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Comparative Analysis of Antioxidant Properties of Cold and Hot Water Leaf Extract of *Rauvolfia vomitoria* Afzel

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Abstract: *Rauvolfia vomitoria* Afzel has attracted significant research interest due to its multiple health benefits. Its antioxidant properties are important in preventing oxidative stress, cancer, heart problems, and neurological disorders. This study compares the antioxidant capacity of *R. vomitoria* leaf extract in both hot and cold water. Standard methods were used to prepare leaf extracts in both hot and cold water, which were subjected to different antioxidant assays. The hot water leaf extract had a better capacity to snare free radicals produced by stable DPPH's free radical at 50% inhibition concentration (3.07 ± 0.54 mg/ml) than the cold water leaf extract (9.82 ± 0.01mg/ml). The nitric oxide inhibitory capacity of *R. vomitoria* at 50% inhibition concentration in cold and hot water leaf extract (3.64 ± 0.38 and 4.24 ± 0.11) showed no significant difference. The hot water leaf extract had higher ferric-reducing antioxidant power (IC_{50} of 7.21 ± 0.07 mg/ml) than the cold water extract (10.85 ± 0.50 mg/ml). The hot water leaf extract showed a higher total antioxidant capacity (IC50 of 6.01 ± 0.21 mg/ml) than the cold water extract (9.01 ± 0.07 mg/ml). These antioxidant evaluations show that *R. vomitoria* hot water leaf extracts were superior to cold water leaf extracts as antioxidants and free radical scavengers. The findings show that *R. vomitoria's* hot-water extract has greater antioxidant activity than its cold-water extract.

Keywords: *Rauvolfia vomitoria, antioxidant, free radicals, inhibition concentration, extract.*

1. Introduction

Rauvolfia vomitoria Afzel, a rainforest tree in the *Apocynaceae* family, grows to approximately 12 meters in height and features a whorled trunk with visible, red, globose fruit. The tree also produces clusters of flowers and oval leaves with straight venation (Ekarika et al., 2020). It is commonly known as serpent wood, swizzle stick, or poison devil's pepper and is widely distributed and cultured in Africa, India, China, and Bangladesh (Yu et al., 2012; Owoade et al., 2021). Yoruba people in Nigeria refer to it as asofeyeje, which implies producing food for birds. The Igbo people in Nigeria refer to it as akanta, while the Ashantes people in Ghana call it pempe (Ajayi, 2021). Over the years, *R. vomitoria* has been generally regarded as a weed, but in recent years, numerous investigations have confirmed its various uses in different countries (Okereke et al., 2015). According to traditional healers in Nigeria and Africa, decoctions of *R. vomitoria* leaves possess strong emetic and anti-inflammatory properties and are used to treat a wide range of ailments, including fever, general weakness, liver issues, digestive illnesses, mental disorders, impotence, piles, rheumatism, gastrointestinal diseases, cancer, hypertension, insanity, snakebite, cholera, diarrhea, jaundice, and venereal diseases (Kumar et al., 2022; Ajayi, 2021; Chinonye et al., 2021; Kumar et al., 2020, 2016, 2015,). Numerous extracts and fractions have been shown to possess anti-inflammatory, anticancer, antibacterial, anti-diabetic, antioxidant, and antipsychotic properties due to their bioactive components (Surendran et al., 2021; Yu & Chen, 2014; Fannang et al., 2011; Bemis et al., 2006).

Oxidative stress is caused by an imbalance in the relative quantities of essential elements involved in oxidative metabolism and is the root cause of many diseases (Fatima et al., 2021). It occurs when a system's capacity to eliminate reactive oxygen species and functional metabolites is exceeded (Sies, 1985; Goodarzi et al., 2018). The delicate balance between pro- and antioxidants in the cellular environment governs an organism's overall health (Fatima et al., 2021). Even at very low concentrations, antioxidants play several physiological roles in the body and contribute to the immune system's defence against illnesses caused by unregulated radical invasion (Pisoschi & Negulescu, 2011; Sunil, 2014). To inhibit oxidative processes, antioxidants act as reducing agents, typically by eliminating reactive oxygen species before they can harm cells (Wolf, 2005).

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They perform a key function in the body's protective system and are essential for preventing diseases and disorders in plants and animals, as well as protecting plants from pollution (Ahmed & Beigh, 2009). Some chronic and degenerative ailments significantly impacted by oxidative damage are heart disease, the nervous system, cancer, ageing, autoimmune disorders, and rheumatoid arthritis (Lien et al., 2008; Goodarzi et al., 2018). Heat treatment can enhance or degrade the nutritional value of food (Chukwu et al., 2010; Oghbaei & Prakash, 2016).

Some people use the cold-water extract of *R. vomitoria Afzel* for medicinal purposes, while others prefer the hot-water extract. Therefore, the goal of this investigation is to compare the antioxidant capabilities of cold and hot water extracts of *R. vomitoria Afze*l to determine which extract has greater medicinal or therapeutic value.

2. Materials and Methods

Collection and Preparation of the Sample

We collected fresh *R. vomitoria* Afzel leaves from Delta State University in Abraka, Delta State, Nigeria. Prof. Aigbokhan Emmanuel Izaka identified the leaves, and Dr Akinnibosun Henry Adewale issued a voucher number (UBH-R421) at the Herbarium Unit of the Plant Biology and Biotechnology Department, University of Benin, Edo State. The fresh leaves were rinsed with distilled water to remove any remaining debris.

Cold Water Extract

A cold-water leaf extract of *R. vomitoria* Afzel was obtained by homogenization (Onyeukwu et al., 2024). 125g of fresh leaves were blended with a mortar and pestle and extracted with 500 mL of distilled water (25% w/v). The extract was subsequently filtered through a double-folded clean sieve cloth, and the cold-water filtrate was employed in the investigation.

Hot Water Extract

Hot water leaf extract of *R. vomitoria* Afzel was obtained by the decoction procedure outlined by Onyeukwu et al. (2024). 125g of fresh leaf was placed in 500 mL of boiling water (25% w/v) and allowed to stand for 15 minutes. The extract was subsequently filtered through double-folded muslin cloth, and the hot-water filtrate was cooled and used in the investigation.

Antioxidant Activity Evaluation

1, 1-diphenyl -2-picryl hydrazyl (DPPH) Assay

The determination of antioxidant capacity followed the methodology of Owoade et al. (2021). To 2 ml of DPPH solution (0.3 mM), 0.2 ml of various *R. vomitoria* aqueous leaf extract concentrations (0.2 - 1.0% w/v) was added, followed by 30 min of incubation in the dark. The absorbance was measured at 517 nm. As a reference, ascorbic acid (0.02-0.10 mg/ml) was employed. Using the following equation, the % inhibition of DPPH radical scavenging was determined: $([A_o - A_1]/A_o) \times 100$ equals the percentage of DPPH radical inhibition. A_1 is the absorbance in the presence of the extract, and A_o is the absorbance of the control (blank, without extract).

Nitric Oxide (NO) Free Radical Scavenging Activity

Using the approach developed by Samuel et al. (2021), the ability of nitric oxide to scavenge free radicals was estimated. Different quantities of the aqueous extract of *R. vomitoria* (0.2 - 1.0% w/v) were combined with 0.5 ml of the 10 mM phosphate buffer saline (pH 7.4), 2 ml of 10 mM sodium nitroprusside, and 0.2 ml of 10 mM sodium nitroprusside. After that, the mixture was incubated at 25^0 °C, and 0.5 ml of the incubated solution was taken out after 150 minutes and combined with 0.5 ml of the Griess reagent [1.0 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthyl ethylenediamine dichloride (0.1% w/v)]. After 30 minutes of incubation at room temperature, the mixture's absorbance at 546 nm was measured against a blank. With the use of the following equation, the percentage inhibition of nitric oxide radical scavenging was determined: % inhibition of NO radical = $([A_0 – A_1]/ A_0) \times 100$. A_1 is the absorbance in the presence of the extract, and A_0 is the absorbance of the control (blank, without extract).

Ferric Reducing Antioxidant Power (FRAP) Assay

The technique of Oborirhovo et al. (2023) was employed to assess the antioxidant activity via ferric reduction. 1.0 ml of the aqueous leaf extract of *R. vomitoria* (0.2 - 1.0% w/v) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v). After 20 minutes of incubation at 50^0 °C, 2.5 ml of 10% w/v trichloroacetic acid is added to the resultant mixture. 0.5 mL of $FeCl_3$ (0.1%, w/v) and 2.5 mL of distilled water were added to the mixture after it was centrifuged at 3000 rpm for 10 minutes, and the upper layer (2.5 mL) was collected. Following that, the absorbance was measured at 700 nm relative to a blank sample containing distilled water and sodium phosphate buffer. 0.02-0.10 mg/ml of ascorbic acid was employed as a reference.

Total Antioxidant Capacity

The approach employed by Oborirhovo et al. (2023) was applied. In screw-capped tubes, 1.0 mL (0.2–1.0% w/v) of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was taken, to which 0.1 mL of the aqueous extract of the leaf of *R. vomitoria* was added and dissolved. The tubes were sealed and incubated at 95^0 °C for 90 minutes in a thermal block. After cooling to room temperature, the absorbance of the aqueous solution in each tube was measured at 695 nm against a blank. The total antioxidant capacity was reported as equivalent to gallic acid (GAE) and was determined using gallic acid standards (0.02-0.10 mg/mL).

Statistical Analyses

Mean ± SD of triplicate values was used to report data. The LSD test was used in analyses of variance (ANOVA) to compare the outcomes. Statistical significance was established at $P < 0.05$. The graph was plotted in Microsoft Excel, and the linear regression of the extract's concentration against the percentage of inhibition was used to determine the IC_{50}.

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3. Results

The IC_{50} values for the leaf extracts in hot and cold water of *R. vomitoria* Afzel for DPPH radical are presented in Figure 1. *R. vomitoria* Afzel hot water leaf extract had a better capacity to snare free radicals produced by stable DPPH's free radical at 50% inhibition concentration (3.07 ± 0.54 mg/ml) than the cold water leaf extract (9.82 ± 0.01mg/ml). $P < 0.05$ indicated a significant difference.

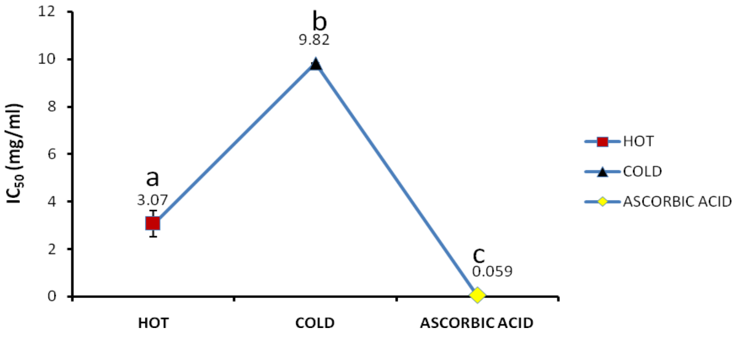

Figure 1. IC_{50} of leaf extract for cold and hot water of *R. vomitoria* Afzel for DPPH radical. Findings presented as Mean ± SD. Data with distinct letters (a-c) exhibit a significant difference ($P < 0.05$).

The IC_{50} values for the leaf extracts in hot and cold water of *R. vomitoria* Afzel showed the ability to scavenge free radicals produced by nitric oxide at IC_{50} of 3.64 ± 0.38 mg/ml and 4.24 ± 0.11 mg/ml, respectively (Figure 2). There was no significant difference between the IC_{50} of leaf extract for cold and hot water ($P < 0.05$). Both extracts had a significantly lower ability to scavenge free radicals produced by nitric oxide than the catechin standard, which had an IC_{50} of 0.076 ± 0.001 mg/ml.

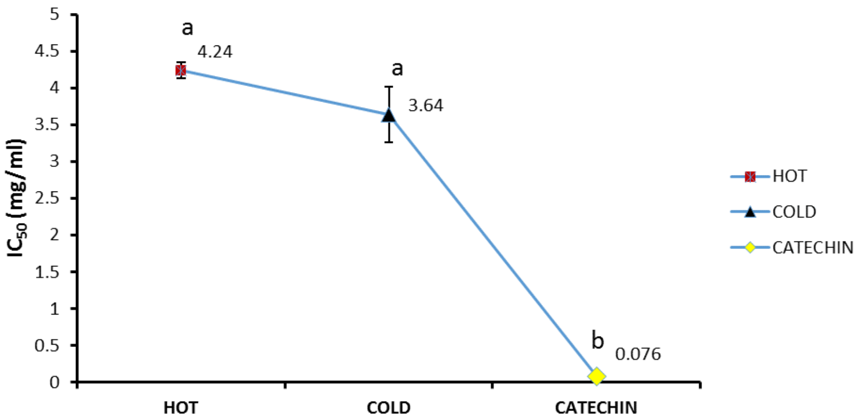

Figure 2. IC_{50} of leaf extract for cold and hot water of *R. vomitoria* Afzel for Nitric oxide radical. Findings presented as Mean ± SD. Data with distinct letters (a-b) exhibit a significant difference ($P < 0.05$).

The IC_{50} of the hot water leaf extracts of *R. vomitoria* Afzel (7.21± 0.07 mg/ml) exceeded the value of the cold extract. (10.85 ± 0.50 mg/ml) However, it was lower than the ascorbic acid standard with an IC_{50} of 0.05 mg/ml for ferric-reducing power (Figure 3). *R. vomitoria* Afzel leaf extracts in standard, hot, and cold water differed significantly ($P < 0.05$).

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Malaysian Journal of Science 45(1): 27-32 (March 2026)

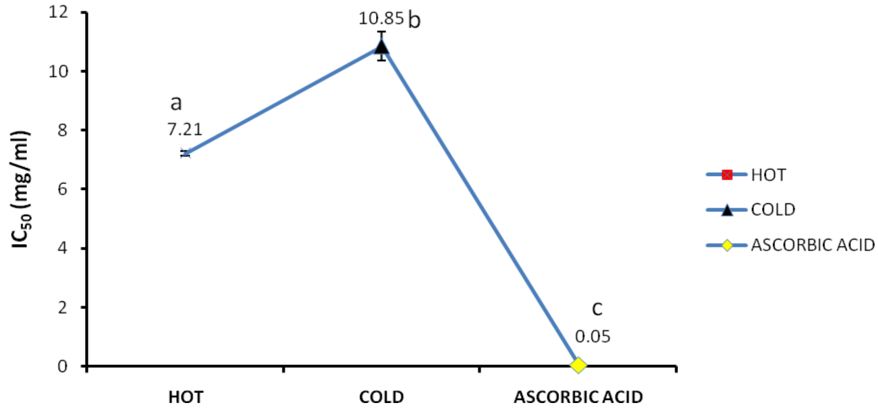

Figure 3. Inhibition concentration at 50% of leaf extract for cold and hot water of *R. vomitoria* Afzel for Ferric reducing power findings presented as Mean ± SD. Data with distinct letters (a-b) exhibit a significant difference (P < 0.05).

The hot-water extract's total antioxidant capacity (IC_{50}: 6.01 ± 0.21 mg/ml) was higher than that of the cold-water extract (9.01 ± 0.07 mg/ml). However, it was lower than that of gallic acid, which had an IC_{50} of 0.051 ± 0.001 mg/mL, exhibiting significant variation (P < 0.05) (Figure 4). The test samples' total antioxidant capability range in this order: Gallic acid > Hot water extract > Cold water extract.

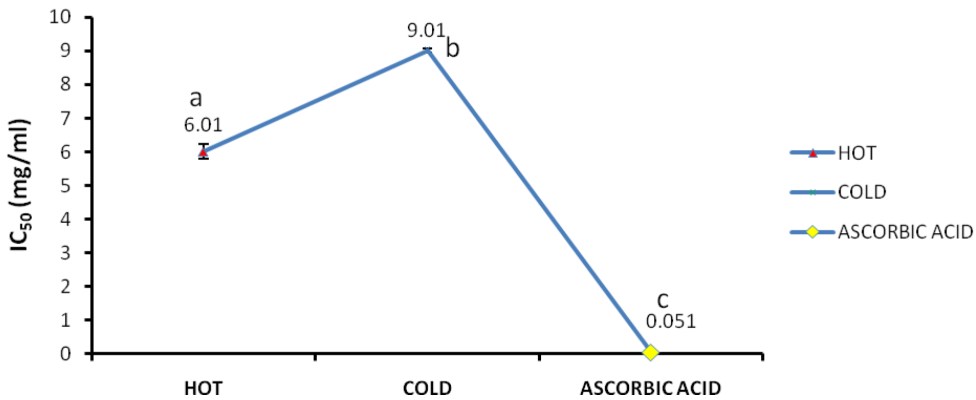

Figure 4. Total antioxidant capacity IC_{50} of leaf extract for cold and hot water of R. vomitoria Afzel. Findings presented as Mean ± SD. Data with distinct letters (a-b) exhibit a signficant difference (P < 0.05).

4. Discussion

It has been established that single assays are typically insufficient for determining the antioxidant ability of natural substances (Rahman et al., 2015). Hence, we evaluated the antioxidant potency of leaf extracts of *R. vomitoria* Afzel in cold and hot water in this study. The results show that *R. vomitoria* Afzel leaf extract, in both cold and hot water, can reduce the effects of free radical chain reactions. *R. vomitoria* Afzel leaf extract from hot water outperformed leaf extract from cold water in its capacity to snare free radicals, producing stable DPPH• free radicals, although lower than standards employed as a benchmark antioxidant. *R. vomitoria* Afzel's hot water leaf extract demonstrated greater nitric oxide scavenging efficacy than cold water leaf extract. Earlier research by Owoade et al. (2021) and Samuel et al. (2021) opined that *R. vomitoria* Afzel leaf extract shows excellent antioxidant capacity. A molecule's ability to give an atom of hydrogen to a radical is one of the processes behind antioxidant action, and the likelihood of hydrogen donation is a key element in neutralizing free radicals (Hu et al., 2000). The capacity of plant extracts to donate hydrogen is likely what makes them effective at scavenging DPPH and nitric oxide. Total flavonoids, total phenols, tannins, and DPPH radical scavenging ability of *R. vomitoria* Afzel showed a positive correlation, according to Agbodjogbé et al. (2022) and Sofidiya et al. (2012), indicating that these compounds were heavily involved in the scavenging activity of *R. vomitoria* Afzel. Yam et al. (2008) revealed that the antioxidant capacity of *R. vomitoria* Afzel may potentially be attributed to non-phenolic compounds.

In a study by Mier and coworkers (1995), they observed that an effective indicator of a compound's possible antioxidant capacity could be its capacity to reduce. Phenolic components in the *R. vomitoria* Afzel leaf extracts may determine the ferric ion-reducing antioxidant ability. This position was supported by Erasto et al. (2011), who observed a strong correlation between

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Malaysian Journal of Science 45(1): 27-32 (March 2026)

reducing the potential of the phenolic content of *R. vomitoria* Afzel. The formation of the green phosphate/Mo(V) complex at an acidic pH after the reduction of Mo(VI) to Mo(V) provided the basis for the total antioxidant capability of the cold and hot water leaf extract of *R. vomitoria* Afzel. Total antioxidant capacity analyses both fat- and water-soluble antioxidants (Aliyu et al., 2013). Our investigation shows that the hot-water leaf extract of *R. vomitoria* Afzel has a greater overall antioxidant capacity, explained by variations in phytochemical components in extracts from plant leaves obtained with both hot and cold water. This corroborates the work of Akpojotor and Ebomoyi (2021) and Chinonye et al. (2021), who documented the phytochemical constituents of *R. vomitoria* Afzel.

Boiling can enhance the extraction of phytochemicals and antioxidants, boosting their bioavailability, or it can reduce substances that are thought to be antagonists of nutrients, like phytic acid and oxalic acid (Southon & Faulks, 2002; Van Boekel et al., 2010). This may be the cause of the hot-water leaf extracts of *R. vomitoria* Afzel having stronger antioxidant activity than the cold-water leaf extract.

5. Conclusion

The results of the antioxidant assays done in this work indicate that *R. vomitoria* Afzel leaf extract from hot water is superior to leaf extract from cold water as a scavenger of free radicals. The results of this investigation demonstrate the antioxidative capacity of the hot-water leaf extract of *R. vomitoria* Afzel, as well as that of the cold-water extract. It is therefore advised to use *R. vomitoria* Afzel hot-water leaf extracts rather than cold-water leaf extracts for therapeutic applications.

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