and other infections such as shigellosis and candidiasis. The findings showed that *S. nodiflora* has the best minimum inhibition concentration of 1 against *E. coli* and *S. typhi* respectively.
This finding also, suggests that *S. nodiflora* can be harnessed as an alternative treatment of infectious diseases caused by *S. aureus*, *E. coli* and *S. typhi* and therefore we hereby conclude that plant-based drugs remain an alternative source of therapeutic agents against multidrug resistant micro-organism of public health importance.

Keywords: Antimicrobial; S. nodiflora; D. velutinum; honey; inhibition zone diameter; minimum inhibition concentration; phytoconstituents.

1. INTRODUCTION

Antimicrobial resistance is a global public health challenge with numerous implications including economic, biological and social implications. This is because it can affect every: age, sex, country, tribe, and continent. Antibiotic resistance is a global health issue [1] hence the reason why it is regarded as the biggest threats to global health, food security, and development [1]. A growing number of infections are becoming harder to treat as the antibiotics used to treat them become less effective due to resistance to antibiotics [2]. This concern has led to search for alternative antimicrobial potent therapeutic agents for treatment of emergent microbial resistance to different micro-organism that with high therapeutic efficacy, reduced cost, available and acceptability.

The use of plant for the treatment of infection that affects humans and animal cannot be traced to its origin because man has since creation depended on herbs for cultural, aesthetics and therapeutic use from the time immemorial till the present day. Naturally plants contained chemicals that are adaptation for their survival against numerous pathogens and predators in nature. These phytochemicals are of public health importance due to their applications in treatment of diseases for plants, animals and man.

Search for Natural antibiotics with little or no side effects and great antimicrobial therapeutic activity necessitated this study in our quest for a treatment alternative with high efficacy.

This study evaluated the antimicrobial profile of *D. velutinum*, *S. nodiflora* and honey against the following: *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella spp*, *Salmonella typhi*, *Staphylococcus aureus* and a fungus; *Candida albicans* which causes different diseases that affects man; therefore, this study has public health implications.

Desmodium velutinum is perennial semi-erect or erect shrub traditionally used for the treatment aches, pains, diarrhoea and preparation with haematuria [3]. It is also used as diuretic, laxative and for treatment of cough, and fever. Previous studies have reported that it is rich in phytochemicals such as tannins, resins, glycosides, saponins and flavonoids [3].

Synedrella nodiflora (L.) Gaertn (Asteraceae) is a small, annual weed of cultivation native to America, found in as invasive weed in farmlands. The leaves of Synedrella nodiflora are used as pollutice for sore rheumatism and juice of the leaves is used for earache. [5]. The aerial part is reported to have insecticidal activity against *Sapodeptera latura* [4]. The chemical analysis reported the presence of alkaloids, flavonoids, flavonols saponins triterpenes [6]. Recent study has shown that *S. nodiflora* reported to have strong antioxidant and antiulcer activity when combined with honey [7].

Honey has been used as food and is regarded as a product of bees' activities in metabolism of plant nectar and therefore contained enzymes and plant chemicals. Honey has been locally used for the treatment of wounds by the people of South eastern Nigeria. The major component of honey is usually sugar and water with traces proteins, enzymes, and nonessential amino acids and enzymes with strong antiseptic property [8-10].

Honey usually have acidic pH of 3.2 to 4.5 therefore can inhibit many pathogenic organisms leading to increased wound healing process through epithelization [11-14]. Honey is also one

of the supersaturated solutions that inhibit bacterial growth primarily due to this high osmolarity and has been reported to be active for wound healing [15].

This study evaluated the antimicrobial Potential of *D. velutinum, S. nodiflora*, and Honey on selected (*Pseudomonas aeruginosa*, *Escherichia coli, Shigella* spp, *Salmonella typhi*, *Staphylococcus aureus* and a *fungus*; *Candida albicans* microbes of public health importance.

2. MATERIALS AND METHODS

2.1 Collection/ Identification of Plant Leaves

Fresh leaves of *D. velutinum* and *S. nodiflora* were collected from its habitat in Awka, Awka South Local Government Area, and Anambra State around July 2018 and was properly identified by a plant taxonomist Mr. Felix Nwafor at the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. A voucher number of PCG/UNN/0336/Fabaceae and PCG/UNN/0338 Asteraceae was obtained. The plant leaves were air dried at room temperature for 14 days, and dry leaves were pulverized to a fine powder.

2.2 Honey Collection

Honey used in this study is a multifloral honey collected from Nsukka Nigeria. Trees around the apiary include almond tree, *Meligna arboreal* tree, *Azadiracta indica* tree etc. Nsuka honey is one of the best honeys used clinically for the treatment of wounds.

2.3 Extraction of Plant Leaf

The plant was extracted with absolute methanol by cold maceration method for 74-hour sand filtrate was concentrated to dryness using the rotary evaporator at 36^0C.

2.4 Micro-Organisms

The test organisms used in this work were collected from the Department Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical sciences Nnamdi Azikiwe University Awka. These organisms were further reconfirmed by subculturing and subjecting pure isolates to specific pure culture identifications techniques. These were standard laboratory cultures whose susceptibility on commonly used antibiotics was already established and were confirmed to be resistant to antibiotics. *Staphylococcus aureus* represent Gram positive bacteria while *Escherichial coli, Pseudomonas aeruginosa, and Salmonella typhi* represents Gram negative bacteria.

2.5 Phytochemical Screening

The Phytochemical analysis was conducted using the methods of [16-20]. The procedures for phytochemical screening were as follows.

2.6 Test for Carbohydrate

Molisch's test (general test): take 2 ml of extract, add two drops of α-naphthol solution in the alcohol and shake, and then add five drops of concentrated H_2SO_4 to the sides of the test tube to observe violet ring at the junction of two liquids.

2.7 Reducing Sugar Test

Fehling's test: in a test tube, add 1 ml Fehling's A and 1 ml Fehling's B solutions and mix and boil for 1 min. Add equal volume (2 ml) of test solution. Heat in boiling water bath for 5 min. Observe for yellow and then brick red precipitate.

2.8 Test for Protein

Millon's test (for proteins): add 2 ml of TS and mix with 4 ml of Millon's reagent; observe for white precipitate. Precipitate if warm, turns brick red or precipitate dissolves giving red color.

2.9 Test for Terpenoids

Salkowski test was used to detect terpenoids. Extract (5 ml) was mixed with chloroform (2 ml), and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish-brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

2.10 Test for Glycoside

Baljet's test: add a test solution with 1 ml of sodium picrate and observe for yellow to orange color.

2.11 Test for Phlobatannins (HCl Test)

Two millilitres (2 mL) of the aqueous solution of the extract were added into dilute HCl and observed for red precipitate that was indicative the presence of phlobatanins.

2.12 Test for Anthraquinone

Borntrager's test: To perform Borntrager's test, 0.1gm of the powdered drug was boiled with 5 ml of 10% Sulphuric acid for 2 minutes. A pink to red colour will confirm the presence of glycoside.

2.13 Tests for Saponin

Foam test: the extract was mixed with water and shaken vigorously. Persistent foam was observed.

2.14 Tests for Anthraquinone Glycosides

Borntrager's test: to 3 ml extract, add dil. H2SO4. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.

2.15 Tests for Flavonoids

Ferric chloride test: to 2 ml of test solution, add few drops of ferric chloride solution, which shows intense green color.

2.16 Tests for Alkaloids

Dragendorff's test: the test solution treated with Dragendoff's reagent (potassium bismuth iodide); reddish brown precipitate was not obtained.

2.17 Tests for Tannins

To 3 ml of extract, add few drops of the following reagents:

5% $FeCl_3$ solution shows deep blue-black coloration. Also add 3 drops of lead acetate solution showed white precipitate.

2.18 Test for Steroids

Liebermann-Burchard reaction: add 2 ml extract with 2 ml of chloroform and add 1–2 ml acetic anhydride and 2 drops of concentration H_2SO_4 from the side of test tube. Observe for first red, then blue, and finally green color.

2.19 Preparation of Extract for Antimicrobial Evaluation

The stock concentrations of each of the extract and honey sample were made by weighing 32 mg /ml each of crude extract and honey respectively into a sterile beaker. Then 2 ml of Dimethyl sulfoxide (organic diluent) was added into each of the samples and reconstituted properly by vortexing. This gives a stock concentration of 16 mg/mL of each extract, thereafter two fold serial-dilutions were made from each of the stock concentrations to get graded concentrations of 8 mg/mL, 4 mg/mL, 2 mg/mL, 1 mg/mL and 0.5 mg/mL for each of the crude extract and honey.

2.20 Antibiotic Susceptibility Testing

Antibiotic susceptibility of pure cultures of the confirmed isolates were performed on diagnostic sensitivity test agar (Mueller Hinton Agar) according to the Kirby Bauer disc diffusion method as described by [21]. The culture media was prepared and each isolate was inoculated into 3 mL sterile nutrient broths in test tubes and incubated at 37 ^{0}C for 24 hours, and was made to match with the 0.5 McFarland turbidity standard. The Mueller Hinton Agar used was prepared based on the manufacturer's specification. It was sterilized by autoclaving, poured into sterile petri dishes and allowed to solidify. These standard inoculums were swabbed under aseptic conditions, on the solidified Mueller Hinton Agar plates and were allowed to soak for about 5 minutes. After this, the antibiotic disks were aseptically placed on the surface of the media and pressed down gently to lap on the culture media. The plates were incubated at 37 ^{0}C for 24 hours. At the end of 24 hours, the inhibition zones of each antibiotic against each isolate were measured and recorded as Inhibition Zone Diameter (IZD). This was performed in duplicates for each antibiotic per isolate and the average value was obtained.

2.21 Determination of Antimicrobial Activity

The antibacterial assay for the crude extract and honey was carried out using the agar well diffusion assay as described by [21] with slight modifications. The antimicrobial activities of the extracts of the plants under study were tested against four standard human pathogenic bacteria species namely *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi*, *Shigella spp* and one pathogenic fungi species namely *Candida albicans*. These were standard laboratory cultures whose susceptibility on commonly used antibiotics was already established. *Staphylococcus aureus* represent Gram positive bacteria while *Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi* represents

Gram negative bacteria. The bacterial suspensions were adjusted to 0.5 McFarland turbidity standard and inoculated onto previously sterilized Mueller-Hinton Agar plates (diameter: 90 mm) while the standardized fungi cultures were inoculated onto Malt Extract Agar plates. A sterile cork borer of eight-millimeter (8 mm in diameter) diameter was used to make five wells on each of the MHA and MEA plates. Aliquots of 80 µl of each extract dilutions, reconstituted in DMSO at concentrations of 8, 4, 2, 1 and 0.5 mg/mL, were applied in each of the wells in the culture plates previously seeded with the test organisms. Ciprofloxacin single disc (10 µg) and miconazole (50 µg/mL) served as the positive controls against the bacteria and fungi organisms respectively. The cultures were incubated at 37 ^{0}C for 24 h (for bacteria) and 25 ^{0}C for 48 h (for fungi) plates respectively. The antimicrobial potential for each extract was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). For the crude and fractions, three replicates were conducted against each organism. Each of the samples was tested against all the test isolates.

2.22 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in triplicate Petri dishes. Here, agar dilution method was adopted. Stock solutions of 16 mg/ml of the various extracts were prepared. Then, two-fold serial dilutions were made to get 8, 4, 2, 1 and 0.5 mg/mL thereafter 2-fold dilutions of each of the concentration was made using 9 mL sterile molten agar this was allowed to solidify. The microbial inoculums which has been standardized to 0.5 McFarland turbidity is streaked on the agar appropriately. The plates are incubated at 37 and 25 ^{0}C for 24 and 48 hours for the bacteria and fungi plates. After incubation the plates were examined for microbial growth by checking for growths using a plus sign (+) indicating growth while a negative sign (-) indicates no growth. * indicates no MIC carried out because there was no antibacterial activity.

2.23 Analysis of Data

All the quantitative data was analyzed using statistical package for social science (SPSS) version 20.0 using one-way analysis of variance (ANOVA). Data were expressed as mean ± standard error of the mean and the means further separated using turkey post-hoc test and Duncan multiple range test to show differences between groups.

3. RESULTS

3.1 Phytochemical Constituents

The result of the qualitative phytochemicals is presented in Table 1. It showed that all the phytochemicals tested (Alkaloids, Glycosides, Flavonoids, Tannins, Phlobatanins, Saponins, Proteins, Carbohydrates and Anthocyanins, Anthraquinones, terpenoids, reducing sugar and steroids) were present in honey but varied across the two plants (*D. velutinum,* and *S. nodiflora*). The result showed the presence of Alkaloids, Glycosides, Flavonoids, Tannins, Phlobatanins, Saponins, Proteins, Carbohydrates and Anthocyanins across the two plants *D. velutinum,* and *S. nodiflora.* While anthraquinones and steroids were absent in *D. velutinum* and *S. nodiflora*. The result further showed that terpenoids was present in *D. velutinum* but absent in *S. nodiflora*. The result further showed the presence of reducing sugar in *S nodiflora* but was absent in *D. velutinum*.

3.2 Antimicrobial Profile

The result of the antimicrobial of *D. velutinum* and *S. nodiflora* and Honey are presented in Table 2. The study was carried out at low concentrations of 8, 4, 2, 1 and 0.5mg/ml *D. velutinum*, *S. nodiflora,* and Honey. The study showed that there was a significant difference ($P<0.05$) in antimicrobial activity between the treatment and control across all the treatment. It revealed that the standard drug had high significant inhibition zone diameter when compared with all the treatment doses of 8, 4, 2, 1 and 0.5mg/ml. The result of the study is presented as follows:

3.2.1 Bactericidal effect against *S. aureus*

The result of the bactericidal effect of *D. velutinum* and *S. nodiflora* and honey against *S. aureus* is presented in Table 2. The study showed that *S. nodiflora* (12.0cm) had the highest diameter of inhibition at 8mg/ml while honey and *D. velutinum* had no activity and this was significantly lower when compared with the standard drug with inhibition diameter of 30 cm.

Table 1. Phytoconstituents of *D. velutinum*, *S. nodiflora*, and *Honey*

SN	**Bioactive compound**	***D. velutinum***	***S. nodiflora***	***Honey***
1	Alkaloids	Present	Present	Present
2	Glycosides	Present	Present	Present
3	Flavonoids	Present	Present	Present
4	Tannins	Present	Present	Present
5	Phlobatanins	Present	Present	Present
6	Saponin	Present	Present	Present
7	Steroid	**Absent**	**Absent**	Present
8	Proteins	Present	Present	Present
9	Terpenoids	Present	**Absent**	Present
10	Carbohydrates	Present	Present	Present
11	Anthraquinones	**Absent**	**Absent**	Present
12	Anthocyanins	Present	Present	Present
13	Reducing Sugar	**Absent**	Present	Present

The study further showed that at 4, 2, 1 and 0.5mg/ml, no inhibition activity was seen in *D. velutinum*, *S. nodiflora*, and Honey. This showed that *S. nodiflora* had the best bactericidal activity against *S. aureus* when compared with honey and *D. velutinum.*

3.2.2 Bactericidal effect against *E. coli*

The result of the bactericidal effect against *E. coli* is presented Table 2. The study showed *D. velutinum*, *S. nodiflora*, and honey had 12.0cm inhibition zone diameter respectively and they were significantly lower when compared with the standard drug with inhibition zone diameter of 18cm. Also, at 4mg/ml the *S. nodiflora* had 12.0cm inhibition zone diameter while honey had inhibition zone diameter of 10.0mg/ml, whereas *D. velutinum* had no activity at 4mg/ml. Also, at 2mg/ml, *S. nodiflora* had inhibition zone diameter of 11.0cm and this was significantly lower when compared with the standard drug with inhibition zone diameter of 18.0cm while honey and *D. velutinum* had no activity. Also, the study showed that at 1.0mg/ml, *S. nodiflora* had 10.5 inhibition zone diameter and they were significantly lower when compared with the control with 18.0 inhibition zone diameter. There was zero inhibition at 0.5mg/ml across all the treatments.

3.2.3 Bactericidal effect against *P. aeruginosa*

The result of the bactericidal effect against *P. aeruginosa* is presented in Table 2. The study showed that *D. velutinum* had 13.0cm (the highest IZD among the treatments) diameter of inhibition at 8mg/ml followed by honey with inhibition zone diameter of 12.0 cm and they were all significantly lower when compared with the standard drug with inhibition zone diameter of 26.0 while *S. nodiflora* had zero inhibition zone diameter. Also, at 4mg/ml *D. velutinum* had 12.0cm while *S. nodiflora* and honey had zero inhibition zone diameter. At 2 and 1 mg/ml and 0.5 mg/ml no inhibition was recorded by all the treatments (*D. velutinum*, *S. nodiflora* and honey had zero inhibition at 2 and 1 mg/ml).

3.2.4 Bactericidal effect against *S. typhi*

The result of the bactericidal effect against *S. typhi* is presented in Table 2. The study showed that *S. nodiflora* had 13cm (the highest IZD among the treatments) diameter of inhibition at 8 mg/ml followed by honey with inhibition zone diameter of 12.0 and they were all significantly lower when compared with the standard drug with inhibition zone diameter of 29.5cm while *D. velutinum* had zero inhibition zone diameter respectively across all the treatment dosage (8, 4, 2, 1 and 0.5mg/ml respectively). The study further showed that at 4 and 2 mg/ml, *S. nodiflora* and honey had 12.5 and 11.5 inhibition zone diameter respectively. The study further showed that at 1 and 0.5 mg/ml that *S. nodiflora* and honey had zero IZD while *S. nodiflora* had 11.5 and 10. 0 inhibition zone diameter respectively while homey had zero IZD. This implied that *S. nodiflora* is very useful medicinal plant for the control of typhoid which is dose dependent and therefore should be exploited for the treatment of typhoid and other related illness caused by *S. typhi.*

3.2.5 Bactericidal effect against *Shigella spp*

The result of the bactericidal effect against *Shigella spp* is presented in Table 2. The study showed that *D. velutinum* had 15cm (the highest IZD among the treatments) diameter of inhibition

at 8mg/ml while honey had 13.0 cm and they were all significantly lower when compared with the standard drug with inhibition zone diameter of 34.5cm. *S. nodiflora* had zero inhibition zone diameter respectively across all the treatment dosage (8,4, 2, 1 and 0.5mg/ml respectively). The study further showed that at 4 and 2 mg/ml, *D. velutinum* had 14.0 and 12.0 inhibition zone diameter. Also, at 1mg/ml *D. velutinum* had 11.0 cm inhibition zones diameters. Also. at 0.5mg/ml all the treatment had zero IZD respectively. This implied that *D. velutinum* and honey had a dose dependent activity against *Shigella spp* but *D. velutinum* had activities till a low concentration of 1 mg/ml and therefore should be taken for treatment of diseases caused by *Shigella spp*.

3.2.6 Fungicidal effect against *C. albicans*

The result of the fungicidal effect against *C. albicans* is presented in Table 2. The study showed that *D. velutinum* and honey with inhibition zone diameter of 15.0 and 14.5 respectively and they were all significantly higher when compared with the standard drug with inhibition zone diameter of 14.0 cm while *S. nodiflora* had zero inhibition zone diameter respectively across all the treatment dosage (8, 4, 2, 1 and 0.5mg/ml respectively). The study further showed that at 4 and 2 mg/ml *D. velutinum* had 14.0 and 13.0 IZD while honey had 12.5 and zero inhibition zone diameters. The study further showed that at 1and 0.5mg/ml that *D. velutinum* had 12.0 and 14.0 IZD respectively while honey had zero inhibition zone diameters at 1 and 0.5 mg/ml respectively. The findings suggest that *D. velutinum* had the highest zone of inhibition against the fungi *C. albicans* and therefore should be harnessed for topicals, suppository and oral therapy for the treatment of candidiasis.

3.3 Mini mum Inhibition Concentration of *D. velutinum, S. nodiflora* and Honey

The Result of the minimum inhibition concentration was presented in Table 3. The result of the findings showed the following:

3.3.1 Minimum inhibition concentration against *S. aureus*

The crude extracts of *D. velutinum* had less than 8mg/ml minimum inhibition concentration against *S. aureus*, *S. nodiflora* had 8 mg/ml, while honey had less than 8 mg/ml minimum inhibition concentration against *S. aureus* (Table 3).

3.3.2 Minimum inhibition concentration against *E. coli*

The crude extracts of *D. velutinum* and honey had 8 mg/ml minimum inhibition concentration against *E. coli* respectively while *S. Nodiflora* had 1 mg/ml minimum inhibition concentration against *E. coli* (Table 3).

3.3.3 Minimum inhibition concentration against *P. aeruginosa*

The crude extracts of *D. velutinum* had 2 mg/ml minimum inhibition concentration against *P. aeruginosa*, *S. nodiflora* had less than 8 mg/ml, while honey had 8 mg/ml minimum inhibition concentration against P. aeruginosa (Table 3)**.**

3.3.4 Minimum inhibition concentration against *S. typhi*

The crude extracts of *D. Velutinum* had less than 8 mg/ml minimum inhibition concentration against *S. typhi*, *S. nodiflora* had 1 mg/ml, while honey had 2 mg/ml minimum inhibition concentration *S. typhi* (Table 3).

3.3.5 Minimum inhibition concentration against *Shigella spp*

The crude extracts of *D. velutinum* had 1 mg/ml minimum inhibition concentration against *Shigella spp*, *S. nodiflora* had less than 8 mg/ml, while honey had 8 mg/ml minimum inhibition concentration against *Shigella spp* (Table 3).

3.3.6 Minimum inhibition concentration against *C. albicans*

The crude extracts of *D. velutinum* had 0.5 mg/ml minimum inhibition concentration against *C. albicans, S. nodiflora* had less than 8 mg/ml, while honey had 4 mg/ml minimum inhibition concentration against *C. albicans* spp (Table 3).

4. DISCUSSION

The findings on the phytoconstituents of S nodiflora is similar to the findings of Hossain et al., [22]. The phytochemicals in *D. velutinum* is similar to the findings of Nkwocha et al., [23] which showed abundance alkaloids, saponin, flavonoids, and tannins. It is reported that the phenolic compounds have been proven to have proven effective in inhibiting the growth and biofilms formation by pathogenic bacteria [24] and this could be as a result of polyvalent action

Table 2. Antimicrobial potentials of *D. velutinum*, *S. nodiflora*, and honey

Plants	8 mg/ml	4 mg/ml	2 mg/ml	1 mg/ml	0.5 mg/ml	mico 50 µg	DMSO	Organisms
D. Velutinum	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	30±1.12b	0.0±0.0a	*S. aureus*
S. nodiflora	12±0.0	10±0.0	0.0±0.0	0.0±0.0	0.0±0.0	30±1.12	0.0±0.0a	
Honey	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	30±1.12	0.0±0.0a	
D. Velutinum	12.0±0.0e	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	18.0±0.0g	0.0±0.0a	*E. coli*
S. nodiflora	12.0±0.0e	12.0±0.0e	11.0±0.0cd	10.5±0.7bc	0.0±0.0a	18.0±0.0g	0.0±0.0a	
Honey	12.0±0.0e	10.0±0.0 c	0.0±0.0a	0.0±0.0a	0.0±0.0a	18.0±0.0g	0.0±0.0a	
D. Velutinum	13.0±0.0b	12.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	26.0±0.0	0.0±0.0a	*P. aeruginosa*
S. nodiflora	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	26.0±0.0	0.0±0.0a	
Honey	12.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	26.0±0.0	0.0±0.0a	
D. Velutinum	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	29.5±0.0f	0.0±0.0a	*S. typhi*
S. nodiflora	13.0±1.0e	12.5±0.5de	11.5±0.5cd	11.5±0.5cd	10.0±0.0b	29.5±0.0f	0.0±0.0a	
Honey	12.0±0.0cde	11.50±0.5cd	11.0±0.0c	0.0±0.0a	0.0±0.0a	29.5±0.0f	0.0±0.0a	
D. Velutinum	15.0±0.0g	14.0±0.0f	12.0±0.0d	11.0±0.0bc	0.0±0.0a	34.5±0.5h	0.0±0.0a	*Shigella* spp
S. nodiflora	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	34.5±0.5h	0.0±0.0a	
Honey	11.50bc	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	34.5±0.5h	0.0±0.0a	
D. Velutinum	15.0±0.0e	14.0±0.0d	13.0±0.0c	12.0±0.0b	14.0±0.0d	14.0±0.0d	0.0±0.0a	*C. albicans*
S. nodiflora	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	
Honey	14.5±0.5de	12.5±0.5bc	0.0±0.0a	0.0±0.0a	0.0±0.0a	14.0±0.0d	0.0±0.0a	

(NB: Means having a common alphabet are not statistically different at the 5% level of significance)

Table 3. Result of MIC of *D. velutinum, M. oppositifolius, S. nodiflora* and Honey

Test orgs.	Extracts / MIC (mg/mL)		
	D. velutinum	***S. nodiflora***	**Honey**
S. aureus	>8	8	>8
E. coli	8	1	8
P. aeruginosa	2	>8	8
S. typhi.	>8	1	2
Shigella spp	1	>8	8
C. albicans	0.5	>8	4

of these abundant flavonoids, tannins and alkaloids. The presence of phenolics in these plants could be the reason why this plant showed antiulcer and antioxidant activity as reported by Onyeka et al. [25]. The research showed that *S. nodiflora*, *D. velutinum* and honey contained tannins, flavonoids and alkaloids and this phytochemical are known for anti-microbial properties. Alkaloids are well known for their antimicrobial properties [26]. Also, high content of saponins and tannins in samples of the extracts and honey are be considered as the basis for its antimicrobial property as previous claims stated that saponin and tannin rich plants have profound antimicrobial activity [27]. The antimicrobial effects of the crude extract of M. oppostifollous showed that this could be as a result of polyvalent action of flavonoids, tannins and alkaloids which are known phenolics with antibacterial activity. Phenolic compounds have been proven to have proven effective in inhibiting the growth and biofilms formation by pathogenic bacteria [28-31].

This research findings showed that the leaf methanol extract of *S. nodiflora* had the best activity against *S. aureus* at a dose dependent rate and this implied that the herb could be harnessed for the treatment of Staphylococcus aureus one of the medically important bacterial infections in humans that causes a variety of diseases in humans which includes gastroenteritis, meningitis, toxic shock syndrome, urinary tract infections and pulmonary infections [32]. The pathogen has adapted different mechanism used for blocking the chemotaxis of leukocytes, production of antiphagocytic agents, and sequestering host antibodies [33-34] leading to resistance to chemotherapy.

Also, the research findings showed that *S. nodiflora* had the best activity against *E. coli* a Gram-negative and rod-shaped facultative anaerobic bacterium notable for diseases such as severe anaemia, kidney failure and urinary tract infection and also causes heamolytic uraemic syndrome (HUS), especially in the young, and the elderly [35].

The findings further showed that *S. nodiflora* had the best activity against *S. typhi* a rod-shaped gram-negative bacterium that causes typhoid fever [36-37] leading to an annual mortality rate of about 200, 000 out of 30 million infection cases globally [38].

Also, the study showed that *D. velutinum* had the best activity against *P. aeruginosa, Shigella spp* and *C. albicans* respectively and this implied that this plant could be harnessed for the treatment of urinary and joint infections caused by P. aeruginosa and other infections such as shigellosis and candidiasis.

5. CONCLUSION

The findings from this study has public health implications. The finding suggests that *S. nodiflora* can be harnessed as an alternative treatment of infectious diseases caused by *S. aureus*, *E. coli* and *S. typhi* such as urinary tract infections, pulmonary infections and typhoid while *D. velutinum* could be harnessed for the treatment of diseases caused by *P. aeruginosa, Shigella spp* and *C. albicans* therefore we hereby conclude that plant based drugs remain an alternative source of therapeutic agents against multidrug resistant micro-organism of public health importance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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