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In vitro comparative antioxidant potentials of cold and hot aqueous extracts of *Asystasia vogeliana* Benth

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Abstract

Oxidative stress and impaired antioxidant system have been involved in the pathophysiology of diverse disease conditions. In recent years, certain medicinal plants utilization as therapeutic agents has drastically increased. The aim of this study was to screen the phytochemicals and antioxidant properties of Asystasia vogeliana. The phytochemical screening and antioxidants property was done in vitro through standard experimental procedure. The antioxidant activity of total phenolic in cold water was 8.75±0.002 mg/mL, and significantly higher than hot water counterpart which exhibited lower total phenolic activity of 1.96±0.050 mg/mL. DPPH radical scavenging activity of cold water of 9.93% was also significantly higher than hot water counterpart that was 0.09%. This study has shown that cold aqueous extract of A. vogeliana leaf has the potential to exhibit better antioxidant activity than the hot water extract. In addition, this study revealed that the leaf of A. vogeliana could be a good source of natural antioxidants which can be implicated in the treatment of several degenerative diseases.

Keywords: phytochemical, antioxidant, pathophysiology, degenerative diseases.

Potentiel antioxydant comparatif in vitro des extraits aqueux froids et chauds *d'Asystasia Vogeliana Benth*

Résumé

Le stress oxydatif et le système antioxydant altéré ont été impliqués dans la physiopathologie de diverses maladies. Ces dernières années, certaines utilisation des plantes médicinales en tant qu'agents thérapeutiques ont considérablement augmenté. Le but de cette étude était de filtrer les phytochimiques et les propriétés antioxydantes d'Asystasia vogeliana. Le dépistage phytochimique et la propriété antioxydants ont été effectués in vitro par procédure expérimentale standard. L'activité antioxydante du phénolique total dans l'eau froide était de 8,75 ± 0,002 mg / ml, et significativement plus élevée que la contrepartie d'eau chaude qui présentait une activité phénolique totale plus faible de 1,96 ± 0,050 mg / ml. L'activité de piégeage des radicaux DPPH de l'eau froide de 9,93% était également significativement plus élevée que la contrepartie à l'eau chaude qui était de 0,09%. Cette étude a montré que l'extrait aqueux froid de la feuille d'A.

Vogeliana a le potentiel de présenter une meilleure activité antioxydante que l'extrait d'eau chaude. De plus, cette étude a révélé que la feuille d'A. Vogeliana pourrait être une bonne source d'antioxydants naturels qui peuvent être impliqués dans le traitement de plusieurs maladies dégénératives.

Mots-clés: phytochimique, antioxydant, physiopathologie, maladies dégénératives.

الباردة المائية لمستخلصات المختبر في مقارنة الأكسدة مضادات إمكانات
Asystasia vogeliana Benth من والساخنة

الملخص

في الأكسدة مضادات نظام وضعف التأكسدي الإجهاد شارك لقد
مرضية متنوعة. في السنوات الأخيرة ، ازداد استخدام لحالات المرضية الفيزيولوجيا
بعض النباتات الطبية كعوامل علاجية بشكل كبير. كان الهدف من هذه الدراسة هو فحص
الخصائص الكيميائية النباتية ومضادات الأكسدة في AsysatsysA anailegov. تم إجراء
المختبر من خلال إجراء تجريبي الفحص الكيميائي النباتي وخصائص مضادات الأكسدة في
ثير من مجم / مل ، وأعلى بكمية 0.002 ± 8.75قياسي. كان النشاط المضاد للأكسدة للفينول الكلي في الماء البارد
حسب الطاشن نالك .لم / مجم 0.050 ± 1.96نظيره في الماء الساخن الذي أظهر نشاطًا فينوليًا إجماليًا أقل من
اء الباردبنسبة 39.9٪ أعلى بكثير من نظيره في الماء الساخن الجذري لـ HPPD من الم
الذي كان 90.0٪. أظهرت هذه الدراسة أن المستخلص المائي البارد لأوراق A. anailegov لديه
القدرة على إظهار نشاط مضاد للأكسدة أفضل من مستخلص الماء الساخن. بالإضافة إلى ذلك
لمضادات الأكسدة يمكن أن تكون مصدرًا جيدًا A. vogeliana باتن قاروأ نأة ساردلا هذه تحضوأ ،
الطبية التي يمكن أن تساهم في علاج العديد من الأمراض التنكسية
الكلمات المفتاحية: الكيمياء النباتية ، مضادات الأكسدة ، الفيزيولوجيا
المرضية ، الأمراض التنكسية.

Introduction

Since the ancient time, humans have been exploring plants as food and medicine through trial and error. Medicinal plants have been indispensable to the development of good health in both developed and developing nations. Information about medicinal plants has long been spread from one generation to other and human knowledge has gradually increased through civilizations and the provision of more facilities (Jamshidi-Kia et al., 2018). Recently, quality and effectiveness of medicinal plants and ensuring their safety as herbal drugs have become a key concern in developing countries and industrialized nations (Komolafe *et al.,* 2021).

The plant *Asystasia vogeliana* Benth belongs to the family Acanthaceae. The *A. vogeliana* is a straggling under shrubs. The leaves are narrowed at either end and up to 0.2 by 0.06 meters they are ovate, simple opposite, and decussate without stipules. The plant is medicinally used against hepatitis in Nsukka, Enugu State, Nigeria. The plant is called natively by the people, 'Ogwu iba ocha n' anya' translating literarily for a drug used for hepatitis (Ugwuanyi *et al*., 2020).

Over the years, bioactivities studies of various plants have described vital position because of the variations in the effectiveness of the plant extracts with the solvent used for extraction, plant part used, temperature of the extracting medium, the plant's age, and geographic origin (Venkatesan *et al*., 2019). Antioxidant property stands to be an indispensible mechanism of beneficial activity of plant-derived extracts and compounds. The aim of this study was to screen the

phytochemicals and antioxidant properties of cold and hot water extracts from *A. vogeliana*.

Materials and Methods

Materials

Collection and identification of plant sample

The fresh sample of *Asystasia vogeliana* was collected in Ile-Ife, Osun State after identification and authentication at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife; where voucher specimen was deposited. The plant sample was then assigned a specimen number (IFE-17776).

Preparation of hot and cold-water extracts of A. vogeliana leaf

The fresh leaves of *A. vogeliana* obtained were rinsed with water after which they were ground with warring blender. The ground leaf samples were then weighed and divided into two portions. A portion of it was soaked with cold water while the other portion was soaked with hot water at boiling temperature (100°C). The two samples were left undisturbed for 24hrs to allow maximum extraction of the various phytochemicals presents in the leaves. Thereafter, the extracts obtained from the two samples (i.e., the cold water and hot water extracts) were concentrated using rotary evaporator. The percentage yield of the extract was then determined.

Estimation of total phenol content

The assay protocol as proposed by Tungmunnithum *et al*. (2018) and modified by Mwihia *et al.* (2017), was used for the determination of the total phenol content of the plant extract, which was based on Folin-Ciocalteu (FC) method. To each of the test tubes containing 1.0, 0.8, 0.6, 0.4, 0.2, and 0.0 ml of 10 µg/ml gallic acid solution (standard) in triplicate, equal volume of distilled water was added to make up to 1 ml of the total. The tests containing 5 µg/ml hot and cold-water extract of *A. vogeliana* leaf instead of gallic acid solution, was also prepared in triplicate with each test tube containing 1 ml of the hot and cold-water extracts. Then, 1.5 ml of Follin Ciocalteu's phenol reagent (1:10) was added to each of the tubes and vortexed for 5 minutes. The mixture was incubated at room temperature for 15 minutes after which 1.5 ml of 7% (w/w) Na_2CO_3 solution was added to give a total volume of 4.0 mL. The reaction mixture was further incubated for 90 minutes after which its absorbance was read at 750 nm against the blank that contains all reagents except Gallic acid and the hot or cold extracts. Average of each absorbance obtained for the gallic standard at varying concentration was determined and plotted against the different concentrations of the gallic acid standard to generate a standard curve from which the concentration of the hot and cold extracts of *A. vogeliana* leaf was later extrapolated using the equation of a straight-line graph of the standard.

Estimation of total flavonoid concentration

Total flavonoid contents were determined using the Dowd method as adapted by Oyetayo *et al*. (2021). 1 mL of 2% aluminum trichloride ($AlCl_3$) in methanol was mixed with the same volume of the methanolic extracts (2000 µg). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 1 mL extract solution with 1 mL methanol without $AlCl_3$. The concentrations of flavonoid compounds were calculated according to the equation obtained from the standard quercetin graph: Absorbance = [0.0333 x quercetin (µg) + 0.0231], with R2 = 0.9961.

Ferric reducing antioxidant power (FRAP) assay

1 ml of sample is dissolved in distilled water, and then addition of 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) according to method described by Oyaizu (Aggrey *et al*., 2019). The mixture was incubated for 20 minutes at a temperature of 50 °C with the addition of 22.5 mL of Trichloro acetic acid (10% w/v) after the completion of the incubation period. An upper layer (2.5 mL) was obtained through a centrifuge 3000 rpm for 10 minutes, and then it was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%, w/v). The absorbance of the final content was measured at 700 nm.

Determination of lipid peroxidation assay (TBARS).

Levels of the thiobarbituric acid reactive species (TBARS) in the tissues and serum homogenates were measured according to the method of Katsiki *et al*. (2016) using standard Fortress from England. 0.4 ml of serum and other tissue homogenates were each mixed with 1.6 ml of Tris-KCl buffer. It was added to 0.5ml of 30% trichloroacetic acid (TCA). After that, 0.5 ml of 0.75% thiobarbituric acid (TBA) was added and incubated for 45 min at 80ºC. The resulting mixture was then cooled on ice and centrifuged at 3000 rpm. The clear supernatant was collected and absorbance measured against a reference blank at 532 nm. The MDA level was calculated according to the method of Katsiki *et al*. (2016).

Total antioxidant capacity

The antioxidant activity was determined by means of DPPH radical scavenging assay (Katsiki *et al*., 2016; ADA, 2017). To 0.2 mL of each extracted samples and the standard Trolox solutions was added 3.8 mL of 0.1 mM DPPH solution in a test tube. The mixtures were shaken for 1 minute and then left in the dark for 30 minutes after which the absorbance was read using spectrophotometer at 517 nm against the blank. Absorbance of a negative control (A control) was taken after adding DPPH radical solution to 0.2 mL of the extraction solvent (distilled water).

% DPPH radical inhibition = A control - A sample × 100%

A control

From equation, the free radical scavenging (antioxidant) activity was expressed as the mean micromole of Trolox equivalent (µMTE/g).

Data analysis

The results are expressed as mean ± SEM (standard error of the mean). Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The results were considered statistically significant for $P<0.05$.

Results

The leaf of *A. vogeliana* plant (78 g) yielded (3.06 g) for hot water extract while (80 g) of the leaf gave (3.22 g) of cold-water extract. These represent 3. 92% and 4.02% of the starting material for cold and hot water extracts, respectively (Table 1). The hot extract yield was more than that of cold water. The hot water extract of *A. vogeliana* leaf had more phenolic content compared to cold water extract but the cold-water extract had more flavonoid content than the hot water extract (Table 2). The % scavenging activity of hot and cold-water extracts of *A. vogeliana* leaf are presented in Table 3. The IC_{50} values (which is the concentration of the extracts or compound needed to scavenge half of the population of free radicals in the given reaction) obtained for the two extracts

showed that the cold-water extract of *A. vogeliana* leaf compared more favourably with the DPPH scavenging activity of ascorbic acid as compared to its hot water counterpart.

Table 1: Percentage yield of hot and cold-water extracts of A. vogeliana leaf

Extract	% Yield
Cold H_2O extract	3.92
Hot H_2O extract	4.02

Table 2: Total phenolic and flavonoid contents of A. vogeliana hot and cold-water leaf extracts

Extracts	Total Phenolic (mg GAE/g)	Total Flavonoid (mg RE/g)
Cold H_2O extract	1.9607±0.002	2.55±0.001
Hot H_2O extract	8.7536±0.001	2.79±0.001

Values are mean ± SEM

Table 3: Percentage (%) DPPH radical scavenging activity of hot and cold-water extract of A. vogeliana leaf and ascorbic acid.

Concentration (mg/ ml)	Standard	Extracts	
	Ascorbic Acid (%)	Cold H_2O extract (%)	Hot H_2O extract (%)
0.05	56.17	3.68	0.01
0.10	63.65	9.93	0.09
0.20	64.38	8.36	4.57
0.40	64.78	19.94	12.06
IC_{50}(mg/ml)	0.15	1.08	1.61

As shown in Figure 1, the cold-water extract of *A. vogeliana* have more ferric reducing antioxidant potential as compared to its hot water counterpart. Nevertheless, both extracts have less activity as compared to ascorbic acid. As shown in Figure 2, the hot water extract of *A. vogeliana* leaf exhibited more lipid peroxidation inhibitory activity than the cold-water extract. Nevertheless, both extracts exhibited little or no inhibitory activity as compared to ascorbic acid. The total antioxidant capacity of the hot and cold extracts of *A. vogeliana* leaf was determined at various concentrations against ascorbic acid standard. As shown Figure 3, the cold water extract has more antioxidant capacity as compared to it hot water counterpart. Although both extracts have relatively low antioxidant capacity with respect to the ascorbic acid standard.

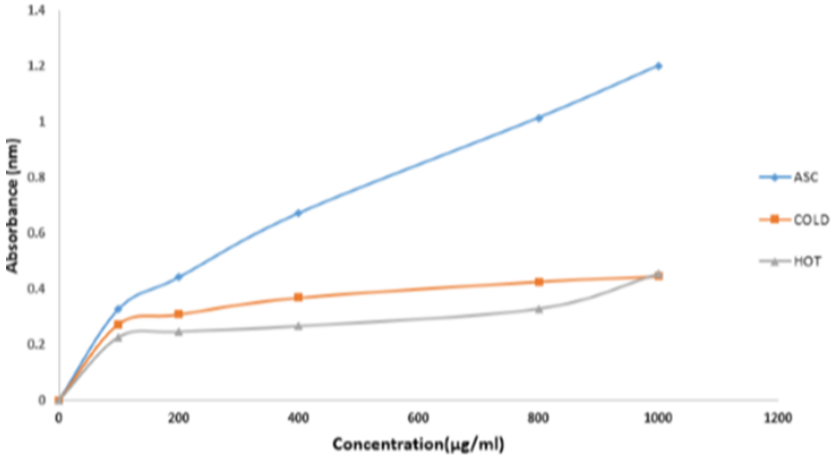

Figure 1: Ferric Reducing Antioxidant Power (FRAP) of hot and cold-water extracts of A. vogeliana leaf. ASC - ascorbic acid (standard); COLD - cold water extract of A.vogeliana leaf ; and HOT – hot water extract of A. vogeliana leaf.

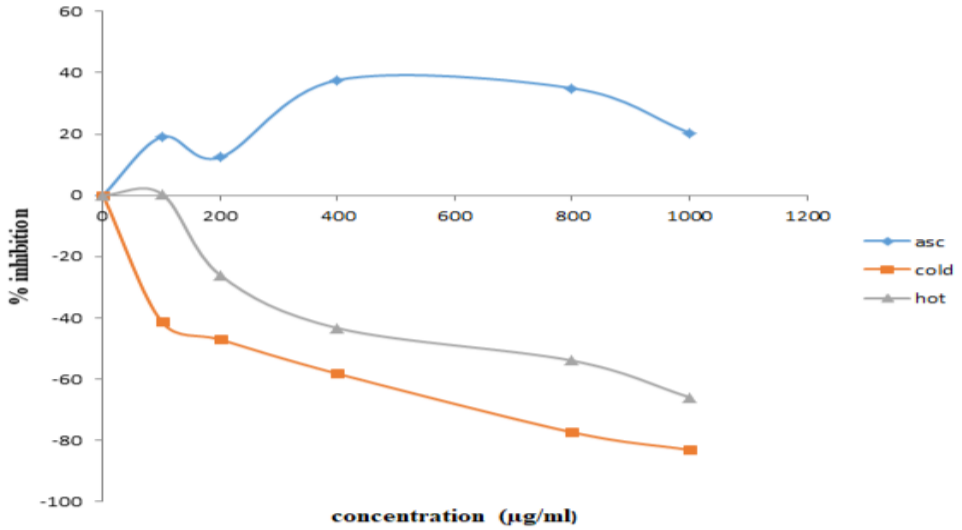

Figure 2. Percentage inhibition of lipid peroxidation of hot and cold-water extract of A. vogeliana leaf in comparison to ascorbic acid standard. Where, ASC – ascorbic acid; COLD – cold water extract of A.vogeliana leaf; and HOT – hot water extract of A. vogeliana leaf.

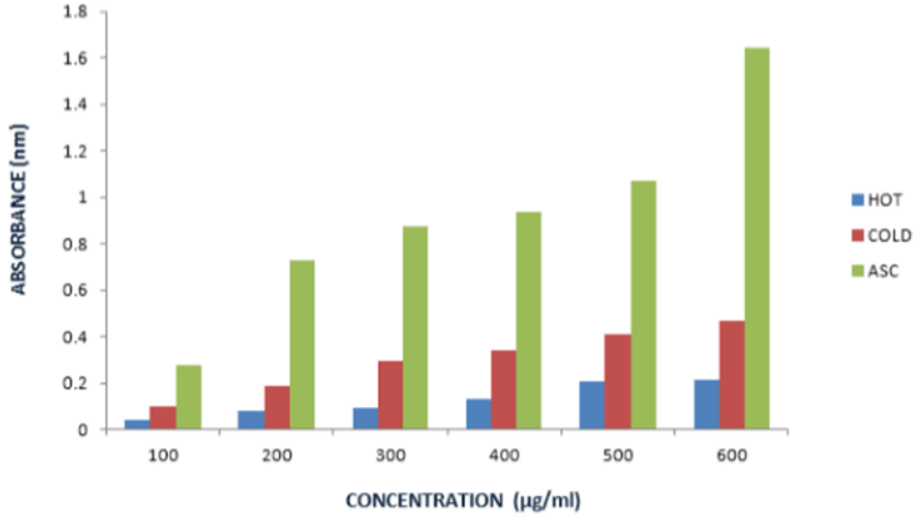

Figure 3: Total antioxidant capacity of hot and cold water extracts of A. vogeliana leaf. Where, ASC – ascorbic acid; COLD – cold water extract of A. vogeliana leaf; and HOT – hot water extract of A. vogeliana leaf.

Discussion

Antioxidants are the compounds responsible for the protection of living organism from the damage caused by the abnormal production of reactive oxygen species concomitant lipid peroxidation, protein damages and others including DNA strand breaking. Antioxidants are generally accepted to be an indispensable line of protection from oxidative damage, which has been indicated in a range of degenerative disorders (Mwihia *et al*., 2017). Biological antioxidants are categorized into inhibitors of radical formation, such as Fenton reaction inhibitor; free radical quenching agents, such as alpha- tocopherol (Vitamins) and enzymes, such as superoxide dismutase. In the same vein, phenolic are good sources antioxidant molecules because of their good redox potentials and stability of the Aryloxy radical (Apak *et al*., 2018).

Numerous in vitro chemical assays with different reaction principles have been frequently used to determine the antioxidant potential of plant extracts. The most common and simplest way, by which we can quickly understand the antioxidant efficiency of any compound, including plant extracts in a short time is DPPH radical assay (Chena *et al*.,2020). This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug and it is commonly used for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay (El Moussaoui *et al*., 2019).

In the present study, DPPH assay was used to determine the free radical scavenging activity and hence the antioxidant potential of the hot and cold-water extracts of *A. vogeliana* leaf. The result from the DPPH assay showed that the cold-water extract of *A. vogeliana* leaf exhibited better scavenging activity than the hot water extract, although both extracts had little radical scavenging potential with respect to ascorbic acid standard which had been well documented in journals as a standard

drug with outstanding antioxidant potential (Siddeeg *et al*., 2021). This result confirms the earlier discoveries documented in the literature on antioxidant potential of the genus Asystasia (Ugwuanyi *et al*., 2020).

The fact that antioxidant activity depends on polarity and the type of the extracting solvent as well as the temperature (Munteanu and Apetrei, 2021) might be the reason for difference in activity of the two plant extracts (i.e. the hot and cold water extract) towards DPPH. Same antioxidant molecule may evidently act differently in the scavenging of radicals of different types. Therefore, additional scavenging assays such as TAC, FRAP, and Lipid peroxidation (TBARS), are needed to fully elucidate the antioxidant potential of a particular plant extract and to this effect this assays were carried out. The two extracts of *A. vogeliana* total antioxidant capacity (TAC) was determined based on the principle of antioxidants reduction of molybdenum (VI) to molybdenum (V) and also the formation of phosphomolybdenum (V) complex at acidic reaction condition. The green color was observed which absorbs light at 695 nm. Total antioxidant capacity of the hot and cold extracts of *A. vogeliana* leaf were determined and it was found that the cold-water extract compared more favourably with the ascorbic acid standard than its hot water counterpart.

FRAP method is used for determination of reducing capacity of the antioxidant compounds through increase in the absorbance of the reaction mixture. The increase in absorbance of the reaction mixture gives a clear picture about the possibility of using these kinds of plants extracts as antioxidants (Bankeu *et al*., 2019). The result obtained for FRAP and TBARS assays showed that the cold-water extract of *A. vogeliana* leaf exhibited better reducing power towards Fe^{3+} ion when compared to the hot water extract. But reverse was the case for the lipid peroxidation assay. In addition to their positive reaction towards the various antioxidant assays conducted on them, the presence of reasonable amount of phenol and flavonoid phytochemical in the two extracts further confirmed their antioxidant potentials and hence their use as a good natural alternative in the treatment of several degenerative diseases.

Hot water extract of *A. vogeliana* leaf had more phenolic content than the cold-water extract. This observation is not unusual as several researches conducted on the extraction efficiency of solvents at varying temperatures had revealed that increase in temperature of extracting solvent will lead to an increase in the quantity of phytochemicals that can be obtained from a particular plant (Munteanu and Apetrei, 2021).

Although the hot water extract of *A. vogeliana* leaf had more phenolic and almost the same flavonoid content relative to the cold-water extract (an attribute which should have qualified it to have better antioxidant activity as compare to it cold water counterpart), it was observed that it exhibited lower antioxidant activity relative to the cold-water extract in all the antioxidant assay carried out in this study with exception of lipid peroxidation assay. This reduced antioxidant potential of the hot water extract of *A. vogeliana* can be attributed to the fact that most metabolites such as phenols and flavonoids tend to lose their activity with increased temperature (Uddin *et al*., 2018).

Conclusion

This study revealed that the leaf of *A. vogeliana* could be a good source of

natural antioxidants which can be implicated in the treatment of several degenerative diseases. In addition, this study has shown that the cold-water extract of *A. vogeliana* leaf had better antioxidant activity compared to its hot water counterpart, which has the tendency to destroy some of the phytochemicals of the plant during extraction process. In conclusion this study had revealed the antioxidant potential of *A. vogeliana* leaf as well as its dependence on extraction conditions. However, there is a need for further research into the characterization and isolation of the various phytochemicals present in the plant as well as the variation of it phytochemical extraction efficiency with solvent type, temperature and duration of extraction.

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