

Comparative Phytochemical Evaluation of *Dissotis rotundifolia* Root and Leaf

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Abstract: *Dissotis rotundifolia* is a common medicinal plant in Nigeria. Quantitative phytochemical analyses were carried out in both dry leaf and root samples using spectrophotometric method. The result of phytochemical analysis of the root revealed the presence of alkaloids (463.20 ± 6.70 mg/100g), polyphenol (179.80 ± 0.66 mg/100g), flavonoids (144.40 ± 5.60 mg/100g), phenol (13100 ± 6.86 mg/100g), tannin (126.80 ± 1.76 mg/100g), cyanogenic glycoside (75.20 ± 2.20 mg/100g), anthraquinone (40.64 ± 1.26 mg/100g), saponin (29.00 ± 0.02 mg/100g), anthocyanin (11.50 ± 1.10 mg/100g) and saponin (0.90 ± 0.60 mg/100g). The result also revealed the presence of alkaloids (155.00 ± 38.32 mg/100g), phenols (110.80 ± 3.42 mg/100g), polyphenols (139.50 ± 8.08 mg/100g), tannins (89.88 ± 1.84 mg/100g), flavonoids (58.80 ± 5.25 mg/100g), cyanogenic glycosides (58.24 ± 1.76 mg/100g), anthraquinones (27.94 ± 6.04 mg/100g), anthocyanin (13.04 ± 0.87 mg/100g), saponins (3.20 ± 0.14 mg/100g) and saponin (3.09 ± 0.04 mg/100g) in the leaf sample. The results indicate that the leaf and seed of *Dissotis rotundifolia* contains appreciable amount of bioactive compounds. Medically the presence of these phytochemicals explains the use of these plant parts in ethnomedicine for the management of ailments.

Key words: *Dissotis rotundifolia* • Phytochemical • Quantitative • Leaf and Root

INTRODUCTION

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal plants” [1]. Some of the drugs used today (e.g., aspirin, codeine, morphine, vinblastine, vincristine, pilocarpine, cocaine, atropine, emetine and ephedrine) have originated from medicinal plants [2]. It has been estimated that approximately one fourth of the prescriptions dispensed from community pharmacies in the United States contain one or more ingredients derived from plant origin [3]. Medicinal plants are generally used in two different ways: as complex mixture made of single plant extract containing a broad range of constituents or multi component mixture comprised of several closely related biologically active compounds. Selection of plant species to be studied when screening for biologically active constituents is a crucial factor for success of the investigation nevertheless, the selection should be mainly based on the ethno-pharmacological selection process that is based on the therapeutic use of plant species by a specific ethnic group [1].

The genus *Dissotis* comprises of 140 species native to Africa [4]. *Dissotis rotundifolia* Triana is a native of tropical West Africa. It belongs to the *Melastomataceae* family and common names include “Pink lady” in English, “Ebafo” in Benin, “Awede” in Yoruba and “Nkpisi-nku” in Igbo [4]. It is a versatile perennial slender creeping herb with prostrate or ascending stems up to 40 cm high, rooting at the nodes and producing from seeds. Traditionally, in various parts of tropical Africa, it has various uses. In Nigeria, the plant is used mainly for the treatment of rheumatism and painful swellings and the leaves decoction is used to relieve stomach ache, diarrhoea, dysentery, cough, conjunctivitis, circulatory problems and venereal diseases [4]. It is used in East Africa for the treatment of bilharzias and in Cameroun, the leaves are used to treat dysentery [5].

Although some plants may have medicinal values, sometimes the medicinal preparations inflicts side effect [6]. Phytochemicals are secondary metabolites of plants which include: alkaloids, flavonoids, saponins, anthraquinones, glycosides, cyanogenic glycosides, phenolic acids, anthocyanins and tannins and others. Phytochemicals have been linked to many positive health

effects in human and animal studies, including coronary heart diseases, diabetes, high blood pressure, inflammation, infections, ulcer, wounds and macular degeneration. It is becoming evident that many phytochemicals may have multiple actions on human health, for example; flavonoids in many fruits and leaves of plants not only have anticancer properties but have also been shown to have anti-allergic, anti-inflammatory properties and cardiovascular protective properties [7]. Alkaloids often have marked pharmacological effect when administered to man and other animals, thus, their presence in plant is of a particular interest [7]. With recent wave of economic meltdown and its attendant effect on the purchasing power of the populace of less developed nations, it becomes vivid that the herb will play an increasing role in health security of the rural people and the increasing urban poor. As common as this herb is in Nigeria, there is still scarce information on the phytochemical constituents. Hence this study was carried out to evaluate the phytochemical constituents of *Dissotis rotundifolia* leaf and seed.

MATERIAL AND METHODS

The fresh leaves and roots of *Dissotis rotundifolia* were collected from Mgbalukwu Inyimagu village in Izzi Local Government Area of Ebonyi State, Nigeria. The plant identified by a taxonomist Prof. S.S.C. Onyekwelu of Applied Biology Department, Ebonyi State University Abakaliki, Nigeria. The fresh leaves and roots were allowed to dry at room temperature and were blended to powdery forms using an electric blender and stored in airtight container.

Quantitative Phytochemical Analysis of *Dissotis rotundifolia* Leaf and Root: The phytochemical constituents of the samples were carried out by the methods modified by the following

Determination of Flavonoids: This was determined by the method of Harborne [8].

Principle: Flavonoids reacts with dilute ammonia (NH_3) to produce a coloured complex which can be measured spectrophotometrically at 470nm.

Procedure: 1g of the samples each were macerated with 20mls each of ethylacetate for 5mins, 5mls each were

transferred into a triplicate tubes and 5mls of dilute ammonia (NH_3) each were added and stirred for 5mins and allowed to stand for some time. The lower layers were collected and the absorbances were read at 470nm against dilute ammonia.

Determination of Phenols: This was determined by the method of Malick and Singh [9].

Principle: Phenols react with phosphomolybdic acid in folin-ciocalteu reagent in alkaline medium to produce a blue coloured complex (Molybdenum blue) which can be estimated spectrophotometrically at 650nm.

Procedure: 1g of the sample was macerated with 20mls of 80% methanol for 10mins and centrifuged for 5mins. Then, 1ml of the supernatant was transferred into triplicate tubes. In the tubes 4mls of distilled water and 0.5ml of folin-ciocalteu were added and mixed properly. After 5mins 2mls of 20% Na_2CO_3 (Sodium carbonate) was also added and stirred and allowed to stand for 30mins. The absorbance was taken at 650nm against the blank. The same procedure was repeated with the second sample.

Determination of Polyphenols: This was determined by the method of Katzung [10].

Principle: The principle is based on the oxidation of molecule containing $-\text{OH}$ groups in the presence of phosphomolybdic acid in an alkaline medium to produce a highly blue coloured solution which is measured spectrophotometrically at 750nm.

Procedure: 1g of the sample was macerated with 20mls of 80% methanol for 10mins and centrifuged for 5mins. Then, 1ml of the supernatant was transferred into triplicate tubes. In the test tubes, 4mls of distilled water and 0.5ml of folin-ciocalteu were added and stirred. After 5mins, 2mls of 20% Na_2CO_3 (Sodium carbonate) was add and allowed to stand for 30mins. The absorbance was taken at 750nm against the blank. The same procedure was repeated with the second sample.

Determination of Alkaloids: This was determined using the method of Harborne [8].

Principle: H_2SO_4 reacts with alkaloids in the presence of formaldehyde to form a coloured complex which is read spectrophotometrically at 565nm.

Procedure: 1g of the sample was macerated with 20mls of methanol and 20% sulfuric acid at the ratio of 1:1 (i.e. 10ml of each) for 5mins and centrifuged for 5mins. Then, 0.5ml of the supernatant was transferred into triplicate tubes. In the tubes 2.5mls of 60% sulfuric acid was added and stirred. After 5mins, 2.5 mls of 0.5% formaldehyde in 60% sulfuric acid was added and allowed to stand for 3hrs. The absorbance was taken at 565nm against the blank. The same procedure was repeated with the second sample.

Determination of Tannins: This was determined by the method of Harborne [8].

Principle: Tannins reduce phosphotungstomolybdic acid in alkaline solution to produce highly coloured blue solution, the intensity of which is proportional to amount of tannins. The intensity is measured in spectrophotometer at 720nm.

Procedure: 1g of the sample was macerated with 20mls of methanol for 10mins and centrifuged for 5mins. Then, 5mls of the supernatant was transferred into triplicate tubes. In the tubes, 0.3ml of 0.1molar ferric chloride in 0.1molar HCl was added and stirred. Then 0.3ml of 8/10000 molar potassium ferricyanide was added and mixed and stood for 5mins. The absorbance was taken at 720 nm against the blank. The same procedure was repeated with the second sample.

Determination of Cyanogenic Glycosides: The method modified by Trease and Evans [11] was used to extract and estimate cyanogenic glycosides.

Principle: Cyanogenic glycosides react to alkaline picrate under boiling temperature to produce a colour that is read spectrophotometrically at 490 nm.

Procedure: 1g of sample was macerated in 20ml of distilled water for 5minutes and was allowed to stand over-night. It was centrifuged for 10minutes. 1ml of the supernatant was transferred into triplicate tube and 4ml of alkaline picrate solution was added into each tube. They were boiled in a water bath for 5minutes and allowed to cool at room temperature. The absorbance was taken at 490nm against the blank. The same procedure was repeated with the second sample.

Determination of Anthocyanin: This was determined using method of Trease and Evans [11].

Principle: Anthocyanin reacts with citrate buffer at pH of 3.4 to give a coloured complex in which the absorbance is read spectrophotometrically at 500nm.

Procedure: 1g of the sample was macerated with 20mls citrate buffer of pH of 3.5 for 5mins and centrifuged for 5mins. Then, 1ml of the supernatant was taken into two set of triplicate tubes, to one set of the triplicate tubes 4mls of citrate buffer at pH 3.4 of 1:1 HCl were added and mixed. They were allowed to stand for 1hr and the absorbances were taken at 500nm against distilled water. The same procedure was repeated with the second sample.

Determination of Anthraquinone: This was determined by the method of Trease and Evans [11].

Principle: Anthraquinone reacts with magnesium acetate to give a pink red color.

Procedure: 1g of sample was macerated with 20ml of 80% methanol and was centrifuged for 5minutes. Then, 1ml of supernatant was transferred into triplicate tubes. To each tube, 1ml of 0.5% magnesium acetate and 1ml of methanol was added and mixed very well before absorbance was taken at 515nm against the blank. The same procedure was repeated with the second sample.

Determination of Saponin: This was determined by the method of Harborne [8].

Principle: Saponin reacts with anisaldehyde and ethylacetate to give a coloured complex which is read spectrophotometrically at 430nm.

Procedure: 0.5g of the sample was macerated with 10mls of methanol for 10mins and centrifuged for 5mins; 2mls of the supernatant was transferred into triplicate tubes. The tubes were placed in water bath to evaporate the methanol and allowed to cool. Then, 2mls of ethylacetate and 1ml of 0.5% anisaldehyde in ethylacetate and 1ml of 5% H₂SO₄ in ethyl acetate were added and placed in a hot water bath at 60°C for 20mins and allowed cool in cold water for 10mins. The absorbance was taken at 430nm. The same procedure was repeated with the second sample.

RESULTS

The result of phytochemical analysis of *Dissotis rotundifolia* leaf and root samples are shown in Figure 1.

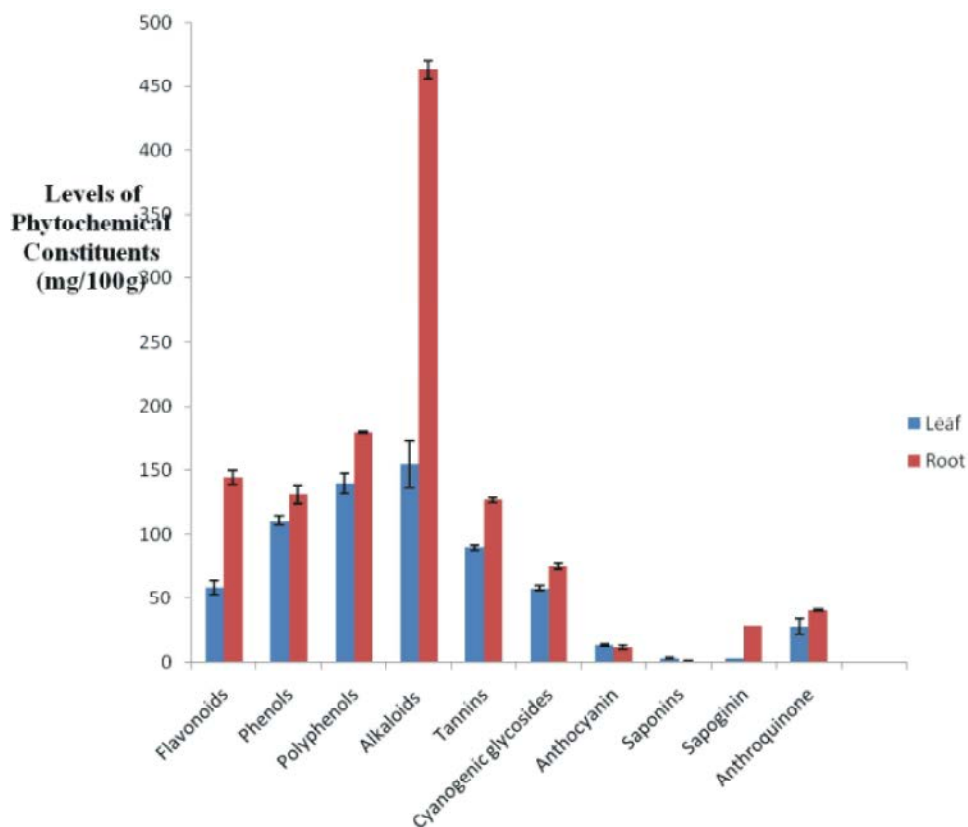


Fig. 1: Phytochemical constituents of leaf and seed of *Dissotis rotundifolia*

The results obtained showed that the root sample of *Dissotis rotundifolia* contained high levels of the phytochemical constituents analyzed than the leaf sample except in anthocyanin (11.50 ± 1.10 mg/100g; 13.04 ± 0.87 mg/100g) and saponins (0.90 ± 0.60 mg/100g; 3.20 ± 0.14 mg/100g) where the leaf sample of *Dissotis rotundifolia* showed high levels of the two phytochemical than the root sample as shown in Figure 1. It was also observed that *Dissotis rotundifolia* leaf and root revealed highest levels of alkaloids (463.20 ± 6.70 mg/100g; 155.00 ± 38.32 mg/100g), in relative to other phytochemical constituents analyzed (Figure 1).

DISCUSSION

Phytochemical analysis is very useful in the evaluation of active biological components of medicinal plants. The quantitative phytochemical analyses were carried out on the dried samples of *Dissotis rotundifolia* leaf and root. Alkaloids, phenols, polyphenols, flavonoids, cyanogenic glycosides, anthocyanins, anthraquinones, tannins, saponin and sapoginin were revealed to be present as shown in Figure 1. The results

obtained revealed that root sample richer in these phytochemical than the leaf sample (Figure 1). According to Aja *et al.* [12], Lawal *et al.* [13] and Wasagu *et al.* [14], phytochemical component are responsible for both pharmacological and toxic activities in plants. Some of these metabolites are said to be useful to both animal and plant itself. But some could be toxic to animals, including man at higher concentration. The presence of these phytochemical constituent in plant indicate that the plant, if properly screened, could yield drug of pharmaceutical significance. This assertion is supported by the fact that plants have been known to be involved in ethno-medicine in the management of various ailments [15, 16].

High levels of alkaloids showed in the samples means that *Dissotis rotundifolia* leaf and root are of pharmacological significance. Many synthetic and semi-synthetic drugs are structural modification of alkaloids which are designed to enhance or change the primary effects of the drug and reduce unwanted side effects. Alkaloids can be used as a central nervous system stimulant and as well as a powerful pain reliever [17]. Alkaloids are known to have antimicrobial activity as well as other physiological activities [18].

Phenols and polyphenols are strong antioxidants which prevent oxidative damage to biomolecules such as DNA, lipids and proteins which plays a role in chronic diseases such as cancer and cardiovascular diseases [19]. Phenols can be used in reduction of risk for infection in minor skin irritations and it also kills germs. Phenols and polyphenols can improve effectiveness at relieving itching and it can be added to lotion meant for the relief of insect bites and sunburn and other painful itching skin conditions [20]. Phenolic compounds are commonly found in both edible and non-edible plants and they have been reported to have multiple biological effects including antioxidant property [20]. The high level of phenols and polyphenols in the leaf and root of *Dissotis rotundifolia* might be the reason behind their traditional uses in the treatment of rheumatism, painful swelling and circulatory problems as reported by Gill [16].

The obtained results revealed that flavonoids are high in both samples (Figure 1). Flavonoids have been shown to have wide range of biological and pharmacological activities such as: anti-allergic, anti-inflammatory, anti-oxidant and antimicrobial activities [20-24]. Flavonoids have been implicated in the inhibition of pro-inflammatory activity of enzymes involved in free radical production, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase [25] and modification of intracellular signaling pathway in cells [25]. Oxidative stress has been linked to cancer, aging, atherosclerosis and inflammation. Flavonoids may help provide protection against these diseases by contributing along with vitamins and enzymes to the total antioxidant defense system to human body [26].

Dissotis rotundifolia leaf and root contain an appreciable amount of tannins which indicates that they have antimicrobial activity [27, 28]. Tannins may offer protection against 6-hydroxydopamine induced toxicity [29]. Foods rich in tannins can be used in the treatment of hereditary hemochromatosis, a hereditary disease characterized by excessive absorption of dietary iron resulting in the pathological increase in total iron store [29].

Natural anthraquinone derivatives tend to have laxative effect [30]. 5-anthraquinone have been shown to inhibit the formation of tau aggregates and dissolve paired helical filaments thought to be critical to Alzheimer's disease progression [31]. Anthraquinone rich substances can cause mutagenicity and potential carcinogenicity [32]. Rhein is an anthraquinone aglycone found in rhubarb that inhibits the activity of cytokines in models of osteoarthritis [33]. This observation led to the development of diacerhein (Diacetylrhein), a synthetic

derivative with better bioavailability. In early clinical studies, oral diacerhein at 100 mg/day improved symptoms in patients with osteoarthritis. Anthraquinones has laxative effect which indicates why plants like rhubarb, senna and cascara has been used since prehistory for such effect [34], nevertheless long term use of anthraquinone laxatives leads to a condition known as melanosis (Or pseudomelanosis) coli [34].

The result showed that *Dissotis rotundifolia* leaf and root contain an appreciable amount of saponin (Figure 1). Saponins bind to bile salt and cholesterol in the intestinal tract. Bile salts from small micelles with cholesterol facilitate its absorption. Saponin causes a reduction of blood cholesterol by preventing its reabsorption [17]. Heffman [35] reported that saponins inhibit sodium ion (Na^+) efflux by the blockage of the entrance of $\text{Na}^+ - \text{Ca}^{2+}$ anti-porter in cardiac muscle, which strengthens the contraction of heart muscle. Considering diosgenin a type of sapogenin which is found to resemble cholesterol in structure and thus interfere with both dietary and endogenous cholesterol absorption, thus leading to increased rate of hepatic and intestinal cholesterol synthesis [36]. Diosgenin also markedly enhance cholesterol secretion into bile which in conjunction with unabsorbed cholesterol, resulted in increased fecal excretion of cholesterol without excretion of bile acid [36].

CONCLUSION

The result of the study revealed that the phytochemicals levels were higher in the root sample of *Dissotis rotundifolia* than the leaf. Phytochemical agents have varying pharmacological potentials that are vital for healthy living conditions, especially in rural areas where access to modern health facilities is limited, medicinal plants/herbs remain the main stay of health care system.

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