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## Phytochemical composition and antioxidant property of *Dissotis rotundifolia* used for malaria management in south Benin

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**Abstract**

Oxidative stress contributes to induction and progression of many diseases such as malaria. The antioxidant activity of ethanolic and aqueous extracts of *Dissotis rotundifolia* were investigated using superoxide anion assay, hydrogen peroxide assay, Ferric Reducing Power method and 2, 2-diphenyl-1-picrylhydrazyl assay. Phytochemicals such as total phenolics and flavonoids were assessed by Folin-Ciocalteu and aluminum chloride methods. Ethanolic extract of *D. rotundifolia* showed the highest phenolic (217.12 ± 3.63mg Gallic Acid Equivalent/g) and flavonoid (22.68 ± 2.14 mg Equivalent Quercetin/g) contents. The same extract showed a higher antioxidant activity with an IC<sub>50</sub> value of 9.93 ± 1.62 µg/mL for 2, 2-diphenyl-1-picrylhydrazyl assay. Superoxide anion radical scavenging (59.69 ± 2.07%), hydrogen peroxide scavenging (81.73 ± 1.29%) and ferric reducing power (910.33 ± 12.52 µmol Ascorbic Acid Equivalent/g) of ethanolic extract were also higher than aqueous extract. *Dissotis rotundifolia* has potent antioxidant activity which probably contributes to its effectiveness in malaria therapy.

**Keywords:** Malaria, antioxidant activity, *dissotis rotundifolia*

**1. Introduction**

In 2017, there were an estimated 435 000 deaths from malaria globally with 219 million cases. Children aged under 5 years are the most vulnerable group affected by malaria. In 2017, they accounted for 61% (266 000) of all malaria deaths worldwide [1]. The WHO African Region accounted for 93% of all malaria deaths in 2017 [1]. People living in developing countries are the most exposed to malaria and are the most dependent on plant resources for the treatment of malaria, especially the people of West Africa [2, 3]. However, do all plants used empirically by local populations have therapeutic properties for treating malaria? The pertinence of this question comes from the fact that some of the plants used by the populations are known to treat other diseases in these same populations. With the emergence of *plasmodium* resistance to some currently available antimalarial drugs [4], it becomes imperative to search for new drugs. The use of plants in traditional medicine for treating various ailments remains an integral part of the culture and traditions of a majority of the world's population. In addition, factors such as the bioavailability and affordability of medicinal plants have led to their massive use. For their defense mechanisms, plants usually produce secondary metabolites such as tannins, saponins, alkaloids, flavonoids and essential oils. These chemical groups are involved in the therapeutic properties of most medicinal plants [5]. Therefore, malaria parasite also stimulates certain cells to produce reactive oxygen species (ROIs) thereby resulting in haemoglobin degradation [6, 7]. The reactive species of oxygen generated by macrophages is a specific example given their probable role in the destruction of parasitized and unparasitized red blood cells. An increase in lipid peroxidation was also noticed in patients with *P. falciparum* malaria [6, 8, 9]. Then, it is evident that ROIs resulting from cellular metabolism during *P. falciparum* infection contribute significantly to the complication of malaria. It is therefore necessary to find therapeutic agents able to reduce impact of the ROIs on host cells during malaria. Recent studies have shown that the use of natural antioxidants alone or in combination with an antimalarial improve antimalarial therapy [10]. Also, biochemical advances have been focused on antioxidants and their potency in minimizing the damaging effects of free radicals, as well as their roles in potentiating drug efficacy [11].

In a previous work, a review of medicinal plants showed the great diversity of plants used by people and practitioners of traditional medicine to treat malaria in southern Benin [12]. Comparative bibliographies of the listed plants lead to select *Dissotis rotundifolia*, one of the most traditionally used. In order to justify its therapeutic use, the chemical compositions as well as the antioxidant potential of the crude extracts of *D. rotundifolia* have been determined.

## 2. Material and methods

### 2.1 Collection and identification of plant materials

*Dissotis rotundifolia* were collected in November 2016 in Southern Benin (Ifangni, Plateau) during an ethnobotanical survey. A voucher specimen was identified and deposited at the National Herbarium of University of Abomey-Calavi, bearing the number YH 313/HNB.

### 2.2 Extraction of plant materials

The plant sample was washed with distilled water, dried at  $22^{\circ}\text{C} \pm 2$  for two weeks and then ground into a fine powder. The ethanol extract were prepared at room temperature by soaking 100 g of dry powder into 1 L of ethanol using an ultrasonic sonicator (Vibra Cell 75115, Biobloc Scientific). The aqueous extract were prepared by heating ( $80^{\circ}\text{C}$ ) 100g of powder in 1 L of distilled water. Each extraction is repeated three times. The mixture were filtered through Whatman filter paper CAT N 1001 150mm, dried and concentrated using a rotary evaporator (BUCHI Rotavapor RII, Switzerland). The obtained extracts were stored at  $4^{\circ}\text{C}$  for later use.

### 2.3 Phytochemical screening

Tests were carried out on both extracts of *Dissotis rotundifolia* using standard procedures to identify the chemical contents such as alkaloids, triterpenes, flavonoids, anthracenes, tannins, saponins, coumarins, essential oils, naphthoquinones, anthocyanes and lignanes [13,14].

### 2.4 Total phenolic content

Total phenolics of extracts were estimated spectrophotometrically using the Folin-ciocalteu assay [15] with gallic acid as the standard solution. The protocol is based on the quantification of the blue color produced when phenolic compounds reacts with mixture  $\text{WO}_4^{2-}/\text{MoO}_4^{2-}$  of Folin reagent. The standard calibration curve was plotted using Gallic acid ( $y = 0.043x - 0.051$ ;  $R^2 = 0.994$ ). The mean of three readings was used and the results expressed as mg of Gallic Acid Equivalents (GAE) /g of extract.

### 2.5 Total flavonoid content

The aluminum chloride colorimetric method [16] was used to determine the flavonoid content of the extracts. Quercetin was used as reference compound to produce the standard curve ( $y = 0.0133x - 0.01$ ;  $R^2 = 0.999$ ) and the results were expressed as mg of quercetin equivalent (QE)/100 mg of extract.

### 2.6 Antioxidant assays

Methods used for antioxidant activity were DPPH free radical scavenging assay, Superoxide scavenging assay, Hydrogen peroxide scavenging assay and Ferric-Reducing antioxidant power (FRAP) assay.

#### 2.6.1 Radical scavenging activity using DPPH method

The ability of the extract scavenge the free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was evaluated. The DPPH assay is a simple, rapid, economical, and widely used method to evaluate antioxidant activity. The antioxidant activity was determined according to the method previously described [17]. Each test sample's stock solution was diluted to final concentrations of 100 - 0.78  $\mu\text{g}/\text{mL}$  in methanol. The radical scavenging activity of the extracts was calculated as the percentage inhibition according to the following formula: scavenging activity (%) =  $[(AB - A_s)/AB] \times 100$ . Where  $A_s$ : is the sample (tested extract solution) absorbance and AB is the blank absorbance.

#### 2.6.2 Superoxide scavenging assay

The measurement of superoxide scavenging potential was determined using the reduction of nitro blue tetrazolium (NBT) method [18]. Quercetin was used as standard. The absorbance value of the reaction mixture is proportional to the superoxide anion scavenging activity of samples. The percent inhibition of superoxide anion generation was calculated using the following equation: Scavenging (%) =  $[(AB - A_s)/AB] \times 100$  where  $A_s$  is the sample (tested extract solution) absorbance and AB is the blank absorbance.

#### 2.6.3 Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) scavenging assay

$\text{H}_2\text{O}_2$  scavenging potential of ethanolic and aqueous extract of the plant were determined using the method described by [19] with a slight modification. Gallic acid was used as standard. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation:  $\text{H}_2\text{O}_2$  radical scavenging activity =  $(A_{\text{control}} - A_{\text{test}})/A_{\text{control}} \times 100$ ; where  $A_{\text{control}}$  is the absorbance of  $\text{H}_2\text{O}_2$ ,  $A_{\text{test}}$  is the absorbance of  $\text{H}_2\text{O}_2$  radical +sample extract or standard.

#### 2.6.4 Ferric-reducing antioxidant power (FRAP) assay

The Ferric Reducing Antioxidant Power was based on iron (III) to iron (II) transformation in the presence of antioxidants. The iron (II) can be monitored by measuring the formation of Perl's blue at 700 nm. Reducing ability was performed using the procedure method described by [20] with some little modifications. Ascorbic acid was used to produce the calibration curve ( $y = 0.0074x + 0.0149$ ;  $R^2 = 0.976$ ). The ability of the plant extracts to reduce ferric ions from the linear calibration curve was performed in triplicate and expressed in micromoles ( $\mu\text{Mol}$ ) of Ascorbic Acid Equivalent per gram (AAE)/g of extract.

### 2.7 Statistical analysis

All assays were carried out in triplicate. Data were expressed as the mean  $\pm$  standard deviation (SD) using Graph Pad Prism Software, version 8.02 (San Diego, California, USA). Statistical analysis was done by student's *t*-test and  $p < 0.05$  considered as significant. The 50% inhibitory concentration ( $\text{IC}_{50}$ ) was calculated from the dose response curve (Graph Pad Prism Version 8.02) obtained by plotting percentage inhibition versus concentrations.

## 3. Results

### 3.1 Phytochemical screening

Phytochemical screening of *Dissotis rotundifolia* leaves revealed the presence of different phytoconstituents (table 1).

**Table 1:** Results of phytochemical screening of the extracts from *D. rotundifolia* whole plant.

Phytochemicals	<i>D. rotundifolia</i>	
	EtOH	H <sub>2</sub> O
Triterpenes	+	+
Flavonoids	+	+
Alkaloids	+	+
Anthracenes	+	-
Naphthoquinones	-	-
Tannins	+	+
Saponins	+	+
Coumarins	+	+
Essential oils	+	-
Anthocyanins	-	-
Lignans	+	-

+: Presence; -: Absence; EtOH: Ethanol; H<sub>2</sub>O: Aqueous; *D. rotundifolia*: *Dissotis rotundifolia*

The ethanolic extract of *Dissotis rotundifolia* contains more secondary metabolites than the aqueous extract; eg. of Anthracenes, essential oils and Lignans.

### 3.2 Total phenolic and flavonoids contents

The total phenol and flavonoids contents of extract from *E.*

*cyrosa* leaves are presented in Table 2. The results showed that phenolic contents of ethanolic extract ( $217.12 \pm 3.63$  mg GAE/g) of *D. rotundifolia* was higher ( $p < 0.05$ ) compared to the aqueous extract ( $139.2 \pm 0.14$  mg GAE/g). Otherwise, the obtained results show that ethanolic extract of *D. rotundifolia* exhibited a higher flavonoids content ( $22.68 \pm 2.14$  mg EQ/g) compared to the aqueous extract ( $12.15 \pm 0.57$  mg EQ/g).

**Table 2:** Total phenolic and flavonoids contents of *D. rotundifolia* extracts.

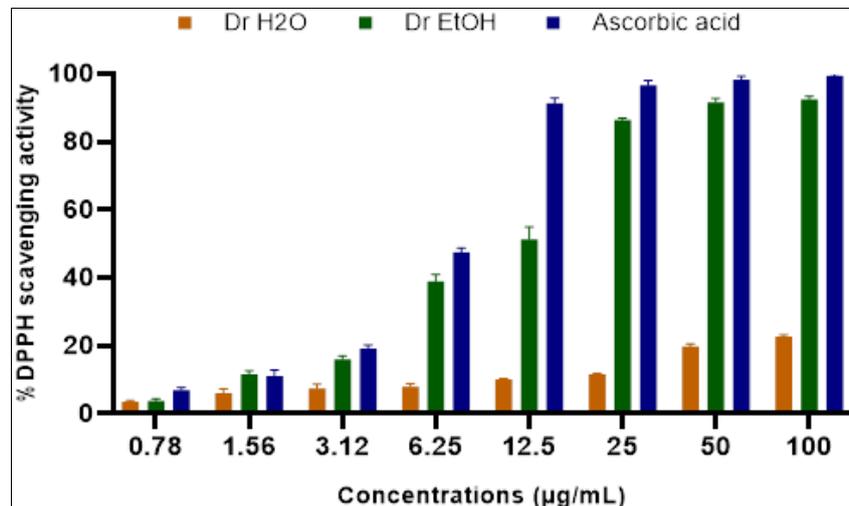
Extracts	Total phenolic (mg EAG/g)	Total flavonoids (mg EQ/g)
EtOH	$217.12 \pm 3.63$	$22.68 \pm 2.14$
H <sub>2</sub> O	$139.2 \pm 0.14$	$12.15 \pm 0.57$

EtOH: Ethanol; H<sub>2</sub>O: Aqueous; *D. rotundifolia*: *Dissotis rotundifolia*. Each value represents a mean  $\pm$  SD ( $n = 3$ ;  $p < 0.05$ ).

### 3.3 Antioxidant assays

#### 3.3.1 Radical scavenging activity assay (DPPH) on plant extracts

DPPH radical scavenging activities of extracts is dose-dependent ( $0.78$ - $100 \mu\text{g/mL}$ ) and the antioxidant activities ranging from  $3.51$  to  $92.66\%$  (Figure 1).

**Fig 1:** Radical scavenging activity of *D. rotundifolia* extracts

Dr. EtOH: *Dissotis rotundifolia* ethanolic extract; Dr. H<sub>2</sub>O: *Dissotis rotundifolia* aqueous extract. Each value represents a mean  $\pm$  SD ( $n = 3$ ;  $p < 0.05$ ).

IC<sub>50</sub> values indicate that the ethanolic extract of *D. rotundifolia* exhibited a higher DPPH scavenging activity ( $p < 0.05$ ) in comparison to the aqueous extract (Table 3).

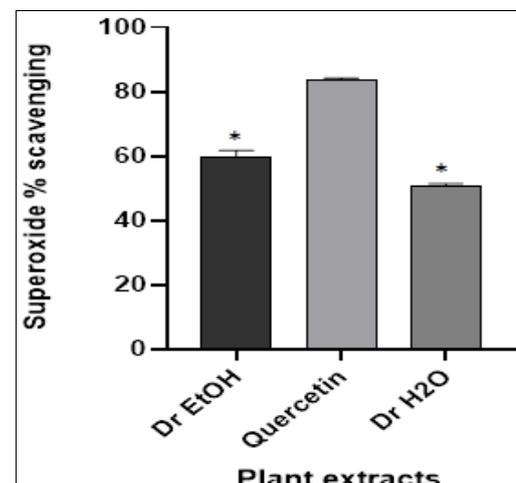
**Table 3:** IC<sub>50</sub> ( $\mu\text{g/mL}$ ) values of *D. rotundifolia* extracts

	<i>D. rotundifolia</i>		Ascorbic acid
	EtOH	H <sub>2</sub> O	
DPPH radical scavenging activity IC <sub>50</sub> ( $\mu\text{g/mL}$ )	$9.93 \pm 1.62$	$58.01 \pm 6.67$	$5.35 \pm 0.29$

Each value represents a mean  $\pm$  SD ( $n = 3$ ;  $p < 0.05$ ).

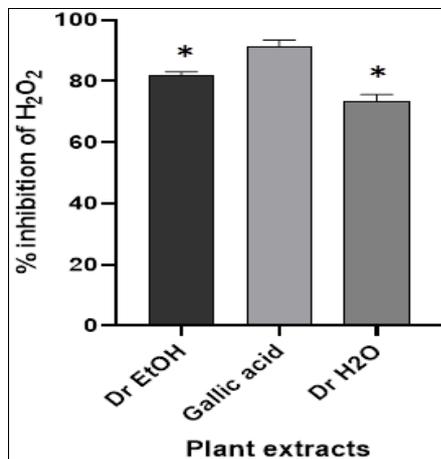
#### 3.3.2 Superoxide anion scavenging activity

In this assay, extracts and standards have moderate superoxide scavenging activities (% scavenging  $< 60\%$ ). The ethanolic extract of *D. rotundifolia* ( $59.69 \pm 2.07\%$ ) exhibited a higher superoxide anion scavenging activity ( $p < 0.05$ ) in comparison to the aqueous extract ( $50.74 \pm 0.71\%$ ).

**Fig 2:** Superoxide radical scavenging activity of ethanolic and aqueous extracts of *D. rotundifolia*. Each value is represented as mean  $\pm$  SD ( $n = 3$ ); \* Significant difference,  $p < 0.05$ . Dr EtOH: *Dissotis rotundifolia* ethanolic extract; Dr H<sub>2</sub>O: *Dissotis rotundifolia* aqueous extract.

### 3.3.3 Hydrogen peroxide scavenging assay

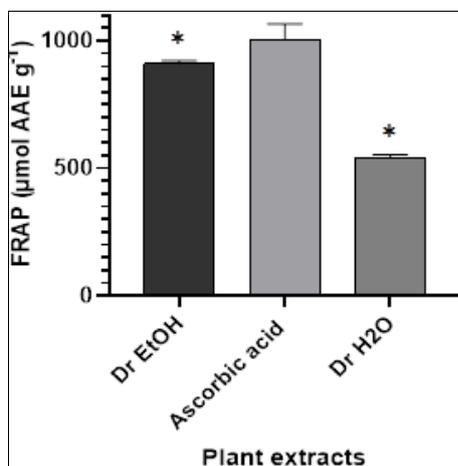
Hydrogen peroxide activity of *Dissotis rotundifolia* extracts was given in figure 3. Gallic acid was used as the positive control. The overall order of the inhibition of H<sub>2</sub>O<sub>2</sub> at 100 µg/mL was as follows: Gallic acid >Dr. EtOH >Dr. H<sub>2</sub>O. The results suggest that ethanol extract of *Dissotis rotundifolia* has a higher H<sub>2</sub>O<sub>2</sub> scavenging ability ( $p < 0.05$ ) in comparison to the aqueous extract (Table 4).



**Fig 3:** Hydrogen peroxide scavenging activity of ethanolic and aqueous extracts of *D. rotundifolia*. Each value is represented as mean  $\pm$  SD ( $n = 3$ ); \* Significant difference,  $p < 0.05$ . Dr EtOH: *Dissotis rotundifolia* ethanolic extract; Dr H<sub>2</sub>O: *Dissotis rotundifolia* aqueous extract.

### 3.3.4 Reducing power of extracts (FRAP)

Reducing power of *D. rotundifolia* extracts was showing in figure 4. In this study, the ethanolic extract ( $960.12 \pm 12.40$  µMol AAE g<sup>-1</sup>) showed a higher capacity of reduction ( $p < 0.05$ ) compared to the aqueous extract ( $540.07 \pm 3.08$  µMol AAEg<sup>-1</sup>).



**Fig 4:** Reducing power of ethanolic and aqueous extracts of *D. rotundifolia*.

Dr. EtOH: *Dissotis rotundifolia* ethanolic extract; Dr. H<sub>2</sub>O: *Dissotis rotundifolia* aqueous extract. Each value represents a mean  $\pm$  SD ( $n = 3$ ), \* Significant difference,  $p < 0.05$ ).

**Table 4:** *In vitro* antioxidant activity of *D. rotundifolia*

	<i>D. rotundifolia</i>	
	EtOH	H <sub>2</sub> O
DPPH (%)	92.66 $\pm$ 0.71	20.57 $\pm$ 0.84
O <sub>2</sub> <sup>-</sup> (%)	59.69 $\pm$ 2.07	50.74 $\pm$ 0.71
H <sub>2</sub> O <sub>2</sub> (%)	81.73 $\pm$ 1.29	73.52 $\pm$ 2.08
FRAP (µMol AAE/g)	910.33 $\pm$ 2.52	540.07 $\pm$ 3.08

All the results show that whatever the method of evaluation of the antioxidant activity used, the ethanolic extract have a better antioxidant activity in comparison to the aqueous extract (Table 4). The antioxidant activity is therefore a function of the extraction solvent.

## 4. Discussion

Phenolic compounds and some of their derivatives are known for their antioxidant and scavenging properties against free radicals. The antioxidant activity of medicinal plants has been linked to their phytochemical compositions [21].

The phytochemical screening of *D. rotundifolia* showed the presence of various secondary metabolites of which the triterpene, flavonoids, alkaloids, anthracenes, tannins, saponins, coumarins, essential oils, and lignanes.

The presence of phytoconstituents such as phenols, flavonoids, tannins and saponins in the ethanolic extract of *D. rotundifolia* whole plant may be a strong factor contributing to the local usage of this plant for the management of diseases. Tannins are polyphenolic compounds that possess anti-diuretic and anti-diarrhoea properties [22]. Saponins have demonstrated potential therapeutic activities as antifungal, antibacterial, and antioxidant agents [23]. The polyphenols present in the extract of the leaves can contribute synergistically to its antioxidant potential. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [24]. Total phenols and flavonoids have demonstrated modulatory effects against lipid peroxidation involved in atherogenesis and carcinogenesis [25].

Various groups of phytochemicals in these extracts of *D. rotundifolia* are believed to possess potent antioxidant activities, which makes them useful in some disease prevention and treatment [26]. The results obtained showed that phenols and flavonoids contents are significantly higher ( $P < 0.05$ ) in the ethanol extract compared to the aqueous extract. This observation could be due to the differences in polarity of the components extracted and that of the solvents used.

Reactive Oxygen/ Nitrogen Species have a main role in the endogenous defensive system, an imbalance in these system leads to oxidative stress and damage to cellular molecules leading to carcinogenesis [27]. Interest in antioxidants of natural origin as food and health supplements has increased much because of their potential to prevent and to reduce the risk of several diseases without any toxic effect [28]. Three main mechanisms would allow antioxidants to confer a defensive role: free electron transfer, hydrogen atom transfer, and metal chelation [29].

Since different antioxidant compounds may act through different mechanisms, no single method can fully evaluate the total antioxidant capacity. For this reason, several methods are used to evaluate the antioxidant activity of molecules, food or plant extracts [30,31]. In this work, we chose four different assays namely 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), superoxide anion (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activity and ferric reducing antioxidant power (FRAP), were used to access the antioxidant activity of *D. rotundifolia*. These methods are significantly representing the main methods of measuring the antioxidant properties of a substance.

The odd electron that contains the free radicals of DPPH is responsible for the deep purple color [32]. When an antioxidant compound gives an electron to the DPPH, it is decolorized and this discoloration can be quantitatively measured from the absorbance variations [33]. This reaction was observed in our

experiment with a color passing from violet to yellow, indicating that the hydrogen of an antioxidant is paired to the odd electron of DPPH radical scavenging the free radicals to form the reduced DPPH-H. Consequently, the absorbencies decreased from the DPPH radical to the DPPH-H formed. The degree of discoloration indicated the scavenging potential of the extracts in terms of hydrogen donating ability.

In this study, the results revealed that at each concentration, ethanolic extract of *D. rotundifolia* showed a higher activity ( $p < 0.05$ ) compared to the aqueous extract (figure 1). These results show that there is a correlation between antioxidant activities of extracts and extraction solvents. Similar observations have been reported in previous studies<sup>[34-36]</sup>.

Although, the reason behind this observation is still not completely understood, it could be attributed to the difference in the degree of solubility of the bioactive ingredients in the two solvents which guaranteed the extraction of more phytochemicals like phenolic compounds and flavonoids in the ethanolic than in the aqueous extract. Meanwhile, these phytochemicals are responsible for the antioxidant properties of plants.

As it is a reactive oxygen species, superoxide has some damaging properties that can be imposed to the cells and DNA and subsequently invites various diseases. Thus, a proposal has been established to gauge the comparative interceptive ability of the antioxidant extracts to scavenge the superoxide radical<sup>[37]</sup> by inhibiting the blue NBT formation<sup>[38]</sup>. The decrease of absorbance in presence of antioxidants indicates consumption of superoxide anion in the reaction mixture. Superoxide has been observed to directly initiate lipid peroxidation. It has also been reported that antioxidant properties of some flavonoids are effective mainly through scavenging of superoxide anion radical<sup>[39]</sup>. The results obtained clearly indicate that the ethanolic extract of *D. rotundifolia* has a noticed superoxide radicals scavenging activity.

Hydrogen peroxide ( $H_2O_2$ ) is an antimicrobial agent known for its cleansing property when it was introduced into clinical practice<sup>[40]</sup>. Its toxicity on human cells has made it lose this favor lately<sup>[41]</sup>.  $H_2O_2$  considered as an alternative for western conventional medicine, is generally used as a liquid form to treat patients. Sometimes, mixed with water it can also treat skin infections or dirty wounds<sup>[42]</sup>. The hydrogen peroxide scavenging activity of ethanol and aqueous extracts of *D. rotundifolia* can be attributed to the proton donating potential of its phytochemical components. Scavenging activity of  $H_2O_2$  by the extracts may be attributed to their phenolics, which can donate electrons to  $H_2O_2$  there by neutralizing it into water<sup>[43]</sup>.

The reducing power of the extract may serve as a significant indicator of its potential antioxidant activity. The presence of antioxidant in the extract might cause the reduction of  $Fe^{3+}$ /ferric cyanide complex to ferrous form. The ethanolic extract of *D. rotundifolia* showed a considerable reducing power indicating that it can act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions. The reducing power of the extract, which potentially serves as a significant reflection of the antioxidant activity, was ascertained using a modified  $Fe^{3+}$  to  $Fe^{2+}$  reduction assay, whereby the color of the test solution, which was yellow, transforms to various hues of green and blue, based on the extent of the reducing power of the samples. The presence of the antioxidants in the samples leads to  $Fe^{3+}$ /ferricyanide complex reduced to the  $Fe^{2+}$  form.

DPPH,  $O_2^-$ ,  $H_2O_2$  and FRAP antioxidant methods may present different results. Then, they showed that the ethanolic extract of *D. rotundifolia* possesses a high antioxidant potential in comparison to the aqueous extract<sup>[44]</sup>. The antioxidant activity of plant species is therefore function of the extraction solvent.

## 5. Conclusion

The results of this study clearly reveal that extracts of *D. rotundifolia* whole plant contain different phytochemical compounds including the appreciable content of phenolic compounds, which possess high antioxidant properties protecting the body against oxidative stress caused by free radicals and can be used to treat many diseases. Thus, with respect to the solvents used for extraction, the ethanolic extract had a higher content of phytochemicals such as phenolic compounds compared to the aqueous extract, an observation which correlated positively with the higher antioxidant activities exhibited by the same extract. The two solvents used in this study have been chosen to represent those commonly used by the local traditional healers in the preparation of this plant for medicinal use. The results of DPPH, superoxide anion radical,  $H_2O_2$  and FRAP, assays showed that the extracts possess not only the antioxidant activities, but also potent free radical scavenger capability. *D. rotundifolia* is a potential free radical scavenger and a useful source of natural antioxidants which may justify its therapeutic effectiveness such as antimalarial property. Further studies are currently ongoing to investigate the antimalarial activity empirically attributed to this plant by populations.

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## 7. Conflict of Interest

The authors declare no competing interest

## 8. References

1. World Health Organization. World Malaria Report, 2018.
2. World Health Organization. World Malaria Report, 2011.
3. Zofou D, Kowa TK, Wabo HK, Ngemenya MN, Titanji VPK. *Hypericum lanceolatum* (Hypericaceae) as a potential source of new anti-malarial agents: a bioassay-guided fractionation of the stem bark. *Malaria J.* 2011; 10:167.
4. Friedrich MJ. Resistance to Malaria Drugs. *JAMA.* 2011; 305:663-663
5. Ghribia L, Ghouilaa H, Omrib A, Besbes M, Janneta HB. Antioxidant and anti-acetylcholinesterase activities of extracts and secondary metabolites from *Acacia cyanophylla*. *Asian Pac J Trop Biomed.* 2014; 4:S417-423.
6. Das BS, Nanda NK. Evidence for erythrocyte lipid peroxidation in acute *falciparum* malaria. *Trans R Soc Trop Med Hyg.* 1999; 93:58-62.
7. Loria P, Miller S, Foley M, Tilley L. Inhibition of the peroxidation degradation of haem as the basis of action of chloroquine and other quinoline antimalarials. *Biochem J.* 1999; 339:363-370.
8. Erel O, Kocyigit A, Avci S, Aktepe N, Bulut V. Oxidative Stress and Antioxidative Status of Plasma and

- Erythrocytes in Patients with Vivax Malaria. Clin Biochem. 1997; 30:631-639.
9. Rath RN, Panigrahi N, Das BK, Das PK. Lipid peroxidation in acute *falciparum* malaria. Indian J Med Res. 1991; 93:303-305.
  10. Al-Obaidi MMJ, Taylor-Robinson AW. Regulation of Expression of Reactive Oxygen Intermediates During *Plasmodium* Infection To Reduce Immunopathology Provides A Possible Antioxidant Adjuvant To Enhance Anti-malarial Drug Therapy. Inter J Clin Microbiol. 2017; 1:8.
  11. Onyesom I, Osioma E, Omoghene O. Total Antioxidant Capacity in Serum of *Plasmodium falciparum* Malarial Infected Patients Receiving Artemisinin-Based Combination Therapy. Am J Med Med Sci. 2012; 2:1-3.
  12. Lagnika L, Djehoue R, Yedomonhan H, Sanni A. Ethnobotanical survey of medicinal plants used in malaria management in South Benin. J Med. Plant Res. 2016; 10:748-756.
  13. Shinde P, Gadekar P, Patil P, Bairagi V. Assessments of pharmacognostic and phytochemical standards of *Convolvulu sarvensis* Linn. Stem. Indian J Pharmacol, 2013, 9-20.
  14. Wagner H, Bladt S. Plant drug analysis. Springer, 2001.
  15. Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 2007; 102:771-776.
  16. Kumar T, Jain V. Appraisal of Total Phenol, Flavonoid Contents, and Antioxidant Potential of folkloric *Lannea coromandelica* using *in vitro* and *in vivo* assays. Scientifica, 2015.
  17. Velázquez E, Tournier HA, Mordujovich De Buschiazzo, P, Saavedra G, Schinella GR. Antioxidant activity of Paraguayan plant extracts. Fitoterapia. 2003; 74:91-97.
  18. Kumar RS, Raj Kapoor B, Perumal P. Antioxidant activities of *Indigofera cassioides* Rottl. Ex. DC. Using various *in vitro* assay models. Asian Pac J Trop Biomed. 2012; 2:256-261.
  19. Mohan SC, Balamurugan V, Salini ST, Rekha R. Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant *Kalanchoe pinnata*. J. Chem. Pharm. Res. 2012; 4:197-202.
  20. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern Med. 2012; 12:221.
  21. Liu X, Zhao M, Wang J, Yang B, Jiang, Y. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. J. Food Compos. Anal. 2008; 21:219-228.
  22. Okwu DE. Phytochemicals and Vitamin content of indigenous species of southeastern Nigerian. J. Sustain. Agric. Environ. 2004; 6:30-37.
  23. Aluko BT, Oloyede OI, Afolayan A. J. Polyphenolic contents and free radical scavenging potential of extracts from leaves of *Ocimum americanum* L. Pak J Biol Sci. 2013; 16:22-30.
  24. Kulkarni AG, Suryakar AN, Sardeshmukh AS Rathi DB. Studies on biochemical changes with special reference to oxidant and antioxidants in malaria patients. Ind. J. Clin. Biochem. 2003; 18:136-149.
  25. Mbaebie BO, Edeoga HO, Afolayan AJ. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. Asian Pac J Trop Biomed 2012; 2:118-124.
  26. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010; 4:118-126.
  27. Polu PR, Nayanbhirama U, Khan S, Maheswari R. Assessment of free radical scavenging and anti-proliferative activities of *Tinospora cordifolia* Miens (Willd). BMC Complement Altern Med. 2017; 17:457.
  28. Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat. Res. 2003; 523-524, 9-20.
  29. Leopoldini M, Russo N, Toscano M. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chem. 2011; 125:288-306.
  30. Stevanato R, Fabris S, Bertelle M, Gregoris E, Momo F. Phenolic content and antioxidant properties of fermenting musts and fruit and vegetable fresh juices. Acta Aliment 2009; 38:193-203.
  31. Wang SY, Lin HS. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J Agric. Food Chem. 2000; 48:140-146.
  32. Gülçin I, Topal F, Çakmakçı R, Bilsel M, Gören A, Erdogan U. Pomological features, nutritional quality, polyphenol content analysis, and antioxidant properties of domesticated and 3 wild ecotype forms of raspberries (*Rubus idaeus* L.). J Food Sci. 2011; 76:C585-593.
  33. Wei SD, Zhou HC, Lin YM. Antioxidant activities of extract and fractions from the hypocotyls of the mangrove plant *Kandelia candel*. Int. J Mol. Sci. 2010; 11:4080-4093.
  34. Abeed AL, Mashkor IM. Phenolic content and antioxidant activity of fenugreek seeds extract. Int J Pharmacogn Phytochem Res. 2014; 6:841-844.
  35. Addai Z, Abdullah A, Sahilah AM. Effect of extraction solvents on the phenolic content and antioxidant properties at two papaya cultivars. J Med Plants Res. 2013; 7:3354-3359.
  36. Kassem A, Abdullah A. Dietary Gum Arabic Supplementation Alter Plasma and Tissue Antioxidant and Free Radical Scavenging Activities in Sprague Dawley Male Rats. J Biol Life Sci. 2015; 6:129-138.
  37. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant Properties of the Ayurvedic Formulation Triphala and Its Constituents. Int J Pharmacogn. 1997; 35:313-317.
  38. Parejo I, Viladomat F, Bastida J, Rosas-Romero, Flerlage N, Burillo J *et al.* Comparison between the radical scavenging activity and antioxidant activity of six distilled and non-distilled mediterranean herbs and aromatic plants. J agric Food Chem. 2002; 50:6882-6890.
  39. Yen GC, Duh PD. Scavenging Effect of Methanolic Extracts of Peanut Hulls on Free-Radical and Active-Oxygen Species. J Agric. Food Chem. 1994; 42:629-632.
  40. Ndip RN, Malange TA, Echakachi CM, Malongue A, Akoachere JF, Ndip LM *et al.* *In-vitro* antimicrobial activity of selected honeys on clinical isolates of *Helicobacter pylori*. Afr. Health Sci. 2007; 7:228-232.
  41. Ghafar MF, Prasad KN, Weng KK, Ismail A. Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species. Afr. J Biotechnol, 2010, 9.
  42. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002; 18:872-879.
  43. Banerjee SK, Bonde CG. Total phenolic content and antioxidant activity of extracts of *Bridelia Retusa* Spreng

- Bark: Impact of dielectric constant and geographical location. J Med Plants Res. 2011; 5:817-822.
44. Alkan FU, Anlas C, Ustuner O, Bakırel T, Sari AB. Antioxidant and proliferative effects of aqueous and ethanolic extracts of *Symphytum officinale* on 3T3 Swiss albino mouse fibroblast cell line. Asian J Plant Sci Res. 2014; 4:62-68.