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METABOLIC SYNDROME: *IN VITRO* ANTIOXIDANT, ANTICANCER, ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITIES OF *DISSOTIS ROTUNDIFOLIA* (SM.) WITH PHYTOCHEMICAL AND NUTRITIONAL COMPONENTS

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Abstract

Background: *Dissotis rotundifolia* (Sm.) Triana is a prostrate herb of the family Melastomataceae. It is used for the treatment of obesity, hypertension, circulatory problems and metabolic syndrome (MS). This study evaluated the antioxidant, anticancer, anti-inflammatory.and antimicrobial activities of the whole plant of *D. rotundifolia* to validate its therapeutic potential in the management and treatment of MS.
Materials and Methods: The plant was screened for proximate, mineral, vitamin and phytochemical components, as well as antioxidant activities using standard laboratory techniques. The anticancer activities of the ethanol plant extract were against kidney (VERO) and breast (MCF-7) cancer cells. The anti-inflammatory activity of the extract was determined using nitric oxide production inhibition assay and the antimicrobial activity was evaluated against five pathogenic organisms. All data were subjected to statistical analysis.
Results: The plant was rich in carbohydrate (47.87%), protein (22.5%), crude fibre (9.57%), Na (206.67 mg/100 g), Ca (183.33 mg/100 g), vitamin C (25.53 mg/100 g), flavonoids (685.00 mg/100 g), alkaloids (616.67 mg/100 g) and saponins (471.67 mg/100 g) contents. The extract exhibited considerable and varied antioxidant activities. The plant extract was very active against VERO and MCF-7 cells at low concentrations (0.145 mg/ml and 0.141 mg/ml respectively) compared to doxorubicin. It also exhibited significant anti-inflammatory action against lipopolysaccharide (LPS)-stimulated RAW macrophage. The extract was active against all test organisms. Overall, *D. rotundifolia* displayed significant antioxidant, anticancer, anti-inflammatory and antimicrobial activities.
Conclusion: The observed bioactivities of the plant could be attributed to its phytocontents and are indications of its potential as an agent for the management and treatment of metabolic syndrome.

Keywords: *Dissotis rotundifolia*, obesity, oxidative stress, inflammation, cytotoxicity, anti-infective.

Abbreviations: MS - Metabolic Syndrome, MCF-7 - Human breast adenocarcinoma cells, VERO - African green monkey kidney cells, LPS – Lipopolysaccharide, RAW – macrophage cell line, NASH - Non-Alcoholic Steato-Hepatitis, DPPH - 2, 2, diphenyl-1-picryl hydrazyl, ABTS - 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), MTT - 3- (4, 5- dimethlthiazol – 2 - yl) -2, 5 –

diphenyltetrazolium bromide, DMEM - Dulbecco's Modified Eargle Media, PBS - Phosphate Buffer Saline, FBS - Fetal Bovine Serum, DMSO – Dimethyl sulfoxide, ATCC – American Type Culture Collection, INT - 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride, MIC – Minimum Inhibitory Concentration.

Introduction

Dissotis rotundifolia (Sm.) Triana (family Melastomataceae) is native to West Africa commonly referred to as Pink Lady (English), Ebafo (Bini), and Awede (Yoruba). The decoction of the whole plant is used to cure rheumatism and yaws. The decoction of the leaves is given to relieve stomachache and diarrhoea in children; it is also used as a vermifuge and as a cure for dysentery. The leaves are slightly warmed in hot ash and put on a boil to hasten suppuration (Gill, 1992). The whole plant is used as an astringent and as a cure for cough, bronchitis, sinusitis, conjunctivitis, circulatory problems and venereal diseases. It forms part of the recipe for the management of obesity, hypertension, rheumatism and circulatory problems (Baba and Onanuga, 2011). The literature is replete with information on the antiplasmodial, antitrypanosomal, antibacterial, antidiarrhoeal, anti-ulcer and antioxidant activity of *D. rotundifolia*, as well as its ovarian and hormonal effects. Also, data from toxicity studies established the relative safety of the plant extract in administration (Yeboah and Osafo, 2017).

Traditionally, *D. rotundifolia* forms part of the recipe used for the management of metabolic syndrome. Metabolic syndrome (MS) is a cluster of conditions that increase the risk of heart disease, stroke, and diabetes. The risk factors of MS are inflammation, oxidative stress, diabetes, hypertension, pro-thrombotic state, low HDL-C (high-density lipoprotein cholesterol), high triglyceride and abdominal adiposity. The diseases that are associated with MS include neurological disorders, Polycystic Ovary Syndrome (PCOS), cancer, cardiovascular disease, type II diabetes, stroke and Non-Alcoholic Steato-Hepatitis (NASH) (Mendrick *et al*., 2018). Studies have shown that there are now clear interactions in particular between gut microbiota as well as chronic bacterial and viral infections; and various aspects of MS such as obesity and inflammation (Sommer and Sweeney, 2010).

Medicinal plants are naturally enriched with vitamins, minerals and phytochemical constituents (Wang *et al*., 2018; Roy *et al*., 2022). Vitamins are essential micronutrients for cellular metabolism and their deficiencies result in diseases. In addition to their role in nutritional function, vitamins are increasingly being recognized as modulators of gene expression and signals transduction when consumed at pharmacological concentrations (Aguilera-Mendez *et al*., 2022). Calcium and phosphorus play an important role in protein synthesis. Calcium is involved in the regulation of nerve impulse transmission, muscle contraction and blood pressure while phosphorus is involved in maintaining homeostasis and acid-base balance (Park and Han, 2021). Flavonoids are secondary metabolites from plants that have been proven to have anti-inflammatory, antioxidant, anticancer, anti-microbial, hepatoprotective, cardioprotective nephroproprotective and renoprotective activities (Wang *et al.,* 2022; Sahraee *et al.,* 2022). The clustering of metabolic abnormality is closely related to oxidative stress and inflammation, as well as the progression of atherosclerosis. Antioxidants are reducing agents such as flavonoids, vitamin C, vitamin E, carotenoids, resveratrol and selenium which are found in food sources, medicinal plants, or in supplement formulations. Antioxidants exhibit the oxidation of other molecules and can be used not only to prevent but to treat health complications of MS and atherosclerosis (Gregório *et al*., 2016).

Given the traditional claim of the potency of *D. rotundifolia* whole plant in the prevention and treatment of MS, coupled with the scarcity of information on its anti-inflammatory and anticancer effects, this study examined the chemical, nutritional and mineral components of the plant. Also, the antioxidants, anticancer, anti-inflammatory and antimicrobial activities of *D. rotundifolia* were evaluated to provide scientific information on its therapeutic potential as an agent for the management and treatment of MS.

Materials and Methods

Collection and identification of *Dissotis rotundifolia*

Dissotis rotundifolia whole plants were collected from a farm at Erunmu, Oyo State, Nigeria. The plant sample was identified by a Curator and deposited at the Herbarium at the University of Ibadan, Ibadan, Nigeria with voucher no. UIH 22906.

Preparation of plant extract of *Dissotis rotundifolia* whole plant

The sample was washed, cut into smaller pieces and air dried at room temperature (27^oC – 30^oC) for four weeks. The sample was powdered and 1000g of the powder was extracted in 10 L of 95% ethanol (analytical grade) for two weeks using cold extraction method. The extract was concentrated at 40^oC using a rotary evaporator and stored in the refrigerator (4^oC) before experimental use.

Proximate analysis of powdered *Dissotis rotundifolia* whole plant

The proximate contents of the sample were determined using standard methods of analysis as described in the ASEAN manual of food analysis (ASEAN, 2011). The ash content was verified using a muffle furnace set at 550 ^oC for 4 hours until a constant weight of ash was obtained. The crude fat was determined by Soxhlet's extraction method using petroleum ether as solvent. The crude protein content of the sample was determined using the micro-Kjeldahl technique. The enzymatic-gravimetric method was used for the determination of crude fibre. The moisture content of the sample was determined using the oven (Gallenkamp) method at 105 ^oC. The carbohydrate content was calculated by subtracting the sum values of the other nutrients from 100 (ASEAN, 2011).

Mineral analysis of powdered *Dissotis rotundifolia* whole plant

The methods described in ASEAN (2011) were used for the digestion of the plant sample. After digestion, calcium (Ca), magnesium (Mg), sodium (Na), zinc (Zn) and iron (Fe) were analysed using Atomic Absorption Spectrophotometer. Phosphorous was

determined using Vanadomolybdate (Yellow method). The percentage transmittance was determined at 400nm using Spectronic 20 Colorimeter (AOAC, 2005).

Vitamin analysis of powdered *Dissotis rotundifolia* whole plant

The protocols described in ASEAN (2011) were used to determine the concentration of vitamins in the sample. Vitamins B1 (Thiamine) and B2 (Riboflavin) were determined using the fluorometric method. Vitamin B3 (Niacin) was determined using a high pressure liquid chromatography method. Vitamin C (Ascorbic acid) was determined using a micro-fluorometric technique. Vitamin A (Carotenoids) was determined using the method of Larsen and Christensen (2005).

Phytochemical screening of powdered whole plant of *Dissotis rotundifolia*

The powdered sample was analysed for the presence of secondary metabolites using quantitative screening methods. The sample was screened for the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycosides and steroids (Sun *et al*. 1998; AOAC, 2005; Harborne, 2005; Ordon-Ez *et al*. 2006).

Antioxidant analysis of *Dissotis rotundifolia* whole plant

The radical scavenging ability of the sample was tested against DPPH (2, 2, diphenyl-1-picryl hydrazyl) radicals (Xiao *et al*., 2020). The ferric ion-reducing power was measured using the methods Katalinic and Chaves (Katalinic *et al*. 2006; Chaves *et al*., 2020). ABTS (2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) scavenging activity of the plant sample was evaluated using the method of Marc *et al*. (2004). The ability of the sample to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Sroka and Cisowski (2003).

***In vitro* anticancer assay of *Dissotis rotundifolia* whole plant**

The anticancer activity of the ethanol extract of *Dissotis rotundifolia* whole plant was tested using the 3- (4, 5- dimethlthiazol – 2 - yl) -2, 5 – diphenyltetrazolium bromide (MTT) assay according to Mosman (1983) with modifications on African green monkey kidney cells (Vero) and the human breast adenocarcinoma cells (MCF – 7). Briefly, the MCF-7 cells were cultured in Dulbecco's Modified Eargle Media (DMEM) (Pan, Separations Scientific) supplemented with 10% Fetal Bovine Serum (FBS) (Pan, Biochom-Africa) and 1% of 100 IUnits/mL penicillin and 100 µg/L streptomycin (Pan, Celtics diagnostic) while the vero cells were cultured in Minimal Essential Medium (MEM) supplemented with 1% gentamycin. Upon confluence, the cells were harvested and seeded 1 x 10^4 in all the wells except the first column of the 96 well plates (Nest, Whitehead scientific) over 24 hours in a humidified incubator with 5% CO_2 at 37^{o}C. Thereafter, the medium was removed and replaced with fresh medium diluted in different concentrations of the extracts (12.5, 25, 50 & 100 mg/mL) and incubated over 48 hours. After 48 hours, the medium was removed and cells in the plate washed with 200 µL of warm Phosphate Buffer Saline (PBS) and then 100 µL of fresh medium was added with 30 µL MTT (stock solution of 5 mg/mL in PBS, Inqaba Biotec) into each well and the plates were further incubated for 4 h at 37 ^{o}C. After 4 hours, the medium with MTT was removed carefully and MTT formazan crystals dissolved by adding 50 µL DMSO to each well. The plates were shaken gently until the amount of MTT reduction was measured immediately by detecting absorbance using a microplate reader (Biotek, SynergyHT, Analytical & Diagnostic Products CC, Johannesburg, South Africa) at a wavelength of 540nm in a micro-titre plate reader. The wells in the first column containing only medium and no cells were used to blank the plate reader. Viability of cells in percentages was calculated using the formula: % Viability = ((Sample Absorbance/control Absorbance) × 100). The experiments were performed in triplicate and repeated three times. Doxorubicin was used as a positive control. A dose-response curve was plotted to enable the calculation of the concentrations that kill 50% of the Vero/ MCF-7 cells (C_{50}) as previously reported by McGaw *et al*. (2007).

Selectivity Index (SI)

The selectivity index (SI) was calculated by dividing the LC_{50} of the tested sample in normal cells by the LC_{50} of cancer cells. The SI values were calculated by applying the formula: SI = LC_{50} normal cell/ LC_{50} cancer cell.

Nitric oxide (NO) production inhibition assay in RAW 264.7 macrophages

Culturing of cells and sample testing: Macrophages were cultured in 75cm^2 flasks in Dulbecco's Modified Eagle's Medium (DMEM; Lonza, South Africa) containing L-glutamine supplemented with 10% fetal bovine serum (FBS; Gibco, Sigma-Aldrich, South Africa) and 1% penicillin/streptomycin/fungizone (PSF; Sigma-Aldrich, South Africa) at 37°C with 5% CO_2. When confluence was reached, the cells were seeded 4 × 10^5 cells/mL in 96-well plates and incubated overnight at 37°C with 5% CO_2 to allow for attachment. Thereafter the medium was removed and replaced with fresh medium, then the cells were activated by addition of 1 µg/mL of lipopolysaccharide (LPS; Sigma-Aldrich, South Africa), followed by addition of different concentrations of the extract (12.5, 25, 50 & 100 µg/mL) (Yang *et al*., 2009). Indomethacin was used as the positive control. Cells were incubated for 24 h at 37°C with 5% CO_2.

Nitrite measurement

Following incubation for 24 h, equal volume of cell supernatants and Griess (Sigma-Aldrich, South Africa) reagent were added to 96-well plate, incubated for 15 min and the absorbance read at 550 nm using microtiter plate reader (Epoch Biotek). The percentage nitric oxide inhibition was calculated based on the ability of the extracts to inhibit nitric oxide formation by macrophages compared with

the control (cells treated with LPS only). The half minimum inhibitory concentration (IC_{50}) was calculated from the equation obtained by plotting the concentrations with corresponding % NO inhibitory values (Belgorosky *et al*., 2014).

Determination of cell viability

In order to ascertain that the NO inhibitory activity observed from the extract was not due to a general toxin on RAW cells, a cytotoxicity assay was done using 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Inqaba biotec, South Africa). Briefly, after removal of supernatant from the RAW macrophages, the cells were washed with PBS and fresh culture medium MTT solution (5 mg/mL) was added to all wells and the plates were incubated further for 4 h. The media were discarded and DMSO (0.5%) was added to each well to dissolve the formed formazan salts (Benov, 2021). The absorbance was read using a microplate reader (Biotek Synergy, USA) at 570 nm. The percentage cell viability for the sample was calculated by comparing the absorbance in the plant extract-treated wells to the negative control (cells treated with LPS only) using a linear regression curve as indicated in the cytotoxicity section. The percentage of cell viability was calculated using the following equation: % Viability = Mean OD sample/ Mean OD blank × 100.

Quantitative antibacterial assay by minimum inhibitory activity

A quick, sensitive serial dilution microplate method (Eloff, 1998) was used to determine the minimum inhibitory concentration (MIC) of the crude plant extract against *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli (ATCC 25922), Bacillus cereus (ATCC 21366*), *Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (ATCC 32045) in triplicates. The cultures were grown overnight in Tryptic soy broth (Sigma Aldrich, SA), and Sabouraud dextrose broth (Sigma Aldrich, SA) for bacteria and fungi respectively and adjusted to McFarland standard 0.5. The extract was dissolved in 50% acetone and 100 µL was added to the first well of a sterile 96-well microtiter plate and a 1:1 serial dilution was done with sterile distilled water. One hundred microtiters of the prepared cultures were added to each well. The microbes were exposed to final extract concentrations of 2.5 to 0.02 mg/ml through two-fold serial dilutions. For the antifungal assay, 40 µl of 0.2 mg/mL tetrazolium salt (MTT) was added to the well prior to incubation. The microplates were then incubated overnight at 37°C under aerobic conditions. After 16-18 h incubation, the presence of bacterial growth was detected by adding to each well 40 µl of 0.2 mg/mL of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) and plates were incubated further at 37°C for 2 h. Microbial growth in the wells appeared as red colour which shows the reduction of INT to a red-coloured formazan. The MIC was determined visually as the lowest concentration inhibiting the growth of the organisms indicated by a reduction in the red colour (Kowalska-Krochmal and Dudek-Wicher, 2021).

Statistical analysis

All values were expressed as mean ± standard deviation (SD) and the test of significance between two groups was estimated with Student's t-test. The One-Way Analysis of Variance (ANOVA) with Tukey's post-hoc test of Graph pad prism 5.0 was also carried out with p-Values < 0.05 considered statistically significant.

Results

The results of the proximate and mineral components of *D. rotundifolia* whole plant are presented in Table 1. The test plant had high contents of carbohydrate (47.87%), protein (22.5%) and crude fibre (9.57%) with low fat (2.73%) content. The plant was rich in minerals. The mineral contents were in the order: Na (206.67 mg/100 g) > Ca (183.33 mg/100 g) > PO_4 (166.67 mg/100 g) > Mg (93.33 mg/100 g) > Fe (12.07 mg/100 g) > Zn (0.53 mg/100 g). *D. rotundifolia* whole plant was rich in vitamin C (25.53 mg/100 g), niacin (2.78 mg/100 g) and carotenoids (2353.33 µg/100 g). Flavonoids content (685.00 mg/100 g) was the highest, followed by alkaloids (616.67 mg/100 g), saponins (471.67 mg/100 g), tannins (278.33 mg/100 g) and cardiac glycosides (11.67 mg/100 g) was the least (Table 2).

The extract exhibited considerable antioxidant activity against DPPH radicals with low IC_{50} of 6.13µg/ml (Table 3). There was correlation between extract concentrations and its ferric reducing activity, as well as hydrogen peroxide scavenging activity. The antioxidant activity of the plant increased along concentration gradients in FRAP and H_2O_2 scavenging assays (Tables 3 & 4). The plant showed significant antioxidant activity against ABTS radicals with low IC_{50} of 0.059mg/ml (Table 4).

The *in vitro* anticancer activity of *D. rotundifolia* extract against kidney cell line (Vero cells) showed that the extract at a low (0.145 mg/ml) concentration, gave the maximum lethal dose when compared to doxorubicin (standard anticancer agent) (Table 5). Also, the *in vitro* anticancer activity of the extract against human breast cancer cell line (MCF-7) revealed a significant cytotoxicity against MCF-7 cell lines at 0.141 mg/ml extract concentration compared to doxorubicin (Table 6). Furthermore, Selective Index (SI) which is used as a marker of safety effectiveness of a drug was used to evaluate selective toxicity against Vero and MCF-7 cell lines. The results showed that the extract had a SI value of 1.031 which was 25% of the value of doxorubicin (4.193) as shown in Table 7.

In another experiment, the anti-inflammatory activity of the plant extract against lipopolysaccharide (LPS)-stimulated RAW macrophage (Table 8) showed that it exhibited anti-inflammatory action well comparable to the standard anti-inflammatory drug (indomethacin). The anti-inflammatory activity of the extract was concentration dependent, the higher the concentration the more effective the extract. At 100µg/ml, *D. rotundifolia* extract exhibited 47.62% inhibition against NO, a value that was 59.50% of the activity of indomethacin (80.02%).

Furthermore, the extract recorded higher percentage (%) cell viability against lipopolysaccharide (LPS)-stimulated RAW macrophage compared to indomethacin (Table 9). The % cell viability increased along concentration gradient from 91.72% at 12.5µg/ml to 93.71% at 100µg/ml. The extract (93.71%) had higher percentage (%) cell viability than indomethacin (80.02%) at 100µg/ml.

The antimicrobial activity of *D. rotundifolia* extract is presented Table 10. The extract was active against all test organisms viz. *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Candida albicans*, *Cryptococcus neoformans*, and *Pseudomonas aeruginosa*,

although it had higher Minimum Inhibitory Concentration (0.62–2.50 mg/ml) than the standard drugs (gentamycin and amphotericin-B). The extract showed antibacterial and antifungal activities.

Table 1: The results of the proximate and mineral compositions of *Dissotis rotundifolia* whole plant

Proximate components of powdered *Dissotis rotundifolia*		**Mineral contents of powdered *Dissotis rotundifolia***	
Parameters	Content (%)	Parameters	Content (mg/100 g)
Moisture content	8.27±0.15	Calcium	183.33±7.63
Protein	22.5±0.2	Magnesium	93.33±7.64
Fat	2.73±0.21	Sodium	206.67±7.64
Ash	9.16±0.15	Iron	12.07±0.21
Crude fibre	9.57±0.15	Zinc	0.53±0.06
Carbohydrate	47.87±21.08	Phosphate	166.67±7.64

Legend: Values are expressed as means ± SD, n = 3.

Table 2: The results of the vitamin and phytochemical compositions of *Dissotis rotundifolia* whole plant

Vitamin composition of powdered *Dissotis rotundifolia*		**Phytochemical composition of powdered *Dissotis rotundifolia***	
Parameters	Content (mg/100 g)	Parameters	Content (mg/100 g)
Vitamin C	25.53±0.21	Alkaloids	616.67±10.41
Thiamin (B1)	0.23±0.24	Flavonoids	685.00±13.23
Riboflavin (B2)	0.21±0.18	Saponins	471.67±10.41
Niacin (B3)	2.78±0.18	Tannins	278.33±10.41
Carotenoids (µg/100 g)	2353.33±5.36	Cardiac glycosides	11.67±2.89
		Steroids	156.67±10.41

Legend: Values are expressed as means ± SD, n = 3.

Table 3: DPPH radical scavenging and Ferric reducing activities of extract of *Dissotis rotundifolia* whole plant

DPPH radical scavenging activity (IC_{50}) in µg/ml			Ferric reducing activity		
Test	*Dissotis rotundifolia*	Ascorbic acid	Concentration (mg/ml)	Absorbance (700nm)	
1	6.10	3.00		*Dissotis rotundifolia*	Ascorbic acid
2	6.30	3.10	0.20	0.44±0.000	0.36± 0.00
3	6.00	3.20	0.40	1.11±0.002	0.47± 0.00
MEAN	6.13	3.10	0.60	1.62±0.000	0.58± 0.00
SD	0.15	0.10	0.80	2.40±0.002	0.62± 0.00
MEAN±SD	6.13±0.15	3.10±0.10	1.00	2.81±0.002	0.71± 0.00

Legend: Values are expressed as means ± SD, n = 3.

Table 4: Hydrogen Peroxide and ABTS Scavenging activities of extract of *Dissotis rotundifolia* whole plant

Hydrogen Peroxide Scavenging Activity			Minimum inhibition concentration (IC_{50}) antioxidant activity against ABTS in mg/ml	
Concentration (µg/mL)	% inhibition		Tests	Values
	Dissotis rotundifolia	Ascorbic acid	1	0.059
500	74.37±1.31	44.63±0.310	2	0.060
400	59.57±1.68	38.57±0.310	Mean	0.059
300	55.38±0.50	33.81±0.270	SD	0.000
200	54.69±0.57	33.13±0.263		
100	51.88±0.44	32.02±0.306		

Legend: Values are expressed as % or means ± SD, n = 2.

Table 5: Half maximal toxicity (LC_{50}) in mg/mL of *Dissotis rotundifolia* extract against VERO cells

Plant Sample and Standard Drug	1	2	3	Mean	SD
D. rotundifolia	0.135	0.138	0.164	0.145	0.016
Doxorubicin	0.014	0.012	0.012	0.013	0.0008

Legend: Values are expressed as means ± SD, n = 3.

Table 6: Half maximal toxicity (LC_{50}) in mg/mL of extract of *Dissotis rotundifolia* against MCF-7 cells

Plant Sample & Standard Drug	1	2	3	Mean	SD
D. rotundifolia	0.148679	0.146406	0.136535	0.141	0.006
Doxorubicin	0.0031	0.0031	0.0031	0.0031	0.000

Legend: Values are expressed as means ± SD, n = 3.

Table 7: Selectivity indices of *Dissotis rotundifolia* extract against VERO and MCF-7 cancer cells

Plant Sample & Standard Drug	VERO	MCF-7	SI
D. rotundifolia	0.145	0.141	1.031
Doxorubicin	0.013	0.0031	4.193

Legend: Values are expressed as means of 3 readings.

Table 8: Activity of *Dissotis rotundifolia* extract on percentage nitric oxide production inhibition (% NO inhibition) in LPS stimulated RAW macrophages

Plant Sample & Standard Drug	Concentration (µg/mL)	1	2	3	Mean	SD
D. rotundifolia	100	48.50	49.12	45.23	47.62	2.08
	50	28.76	27.83	28.60	28.40	0.49
	25	20.67	15.86	17.72	18.09	2.43
	12.5	10.72	13.06	11.04	11.61	1.26
Indomethacin	100	78.65	80.27	81.15	80.02	1.26

Legend: Values are expressed as means ± SD, n = 3.

Table 9: Percentage (%) cell viability of LPS-activated RAW macrophages treated with *Dissotis rotundifolia* extract

Plant Sample & Standard Drug	Concentration (µg/mL)	1	2	3	Mean	SD
D. rotundifolia	100	91.77	93.97	95.39	93.71	1.82
	50	96.04	88.72	91.33	92.03	3.70
	25	85.20	82.64	88.90	85.58	3.14
	12.5	92.27	88.93	93.97	91.72	2.56
Indomethacin	100	78.65	80.27	81.15	80.02	1.26

Legend: Values are expressed as means ± SD, n = 3.

Table 10: Minimum inhibitory concentration of *Dissotis rotundifolia* extract against test organisms

Plant Sample & Standard Drug	Organism/MIC (mg/mL)					
	E. coli	*B. cereus*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*C. neoformans*
D. rotundifolia	1.25	1.25	0.15	2.5	1.25	0.62
Gentamycin	0.0008	0.0005	0.0003	0.00013	NA	NA
Amphotericin-B	NA	NA	NA	NA	0.00625	0.025

Legend: Values are expressed as means of 3 readings. NA = Not Active.

Discussion

Dissotis rotundifolia whole plant is very rich in proximate components especially protein and crude fibre. The body needs protein to build muscle cells, cells of major organs, skin and red blood cells. One of the major functions of protein in MS is to slow down the absorption of carbohydrates (Miglani and Bains, 2017). The slow release of sugar from carbohydrate prevents spikes in both blood sugar and insulin (Miglani and Bains, 2017). Crude fibre is an important part of diet and it decreases serum cholesterol level, risk of coronary heart disease, hypertension, diabetes, colon and breast cancer that are reportedly associated with MS (Lepping *et al*., 2022).
The test plant contains significant contents of sodium, calcium, phosphorus and iron. *Dissotis rotundifolia* whole plant could be effective as analgesic and anti-inflammatory agents based on its mineral composition and chronic inflammation has been listed as one of the major features of MS (Sharma, 2011). Iron is an immune booster with Phosphorus being a critical component of every cell. It works with the B vitamins to generate energy and is necessary for growth of the bones and teeth, which are 85% phosphorous. It works with sodium and potassium to maintain acid-base balance, and assist in muscle contraction, kidney function, heartbeat regulation, and in nerve conduction (Serna and Bergwitz, 2020). *Dissotis rotundifolia* whole plant is very rich in vitamins. Vitamin C is a strong antioxidant and anti-hypertensive agent (Padayatty *et al*., 2003; Chiu *et al*., 2021). Niacin (vitamin B3) lowers low-density lipoprotein (LDL) cholesterol (it is used to treat high cholesterol); it increases high-density lipoprotein (HDL) cholesterol and lowers triglyceride which may help prevent heart disease (Nurmohamed *et al*., 2021). It may also help treat Type 1 diabetes and boost brain function (Shi *et al*., 2020). It improves skin function and may reduce symptoms of arthritis (Penberthy and Kirkland 2020). Riboflavin (vitamin B2) helps in energy metabolism, ocular function, antibody and red blood cells formation and mucosal maintenance (Hanna *et al*., 2022). Thiamine (vitamin B1) helps prevent complications in the nervous system, brain, muscles, heart, stomach and intestines (Chawla and Kvarnberg, 2014). It is also involved in the flow of electrolytes in and out of muscles and nerve cells (Pacei *et al*., 2020). Dietary carotenoids decrease the risk of diseases, particularly cancers and eye disease (Ali *et al*., 2020). They are strong antioxidants and prevent cardiovascular diseases.Overall, vitamins C and B3 could be useful in preventing risks such as high cholesterol, diabetes and cardiac disease associated with MS (Uribe *et al*., 2017). *D. rotundifolia* extract is abundantly enriched with alkaloids, flavonoids, tannins, phenols, and saponins. Therefore, the antioxidant and anti-inflammatory activities of the plant could be attributed to its phytochemical contents. Previous studies have reported that the phytochemicals in *D. rotundifolia* might be responsible for its antidiarrhoeic and other biological activities (Abere *et al.,* 2010). Adinortey *et al.* (2020) also reported the gastro-protective effect of flavonoid-rich extract of *D. rotundifolia* whole plant against ethanol-induced gastric mucosal damage in experimental animal model.

D. rotundifolia extract exhibits significant antioxidant activity against DPPH and ABTS radicals. It also shows strong ferric reducing and hydrogen peroxide (H_2O_2) scavenging activities. Oxidative stress has been implicated in chronic diseases such as atherosclerosis, cancer, diabetes, hypertension, hyperlipidemia, rheumatoid arthritis, myocardial infarction, stroke, aging and other degenerative diseases (Sharifi-Rad *et al*., 2020; Francini-Pesenti *et al*., 2019). *D. rotundifolia* has strong antioxidant activity and could be useful in the management and treatment of MS.

D. rotundifolia shows *in vitro* anticancer activity against kidney cell line (Vero cells). It displays a dose- dependent and strong anticancer activity that was comparable to that of the standard anticancer agent (doxorubicin). Many of the synthetic anticancer agents are highly cytotoxic with avalanche of toxic effects to various vital organs in the body (Remesh, 2020). Therefore, maximum advantage should be deployed for the identification and isolation of bioactive principles that might be responsible for the anticancer property of the plant. Chronic inflammation has been linked to multi-stage carcinogenesis (Wang *et al.,* 2022). It has been reported that that chronic inflammation plays a major role in oncogenesis, promoting genome instability, epigenetic alterations, proliferation and dissemination of cancer cells (Muller *et al.,* 2022). From this study, *D. rotundifolia* whole plant exhibits strong anti-inflammatory activity against lipopolysaccharide (LPS)-stimulated RAW macrophage cell line. In fact, there is scarcity of information on the anti-inflammatory action of this plant. The anti-inflammatory activity of *D. rotundifolia* as demonstrated in this study is an indication that it could be used for the management of disease conditions such as cancer and inflammatory bowel disease associated with chronic inflammation. The results on the anticancer and anti-inflammatory properties of *D. rotundifolia* as recorded in this study also correlate with its activity against human breast cancer cell line (MCF-7), high percentage (%) cell viability, and selective index (SI) which is used as a marker of drug safety. The high selective index of *D. rotundifolia* is of great advantage compared to most of the synthetic chemotherapeutic agents. Therefore, the use of the plant as an anticancer agent could open a novel therapeutic window for the management of cancer in developing countries such as Sub-Saharan African where the resources are limited for effective management of cancer patients. Furthermore, cancer has been reported as a component of MS. (Mendrick *et al*., 2018).

The extract of *D. rotundifolia* demonstrates inhibitory effect against *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Candida albicans, Cryptococcus neoformans,* and *Pseudomonas aeruginosa* compared to gentamycin and amphotericin-B, indicating antimicrobial activity. Therefore, *D. rotundifolia* as a medicinal plant might be useful for the prevention and treatment of diseases, especially bacterial infections associated with obesity and inflammation in MS (Sommer and Sweeney, 2010).

Conclusion

Overall, *Dissotis rotundifolia* whole plant exhibited significant *in vitro* antioxidant, anticancer, ant-inflammatory and antimicrobial activities. The observed bioactivities of the plant could be attributed to its phytochemical and nutritional components. The plant might be a potential agent for the management and treatment of metabolic syndrome. Furthermore, the plant could be utilized in developing countries such as Nigeria due to its availability, accessibility and potency.

Conflict of interest

The authors declare no conflict of interest.

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