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In Vitro Antioxidant and Oxidative DNA Damage Protective Properties of Methanol and Aqueous Extracts of *Dissotis rotundifolia* Whole Plant

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Abstract:
Antioxidants present in natural sources helps to scavenge free radicals and thus, provide health benefits. The study report the in vitro radical scavenging capacity of methanol and aqueous extracts of *Dissotis rotundifolia* whole plant. The main objective for this research was to use standard procedures to determine phenolic content, nitric oxide scavenging activity, hydroxyl radical scavenging activity, DPPH-scavenging activity and DNA damage protecting activity to evaluate the antioxidant potential of various extracts. Concentrations of plant extracts ranging from 0.02 to 0.10 mg/ml were prepared and mixed with appropriate volumes of reagents and the respective absorbances read at the respective wavelength. 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 cancer prevention.

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

I. 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 ($OH^{\bullet}$), peroxyl ($ROO^{\bullet}$), peroxynitrite ($^{\bullet}ONOO^{\bullet}$) and nitric oxide ($NO^{\bullet}$) radicals as well as non-free radical species such as hydrogen peroxide (H2O2), ($1O^2$)nitrous acid (HNO2), 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, 4]. Biological systems are protected against free radical induced cell damage by the activity of inherent antioxidants 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 [5, 6]. 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, phenolics, 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 ccc[5, 7, 8]. Many studies have shown positive 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 [7, 9, 10]. Despite extensive research on the antioxidant properties of most plants, little is known about the many tropical underutilised 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 [11]. 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.

II. 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 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 screening of the methanolic and aqueous extracts of *D. rotundifolia* was carried out as per the standard protocols [12, 13] to determine the presence of glycosides, terpenoids, flavonoids, carbohydrates, protein, alkaloids, phenolic compounds, tannins, saponins and Phytosterols.

Determination of total phenolic content
The total phenolic content was determined using the Folin-Ciocalteu assay described by Meda et al [14]. 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 free radical scavenging activity

Determination of Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extracts was determined according to the method reported by Pavithra and Vadivukkarasi [15] 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 mol/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 minutes. 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 A0 was the blank absorbance and A1 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 [16]. 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 minutes. 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[17]. 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 hours. Thereafter, 1.5ml of Griess reagent [1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylene diamine dihydrochloride] was added. The absorbance was measured at 540nm after 30 minutes against a phosphate buffer blank. Control was maintained with all chemicals excluding extract. The % scavenging activity of nitric oxide was calculated using the formula:

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

Cowpea seeds (*Vigna unguiculata*) purchased from a market at Cape Coast, Ghana, were sowed and allowed to germinate. After one week, the leaves of the germinated seeds were collected.

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[18]. Oxidative damage to DNA was induced using hydroxyl free-radical generating system (H2O2/UV) described by Russo et al [19] 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% H2O2. 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 H2O2. 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.

III. RESULTS AND DISCUSSION

Phytochemical Analysis

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). 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 [20-22]. Phenolics are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants or plant products. The total phenolic content of methanol extract of *D. rotundifolia* (5.0725 mg GAE/g) was higher than the aqueous extract (3.272 mg GAE/g). 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[23].

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

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

Present (+), absent (-)

Antioxidant 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 1. 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. 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 [24, 25]. Furthermore, this result is consistent with other findings [25, 26] where methanol extract showed higher antioxidant activity over aqueous extract.

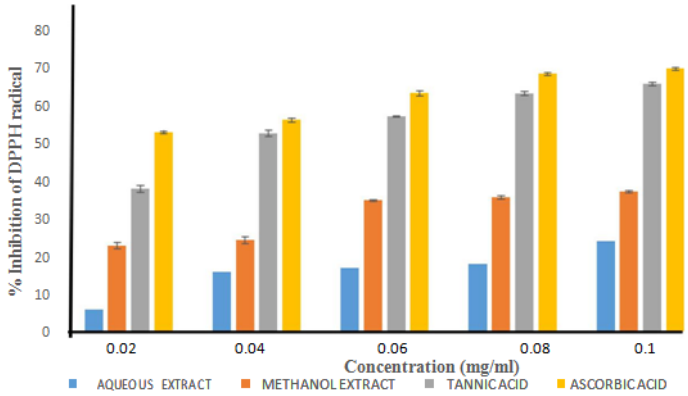

Figure.1. DPPH scavenging activities of methanol and aqueous extracts from *Dissotis rotundifolia* whole plant and standard tannic acid and ascorbic acid

Hydroxyl radical is the most reactive oxygen centered species and causes severe damage to adjacent biomolecule. Figure 2 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. Similarly to reactive oxygen species, NO is also implicated in inflammation, cancer and other pathological conditions [27]. NO is a very unstable species under aerobic conditions. 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. 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.

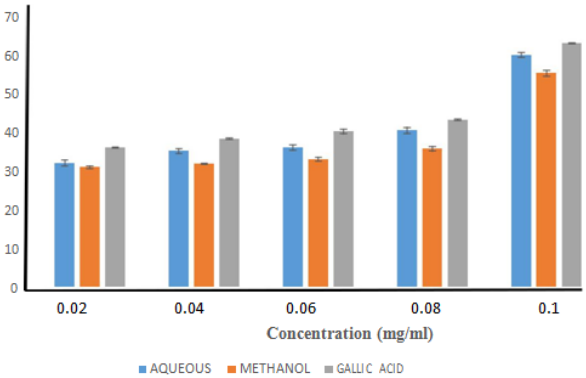

Figure.2. Hydroxyl scavenging activities of both methanol and aqueous extracts from *Dissotis rotundifolia* whole plant and standard gallic acid.

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 (fig3). Gallic acid recorded the highest % radical scavenging ability, followed by aqueous extract and methanol extract, i.e. 42.48%, 24.88%, and 23.18% 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

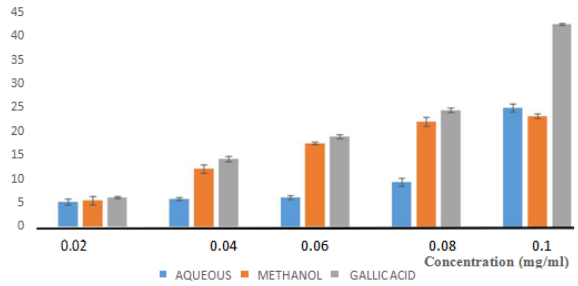

Figure.3. Nitric oxide scavenging activities of methanol and aqueous extracts from *Dissotis rotundifolia* whole plant and standards, tannic acid and gallic acid.

DNA damage protection

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 H2O2 generates OH radicals, which cause oxidative damage. OH bound to DNA leads to strand breakage, deoxysugar fragmentation, and base modification [28]. Figure 4 shows the electrophoretic pattern of DNA after UV-photolysis of H2O2 (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). 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 H2O2 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 H2O2. The addition of methanol and aqueous extracts of *D. rotundifolia* to the reaction mixture of H2O2 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. 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. The result agrees with other reports which indicate the potential of plants to protect against free radical-mediated DNA damage [28, 29].

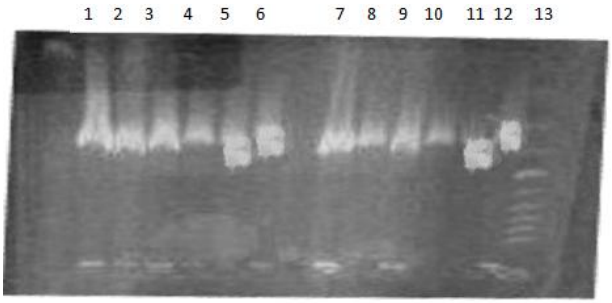

Figure.4. Electrophoretic pattern of DNA after UV-photolysis of H2O2 in the presence or absence of methanol or aqueous extracts from *Dissotis rotundifolia* whole plant.). Lane 6&12: control (DNA only), lane 5&11: treated (DNA+H2O2), lane 4&10: untreated (DNA+ extracts), lane 3&9: treated (DNA+10mg/ml extracts+H2O2), lane 2&8: treated (DNA+0.1mg/ml exracts+H2O2) and lane 1&7: treated (DNA+ tannic acid+H2O2) and lane 13: marker.

IV. 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 cancer prevention. The antioxidant activity of *D. rotundifolia* may justify further investigation of its other beneficial biological properties and determine its safety.

V. REFERENCES

[1]. B. Halliwell, J.M. Gutteridge, Free radicals in biology and medicine, Oxford University Press, USA, 2015.

[2]. T. Ak, I. Gulcin, Antioxidant and radical scavenging properties of curcumin, Chemico-biological interactions, 174 (2008) 27-37.

[3]. C. Guo, J. Yang, J. Wei, Y. Li, J. Xu, Y. Jiang, Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay, Nutrition Research, 23 (2003) 1719-1726.

[4]. B. Uttara, A.V. Singh, P. Zamboni, R.T. Mahajan, Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options, Current neuropharmacology, 7 (2009) 65-74.

[5]. A.M. Pisoschi, M.C. Cheregi, A.F. Danet, Total antioxidant capacity of some commercial fruit juices: electrochemical and spectrophotometrical approaches, Molecules (Basel, Switzerland), 14 (2009) 480-493.

[6]. A. Boakye, F. Wireko-Manu, J. Agbenorhevi, I. Oduro, Antioxidant activity, total phenols and phytochemical constituents of four underutilised tropical fruits, International Food Research Journal, 22 (2015) 262-268.

[7]. K.N. Prasad, L.Y. Chew, H.E. Khoo, K.W. Kong, A. Azlan, A. Ismail, Antioxidant Capacities of Peel, Pulp, and Seed Fractions of Canarium odontophyllum Miq. Fruit, Journal of Biomedicine and Biotechnology, 2010 (2010) 8.

[8]. B. Halliwell, J. Gutteridge, Oxygen toxicity, oxygen radicals, transition metals and disease, Biochemical journal, 219 (1984) 1.

[9]. G. Piluzza, S. Bullitta, Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area, Pharmaceutical biology, 49 (2011) 240-247.

[10]. B. Halliwell, Dietary polyphenols: good, bad, or indifferent for your health, Cardiovascular research, 73 (2007) 341-347.

[11]. A. Mann, E.C. Egwim, B. Banji, N. Abdukadir, M. Gbate, J. Ekanem, Efficacy of Dissotis rotundifolia on Trypanosoma brucei brucei infection in rats, Afr J Biochem Res, 3 (2009) 5-8.

[12]. A. Harborne, Phytochemical methods a guide to modern techniques of plant analysis, Springer Science & Business Media, 1998.

[13]. G. Trease, W. Evans, A text book of pharmacognosy Academic press, London, 198 (1989) 22-40.

[14]. A. Meda, C.E. Lamien, M. Romito, J. Millogo, O.G. Nacoulma, Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity, Food Chemistry, 91 (2005) 571-577.

[15]. K. Pavithra, S. Vadivukkarasi, Evaluation of free radical scavenging activity of various extracts of leaves from Kedrostis foetidissima (Jacq.) Cogn, Food Science and Human Wellness, 4 (2015) 42-46.

[16]. K. Shimada, K. Fujikawa, K. Yahara, T. Nakamura, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, Journal of Agricultural and Food Chemistry, 40 (1992) 945-948.

[17]. G.C. Jagetia, S.K. Rao, M.S. Baliga, S.B. K, The evaluation of nitric oxide scavenging activity of certain herbal formulations in vitro: a preliminary study, Phytotherapy research : PTR, 18 (2004) 561-565.

[18]. J.J. Doyle, J.L. Doyle, A rapid DNA isolation procedure for small quantities of fresh leaf tissue, Phytochemical Bulletin, 19 (1987) 11-15.