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Original Article

Total Phenolic, Flavonoid and Alkaloid Contents, Oxidative DNA Damage Protective and Antioxidant Properties of Methanol and Aqueous Extracts of *Dissotis rotundifolia* Whole Plant

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ABSTRACT

Background: Antioxidants present in natural sources helps to scavenge free radicals and thus, provide health benefits. The main objective for this research is to determine phenolic, flavonoid, and alkaloid contents, nitric oxide scavenging activity, hydroxyl radical scavenging activity, and DPPH-scavenging activity, ferric reducing power of the extracts and DNA damage protecting activity to evaluate the antioxidant potential of various extracts. **Materials and Methods**: *In vitro* antioxidant potential of the *D. rotundifolia* extract was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), and hydroxyl (OH) radical scavenging assays. Ferric reducing power ability of the extract was also examined using tannic acid, and ascorbic acid as standard. Concentrations of plant extracts ranging from 0.02 to 0.10 mg/ml were prepared and mixed with appropriate volumes of reagents. **Results:** Methanol extract of *D. rotundifolia* exhibited higher content of phytochemical compounds (alkaloid = 12.4 mg QE/g; flavonoids = 19.3 mg QE/g; and phenols = 18 mg QE/g) at concentration 0.1 mg/ml compare to the aqueous extract (alkaloid = 9.1 mg QE/g; flavonoids = 10.5 mg QE/g; and phenols = 16.5 mg QE/g). An over-all trend found in the present study highlights the fact that the methanol extract have better antioxidant capacities (DPPH, NO, OH and FRAP) than the aqueous extract. *D. rotundifolia* extracts exhibited considerable protection to the damage of native supercoiled circular at concentrations, 0.1 mg/ml and 10 mg/ml. **Conclusion:** The study showed that the extracts can competently protect the body against oxidative stress, therefore can be used as a source of potent natural antioxidant compounds.

Key words: *Dissotis rotundifolia* whole plant, Reactive Oxygen Species (ROS), Reactive Nitrogen Species, DNA damage protecting activity and Antioxidant activity.

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INTRODUCTION

Reactive oxygen and nitrogen species (ROS/RNS) are central to biochemical process and represent an essential part of aerobic life and metabolism. Various metabolic processes and external factors such as tobacco smoke, UV radiation and other environmental pollutants, trigger the production of ROS/RNS.[1] ROS/RNS include free radicals such as superoxide ($O^{2\bullet-}$), hydroxyl ($OH^{\cdot}$), peroxyl ($ROO^{\cdot}$), peroxynitrite ($^{\cdot}ONOO^{\cdot}$) and nitric oxide ($NO^{\cdot}$) radicals as well as non-free radical species such as hydrogen peroxide (H_2O_2), ($1O^2$)nitrous acid (HNO_2), singlet oxygen and hydrochlorous acid (HOCl).[2] Excessive production of these reactive species causes oxidative stress/damage to biomolecules such as lipids, enzymes, proteins and DNA in cells and tissues. The increased production of ROS/RNS is considered a universal feature of stress conditions. Oxidative stress has been linked to many chronic diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke, aging and other degenerative diseases in humans.[3]

Biological systems are protected against free radical induced cell damage by the activity of inherent anti-oxidants which may be enzymes or non-enzymatic compounds. Most human cells unlike plant cells do not generate adequate amounts of antioxidants to protect against oxidative stress/damage.[4] Hence antioxidants may be given as supplements. Hence, a growing interest to search for alternative natural and safer sources of antioxidants. Plant-sourced natural antioxidants such as vitamin C, vitamin E, carotenes, phenolic, flavonoids, phytates and phytoestrogens are believed to play a potential role in interfering with the oxidation process by reacting with free radicals, chelating catalytic metals and scavenging oxygen in biological systems.[4,5] Many studies have shown positive

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correlation of the increased dietary intake of natural phenolic antioxidants with the reduced coronary heart disease and cancer mortality, as well as with longer life expectancy.[5]

Despite extensive research on the antioxidant properties of most plants, little is known about the many tropical underutilized plants in developing nations especially Ghana. One such plant is *Dissotis rotundifolia* which is a versatile perennial slender creeping herb. In its native range in Africa, *D. rotundifolia* is used to treat several illnesses such as dysentery, rheumatism, circulatory problems, conjunctivitis, venereal disease, peptic ulcer and hookworm infestation; it is also used to prevent miscarriages. Extract of *D. rotundifolia* has also been shown to be effective against Trypanosoma brucei, parasite that causes African sleeping sickness.[6] Systematic investigation of extracts of this plant for its medicinal properties could provide an important input to pharmaceutical industry. Therefore, in this study, the phytochemical constituent, antioxidant property and protection from oxidative DNA damage by methanol and aqueous extracts of *D. rotundifolia* plant were investigated to assess the potential protective benefits of this plant against degenerative reactions induced by free radicals.

MATERIALS AND METHODS

Plant Material

Fresh whole plant of *Dissotis rotundifolia* was collected from the University of Cape Coast botanical garden, Ghana. The taxonomic identity of the plant was determined with voucher number UCCBG000435, by a plant taxonomist at the Department of Botany, University of Cape Coast, Ghana. The plant sample was washed under running tap water to remove unwanted dirt and other foreign materials. The sample was air dried under shade until no moisture left. The dried sample was ground into powder using a blender

Preparation of plant extracts

Methanol extraction

The methanol extract was prepared by soaking 60 g of powdered sample of *Dissotis rotundifolia* in 210 ml of methanol (70 %) for 72 h at room temperature (35°C). The mixture was then filtered using Whatman filter paper No 1. The filtrate was concentrated under reduced pressure using rotary evaporator at temperature of 46°C. The resulting extract was weighed and stored in airtight bottles at room temperature for further used.

Aqueous extraction

The aqueous extract was prepared by soaking 60 g of the powdered sample in 600 mL of sterile distilled water for 2 h in 90°C water bath. The mixture was then filtered using Whatman filter paper No 1. The filtrate was concentrated under reduced pressure using a rotary evaporator at a temperature of 90°C. The resulting extract was weighed and stored in airtight bottles at room temperature for further use.

Phytochemical screening

Qualitative analysis

Phytochemical qualitative test of the Methanol extracts of *Dissotis rotundifolia* was performed as per standard protocols[7] to reveal the existence of reducing sugars, proteins, phenolic compounds, flavonoids, tannins, saponins, alkaloids, terpenoids and cardiac glycosides.

Quantitative Analysis

Determination of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu assay described by Meda *et al*.[8] Briefly, 0.5 mL of extract (1 mg/mL) and 2.5 mL of 10 % Folin-Ciocalteu's reagent solution were mixed. After incubation for 2 min at room temperature, 2.5 mL of 7.5 % sodium carbonate solution was added. The mixture was incubated at 45^{O}C for 45 min and subsequently photometrically measured at 760 nm. Gallic acid (0–100 µg/mL) was used as standard to produce the calibration curve. The mean of three readings was used and the total phenolic content was expressed in milligrams of Gallic acid equivalents (GAE) per gram of plant extract.

Determination of total alkaloids

Exactly 1g of the sample was weighed into a 250 ml beaker and 40 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.[7]

Determination of total flavonoids

Exactly 1g of the plant sample was extracted repeatedly with 10 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transported into a container and evaporated into dryness over a water bath and weighed to a constant weight.[9]

Determination of free radical scavenging activity

Ferric reducing antioxidant property (FRAP assay)

Ability of the sample extracts to reduce ferric ions was measured according to the modified method described by Oyaizu.[10] A mixture containing 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 mL of various concentrations (0.02–0.10 mg/ml) of extract and standards prepared in distilled water. The resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 mL of trichloroacetic acid (TCA) (10% w/v), which was then centrifuged at 3000 rpm for 10 min. About 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1% w/v). The absorbance was then measured at 700 nm against a blank sample containing a phosphate buffer. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

Determination of Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extracts was determined according to the method reported by Pavithra and Vadivukkarasi[11] with slight modification. The reaction mixture contained 0.5 mL of various concentration (0.02-0.10 mg/mL) of extracts and standard (Gallic acid), 1.0 mL iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of 0.018% EDTA and 1.0 mL DMSO (0.85% in 0.1 mole/L phosphate buffer pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22 %) and incubated at 80^{O}C – 90^{O}C for 5 min. The reaction was terminated by adding 0.1 mL of ice cold TCA (17.5%). A 3.0 mL Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid and 2.0 mL of acetyl acetone were mixed and distilled water was added to a total volume of 1 L) was added and incubated at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured at 412 nm against a

reagent blank which contains all constituents except ascorbic acid. All experiments were performed in triplicates. The % inhibition of hydroxyl radical scavenging was calculated by using the formula:

$$OH^{-}scavenged\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where A_0 was the blank absorbance and A_1 was the mixture containing the extract absorbance or the standard absorbance.

Determination of 1, 1 Diphenyl-2, picrylhydrazine (DPPH) - free radical scavenging activity

DPPH radical scavenging activity was assessed according to the method of Shimada *et al*.[12] The reaction mixture contained 1.0 mL of various concentrations (0.02-0.10 mg/mL) of extracts and standard (Gallic acid) and 1.0 mL of DPPH solution (0.135 mM). The mixture was shaken vigorously and left in a dark for 30 min. The absorbance was measured at 517 nm against a reagent blank containing only methanol. All experiments were performed in triplicates. The inhibition percentage for scavenging DPPH radical was calculated according to the equation:

$$DPPH\ scavenging\ activity\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Determination of Nitric oxide scavenging activity

Nitric oxide radical scavenging activity was measured spectrophotometrically according to the method described by Jagetia *et al*.[13] A volume of 2ml sodium nitroprusside in phosphate buffer (0.02M, pH 7.4) was mixed with different concentrations (0.02-0.10 mg/ml) of the extracts and standard (ascorbic acid). The reaction mixture was at 25^{O}C for 2 h. Thereafter, 1.5 ml of Griess reagent [1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride] was added. The absorbance was measured at 540nm after 30 min against a phosphate buffer blank. Control was maintained with all chemicals excluding extract. The % scavenging activity of nitric oxide was calculated using the formula:

$$scavenging\ activity\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Genomic DNA extraction

Cowpea seeds (*Vigna unguiculata*) purchased from Kotokuraba market, Cape Coast, in the Central region of Ghana, were sowed and allowed to germinate. After 1 week, the leaves of the germinated seeds were collected. Genomic DNA was extracted adopting a modified cetyltrimethylammonium bromide protocol described by.[14] The integrity of the extracted DNA was tested by running a 5 µl DNA sample on gel electrophoresis at 100 V for 1 h 20 min. The purity was also determined by measuring the absorbance of each DNA sample at 260 nm and 280 nm and the ratio computed. A ratio ≥1.8 ≤2.0 implied a pure nucleic acid sample; however, a ratio <1.8 indicates impurity depicting the presence of protein. Meanwhile, a ratio >2.0 indicates that the samples were contaminated with phenol or chloroform. To standardize the samples, the concentration of the DNA was also determined using the formula:

$$\text{DNA concentration } (\mu g / ml) = \left(\frac{A_{260} * D * 50\mu g/ml}{1000}\right)$$

Where A_{260} represents the absorbance at 260 nm and D is the dilution factor. The determination of the concentrations enabled the standardization to 100 ng/µl. Samples that were highly concentrated were diluted with sterile Tris-EDTA (TE) buffer, whereas those with lower concentrations, the extraction process was repeated.

DNA damage protective potential of methanolic and aqueous extracts of *Dissotis rotundifolia*

DNA protection potential of the extracts were evaluated using cowpea (*Vigna unguiculata*) genomic DNA. Genomic DNA was extracted following the cetyl trimethyl-ammonium bromide (CTAB) procedure as described by Doyle and Doyle.[15] Oxidative damage to DNA was induced using hydroxyl free-radical generating system (H_2O_2/UV) described by Russo *et al*[16] in the presence of the extracts. Briefly, 10µl aliquot of cowpea DNA was added to microfuge tubes containing 10µl of different concentrations of extracts (0.02, 0.1, and 10 mg/ml) and 10µl of 30% H_2O_2. Tannic acid (10 mg/ml) was used as a positive control instead of the extract. The normal control contained only the DNA extract while the negative control contained DNA plus H_2O_2. The tubes were UV irradiated using UV transilluminator (UVP Upland, CA 91786 U.S.A.) for 12 h at room temperature. After irradiation, 5µl of X6 bromophenol blue was added to each tube. All samples were analyzed by gel electrophoresis on 1% agarose gel (containing ethidium bromide) in TAE buffer (pH 8). Untreated, un-irradiated cowpea DNA was run along with untreated UV-irradiated DNA and an extracts treated UV-irradiated sample.

Statistical Analysis

All tests were conducted in triplicate. Data are reported as means ± standard error (SE). Results were analyzed statically by using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Results

Phytochemical analysis

Accurately, methanol and aqueous extracts from *D. rotundifolia* whole plant retains a percentage yield of 5.602 % and 10.003 % respectively. It was recognized that aqueous extract had greater % yield than that of the methanol extract. Phytochemical analysis of both the methanol and aqueous extracts of *D. rotundifolia* revealed the presence of reducing sugars, proteins, phenolic compounds, flavonoids, tannins, saponins, alkaloids, terpenoids and cardiac glycosides, but no Phytosterols (Table 1).

Total alkaloid, phenol and flavonoid Contents of aqueous and methanol extracts of *D. rotundifolia*.

There were different compositions of phytochemicals in the extracts of *D. rotundifolia* (Table 1 and Figure 1). There were no significant differences statistically in the levels of flavonoids, and phenols between the aqueous and methanol extracts of *D. rotundifolia* (Table 2). There was a statistically significant difference in the levels of alkaloids between the

Table 1: Phytochemical constituents of methanol and aqueous extracts of *D. rotundifolia*.

Phytochemicals	Methanol extract	Aqueous extract	AA
Alkaloids	+++	+	+
Tannins	++	+ ++	++
Saponnins	++	+	+++
Glycosides	++	+ +	++++
Reducing sugars	++++	++	+++
Flavonoids	++	+	+
Terpenoids	+	+	++
Phenols	+++	+ +	+++
Proteins	++	+++	+++
Phytosterols	_	_	+++

Present (+), absent (-) and AA represents Ascorbic Acid

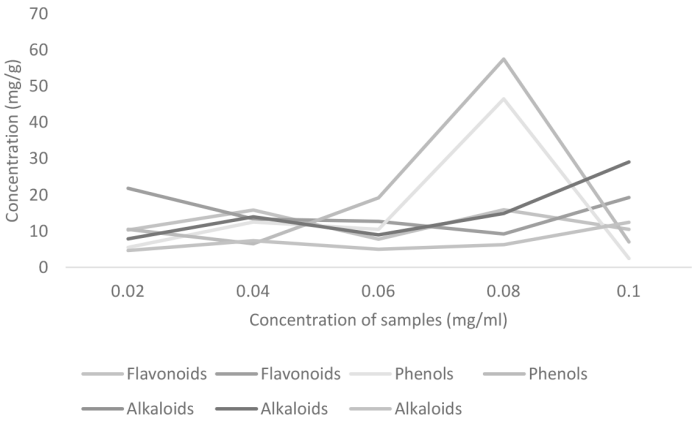

Figure 1: Quantitative contents of phytochemicals in *D. rotundifolia* (mg/g)

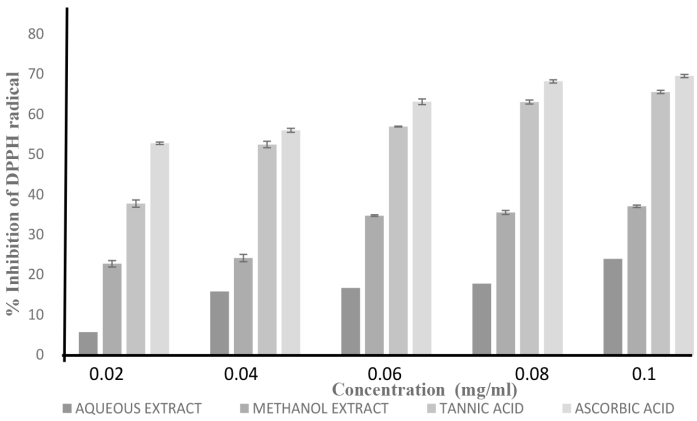

Figure 2: DPPH scavenging activities of methanol and aqueous extracts from *Dissotis rotundifolia* whole plant and standard tannic acid and ascorbic acid.

Table 2: Quantitative contents of phytochemicals in *D. rotundifolia* (mg/ QE/g)

Samples	Flavonoids		Phenols		Alkaloids	
Conc.mg/ml	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
0.02	10.3	11.8	5.5	10.5	7.9	4.7
0.04	10.8	13.4	12.5	6.5	14	7.4
0.06	7.8	12.7	7.5	9.2	9	5
0.08	15.9	9.2	10.5	17.5	14.9	6.3
0.10	10.5	19.3	16.5	18	9.1	12.4

methanol and aqueous extracts of *D. rotundifolia* (Table 2). The alkaloids begin to reduce and their concentrations weaken as the plants mature.

Antioxidant activity

DPPH scavenging activity

The *in vitro* antioxidant assays performed on this plant reveal significant antioxidant potential. DPPH is a stable free radical commonly used to investigate the scavenging activity of phytochemicals. The results of the DPPH scavenging activity of the two extracts, along with ascorbic acid and tannic acid (reference standards), are shown in Figure 2. Although both extracts shows DPPH scavenging activity, the activity is lower compared to the standards (tannic acid and ascorbic acid). The methanol extract shows higher activity (37.03%) compared to the aqueous extract which shows 23.09% inhibition at the highest concentration of 0.1 mg/mL

Hydroxyl radical scavenging activity

Figure 3 represents the hydroxyl radical scavenging activity of methanol and aqueous extracts as well as standard Gallic acid. Gallic acid exhibited highest hydroxyl radical scavenging activity compared to the extracts. The extracts exhibited strong NO scavenging activity compared with standard Gallic acid (Figure 3). Both standard Gallic acid and extracts show dose dependent inhibition of the NO radicals. The aqueous extract again shows higher activity (59.87 %) compared to methanol the extract which shows 51.16% inhibition at the highest concentration of 0.1 mg/ml.

NO radical scavenging activity

Gallic acid recorded the highest % radical scavenging ability, followed by aqueous extract and methanol extract, i.e. 42.48%, 24.88%, and 23.18% (Figure 4) respectively for the highest concentration tested (100µg/ml). The NO radical scavenging activity of *Dissotis rotundifolia* whole plant may help arrest chain reactions initiated by excess generation of $ONOO^-$ that is detrimental to human health.

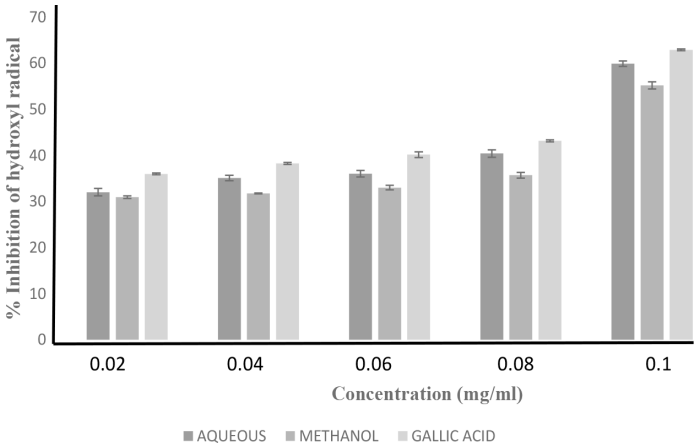

Figure 3: Hydroxyl scavenging activities of both methanol and aqueous extracts from *Dissotis rotundifolia* whole plant and standard Gallic acid.

FRAP assay

Figure 5 shows the results of the FRAP assay of methanol and aqueous extracts of *Dissotis rotundifolia* whole plant. The methanol extracts of the *D. rotundifolia* showed significantly higher FRAP values compared to all of the aqueous extracts. An over-all trend found in the present study climaxes the fact that the methanol extract have better antioxidant capacities than the aqueous extract.

DNA damage protection

Figure 6 shows the electrophoretic pattern of DNA after UV-photolysis of H_2O_2 (100 mM) in the absence and presence of different concentrations of methanol and aqueous extracts of *D. rotundifolia* whole plant (0.02, 0.1, and 10 mg/ml).

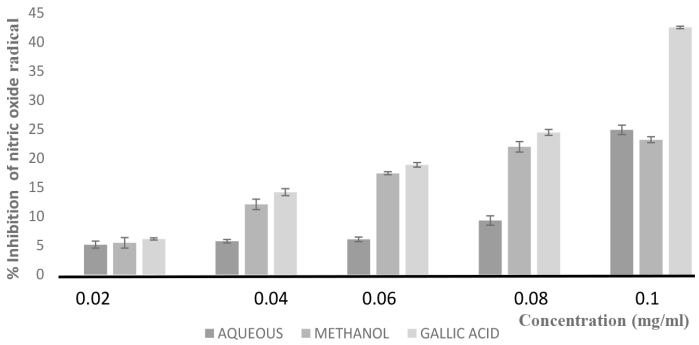

Figure 4: Nitric oxide scavenging activities of methanol and aqueous extracts from *Dissotis rotundifolia* whole plant and standards, tannic acid and Gallic acid.

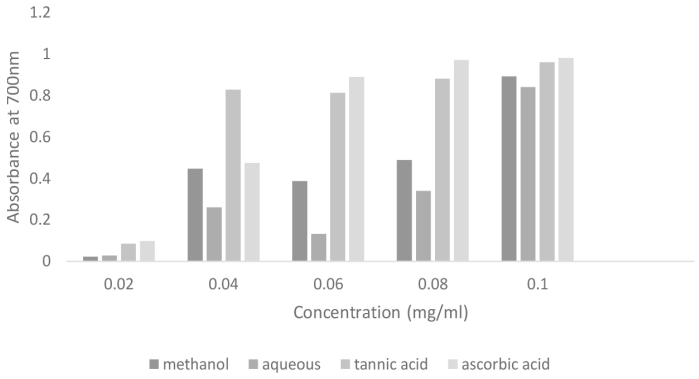

Figure 5: Ferric Reducing power of methanol and aqueous extracts from *Dissotis rotundifolia* whole plant and standards, tannic acid and ascorbic acid.

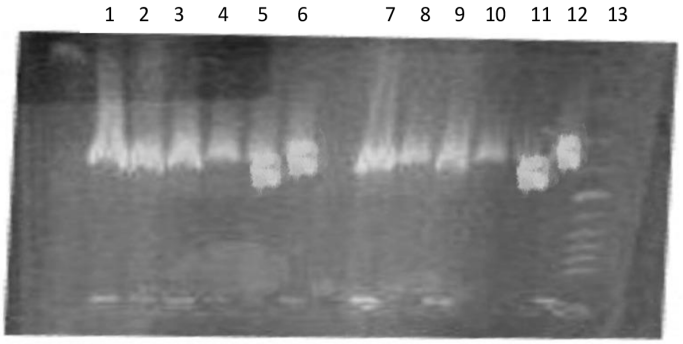

Figure 6: Electrophoretic pattern of DNA after UV-photolysis of H_2O_2 in the presence or absence of methanol or aqueous extracts from *Dissotis rotundifolia* whole plant.). Lane 6and12: control (DNA only), lane 5and11: treated (DNA+H_2O_2), lane 4and10: untreated (DNA+ extracts), lane 3 and 9: treated (DNA+10mg/ml extracts+H_2O_2), lane 2and8: treated (DNA+0.1mg/ml exracts+H_2O_2) and lane 1and 7: treated (DNA+ tannic acid+H_2O_2) and lane 13: marker.

DNA derived from leaves of cowpea showed two bands on agarose gel electrophoresis (line …), the faster moving prominent band corresponding to the native supercoiled circular DNA and the slower moving very faint band is the open circular form. The UV irradiation of DNA in the presence of H_2O_2 resulted in the scission of supercoiled DNA to give prominent open circular DNA and a faint linear DNA (lanes 5 and 11), an indication that $OH^{\cdot}$ was generated from UV photolysis of H_2O_2. The addition of methanol and aqueous extracts of *D. rotundifolia* to the reaction mixture of H_2O_2 displayed considerable protection to the damage of native supercoiled circular DNA (lanes 2,3, 8 and 9) with the aqueous extract doing better than the methanol extract.

Effects of the solvent system

In the present study, we used 2 different extracting solvents (methanol, and water), and we found out that they differed considerably in their capability to extract antioxidants. Conventionally, solvents such as methanol, ethanol, and acetone have been routinely used to extract phenolic/antioxidant compounds from fresh plants at different concentrations in the presence of water[17] used plays a substantial character in the precise quantification of antioxidants, it is very tough to compare the data available in the literature.[17] Nevertheless, generally, solvents such as methanol, ethanol, and acetone have different polarities, vapor pressures, and viscosities; solvents with low viscosity have low density and high diffusivity, which can allow them to with no trouble diffuse into the pores of the plant materials to leach out the active constituents.[18] Furthermore, dissimilarities in antioxidant activity and the yield of phenolic compounds have been testified to be influenced by extracting solvents.[19] Considering the variable results which can be obtained during extracting of antioxidants using various solvents, it is quite difficult to develop a standard extraction protocol that can provide better or more consistent results. From the toxicological point of view water is considered to be safer than methanol,[20] particularly with regard to applications or use in the food industries. However, in general, the least polar solvents are considered to be most suitable for the extraction of antioxidant compounds, unless very high pressure is employed.

DISCUSSION

The presence of these bioactive compounds in all the extracts indicates the potential health benefits of the plant. The presence of flavonoids and phenolic compounds enhances the possibility of antioxidant activity, as many studies have reported a strong positive correlation between these compounds and the antioxidant activity of extracts.[21] Phenolics are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants or plant products. Generally, the total phenolic content of methanol extract of *D. rotundifolia* was higher than the aqueous extract (Table 2 and Figure 1). The variations may be attributed to the different solvents employed as phenolic constituents of plants have been reported to be either hydrophilic and/ or lipophilic.[22] Environmental influences such as soil type, growing season, geographic location, and mineral status are known to impact intensities of plant secondary metabolites.[23] *D. rotundifolia* extracts that exhibited great altitudes of phytochemicals may be strong to insects and diseases using the phytochemicals as protective chemicals.[24] Vaclavikova *et al.* (2008)[25] in recent times demonstrated that anti-oxidant possessions of flavonoids and their ability to chelate free metallic ions could be operational in reducing the poisonousness of Doxorubicin (DOX) 39. The presence of flavonoids and phenols in *D. rotundifolia* makes the plant a very potent antioxidant.

Although both extracts shows DPPH scavenging activity, the activity is lower compared to the standards (tannic acid and ascorbic acid). The methanol extract shows higher activity (37.03%) compared to the aqueous extract which shows 23.09% inhibition at the highest concentration of 0.1 mg/ml. DPPH is a stable free radical commonly used to investigate the scavenging activity of phytochemicals. This result agrees well with the result on total phenolic content which is higher in the methanol extract. Usually, high total phenol contents lead to better DPPH-scavenging activity.[26,27] Furthermore, this result is consistent with other findings[26] where methanol extract showed higher antioxidant activity over aqueous extract.

Hydroxyl radical is the most reactive oxygen centered species and causes severe damage to adjacent biomolecule. Similarly to reactive oxygen species, NO is also implicated in inflammation, cancer and other pathological conditions.[28] NO is a very unstable species under aerobic conditions.

The extracts exhibited strong NO scavenging activity compared with standard Gallic acid (Figure 3). The DPPH, hydroxyl and NO radicals scavenging activity of the extracts may be due to the presence of hydrogen donating ability of phenolic compounds in the extracts.

In addition to the presence of phytochemicals, the extracts also showed nitric oxide scavenging activity. The toxicity and damage caused by NO and superoxide anion is heightened as they react to produce reactive peroxynitrite ($ONOO^-$), which leads to serious toxic reactions with biomolecules such as proteins, lipids and nucleic acids (Jagetia *et al*., 2004). In this study, the scavenging ability increased with increasing concentration of the extracts or standards though the activity of the standard (Gallic acid) were relatively more pronounced than that of the extracts (Figure 3). The NO radical scavenging activity of *Dissotis rotundifolia* whole plant may help arrest chain reactions initiated by excess generation of $ONOO^-$ that is detrimental to human health

The FRAP assay is mostly labored to measure the antioxidant influence of any substance in the reaction medium, as its reducing ability. Naturally present antioxidants are deliberated as reductants, and the inactivation of oxidants by reductants is described as a redox-reaction.[29] An overall trend found in the present study climaxes the fact that the methanol extract have better antioxidant capacities than the aqueous extract. A conceivable reason for this dissimilarity is that methanol and aqueous accumulate different quantities of phytochemicals, which in turn can affect the level of antioxidants present.

Hydroxyl free radicals are well known to damage cellular DNA in humans, and even partial damage to DNA can make a cell cancerous. UV-photolysis of H_2O_2 generates OH radicals, which cause oxidative damage. OH bound to DNA leads to strand breakage, deoxysugar fragmentation, and base modification.[30] The intensity of the DNA damage was reduced on a concentration dependent manner of the extracts towards DNA which shows the protective effect of the extract towards hydrogen peroxide induced damage. The result suggests that extracts of *D. rotundifolia* protect DNA through antioxidant activity and may be used in future to prevent cancer and related diseases. The result agrees with other reports which indicate the potential of plants to protect against free radical-mediated DNA damage.[31]

CONCLUSION

The results of this study indicate that extracts of *D. rotundifolia* whole plant contain a variety of phytochemical compounds that can competently protect the body against oxidative stress caused by free radicals and might therefore be used as a source of potent natural antioxidant compounds. The study also showed the DNA damage protective potential of the extracts, which could be used in disease therapy, thus, cancer prevention and related. The antioxidant activity of *D. rotundifolia* may justify further investigation of its other beneficial biological properties and determine its safety.

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CONFLICT OF INTEREST

The Authors declares that they have no conflict of interest.

ABBREVIATIONS

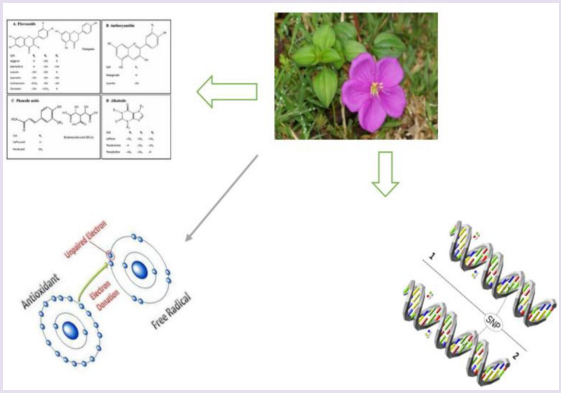
CTAB: cetyl trimethyl-ammonium bromide; FRAP: Ferric reducing antioxidant property; DPPH:1, 1 Diphenyl-2, picrylhydrazine; OH: Hydroxyl; NO: Nitric oxide.

REFERENCES

1. Halliwell B, Gutteridge JM, Free radicals in biology and medicine, Oxford University Press, USA. 2015.
2. Gulcin TI. Antioxidant and radical scavenging properties of curcumin. Chemico-biological Interactions. 2008;174(1):27-37.
3. Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition Research. 2003;23(12):1719-26.
4. Pisoschi AM, Cheregi MC, Danet AF. Total antioxidant capacity of some commercial fruit juices: electrochemical and spectrophotometrical approaches. Molecules (Basel Switzerland). 2009;14(1):480-93.
5. Prasad KN, Chew LY, Khoo HE, Kong KW, Azlan A, Ismail A. Antioxidant Capacities of Peel, Pulp, and Seed Fractions of Canarium odontophyllum Miq Fruit. J Biomed and Biotech. 2010;8.
6. Mann A, Egwim EC, Banji B, Abdukadir N, Gbate M, Ekanem J. Efficacy of *Dissotis rotundifolia* on Trypanosoma brucei brucei infection in rats. Afr J Biochem Res. 2009;3:5-8.
7. Harborne A. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science and Business Media, 1998.
8. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry. 2005;91(3):571-7.
9. Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium.* Pacific Science. 1974;48:458-63.
10. Oyaizu M. Studies on products of browning reaction – Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr. 1986;(44):307-15.
11. Pavithra K, Vadivukkarasi S. Evaluation of free radical scavenging activity of various extracts of leaves from Kedrostis foetidissima (Jacq.) Cogn. Food Science and Human Wellness. 2015;4(1):42-6.
12. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry. 1992;40(6):945-8.
13. Jagetia GC, Rao SK, Baliga MS. The evaluation of nitric oxide scavenging activity of certain herbal formulations *in vitro*: a preliminary study. Phytotherapy Research PTR. 2004;18(7):561-5.
14. Devi KD, Punyarani K, Singh NS, Devi HS. An efficient protocol for total DNA extraction from the members of order Zingiberales-.suitable for diverse PCR based downstream applications. Springer Plus. 2013;2:669.
15. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. 1987;19:11-5.
16. Russo A, Izzo AA, Borrelli F, Renis M. Vanella, Free radical scavenging capacity and protective effect of Bacopa monniera L. on DNA damage. Phytotherapy Research PTR. 2003;17(8):870-5.
17. Alothman M, Bhat R. Karim AA Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem. 2009b;115(3):785-8
18. Hemwimol S, Pavasant P, Shotipruk A. Ultrasound-assisted extraction of anthraquinones from roots of Morinda citrifolia. Ultrason Sonochem. 2006;13(6):543-8.
19. Vinholes J, Vizzotto M. Synergisms in Alpha-glucosidase Inhibition and Antioxidant Activity of Camellia sinensis L. Kuntze and Eugenia uniflora L. Ethanolic Extracts. Pharmacognosy Res. 2017;9(1):101-7.
20. Oktay M, Gulcin I, Kufrevioglu OI. Determination of *in vitro* antioxidant activity of fennel (Foeniculum vulgare) seed extracts. LWT Food Sci Technol. 2003;36(2):263-71.
21. Mustafa RA, Abdul Hamid A, Mohamed S, Bakar FA. Total phenolic compounds, flavonoids, and radical scavenging activity of 21 selected tropical plants. Journal of Food Science. 2010;75:C28-35.
22. Farmani F, Moein M, Khoshnood MJ, Sabahi Z. Anthocyanin Isolation from *Berberis integerrima* Bunge Fruits and Determination of Their Antioxidant Activity. Free Radicals and Antioxidants. 2018;8(1):1-5.
23. Davis DR, Riordan HD. "Changes in USDA food composition data for 43 garden crops." J Am Coll Nutr. 2004;23(6):669-82.
24. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. "Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study." Lancet. 1993;342(8878):10-11.
25. Vaclavıkova R, Kondrova E, Ehrlichova DM, Boumendjel A, Kovar J, Stopka P. "The effect of flavonoid derivatives on doxorubicin transport and metabolism."

Bioorg Med Chem. 2008;16(4):2034-42.
26. Nandhakumar E, Indumathi P. *In vitro* antioxidant activities of methanol and aqueous extract of Annona squamosa (L.) fruit pulp. Journal of Acupuncture and Meridian Studies. 2013;6(3):142-8.
27. Ebrahimzadeh M, Bahramian F. Antioxidant activity of Crataegus pentaegyna subsp. elburensis fruits extracts used in traditional medicine in Iran. Pakistan Journal of Biological Sciences. 2009;12(5):413.
28. Moncada S, Palmer R, Higgs E. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological Reviews. 1991;43(2):109-142.
29. Sun T, Ho CT. Antioxidant activities of buckwheat extracts. Food Chem. 2005;90(4):743-9.
30. Guha G, Rajkumar V, Mathew L, Kumar RA. The antioxidant and DNA protection potential of Indian tribal medicinal plants, Turkish Journal of Biology. 2011;35(2):233-42.
31. Kalita S, Kumar G, Karthik L, Rao KV. *In vitro* antioxidant and DNA damage inhibition activity of aqueous extract of *Lantana camara*L. (Verbenaceae) leaves. Asian Pac J Trop Biomed. 2012;(2):S1675-9.

GRAPHICAL ABSTRACT



SUMMARY

- Antioxidants are natural sources that help to scavenge free radicals.
- Concentrations of plant extracts ranging from 0.02 to 0.10 mg/ml were prepared
- Extracts of *D. rotundifolia* whole plant contain a variety of phytochemical compounds in appreciable quantities.
- Methanol extract exhibited better antioxidant capacities (DPPH, NO, OH and FRAP) than the aqueous extract.
- *D. rotundifolia* extracts exhibited considerable protection to the damage of native supercoiled circular.
- herefore *D. rotundifolia* extracts can be used as a source of potent natural antioxidant compounds.

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