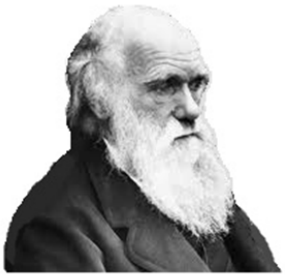
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Phytochemical Screening and Assessment of Antioxidant and Hypertensive Activities of *Phyllanthus amarus* Schumach. & Thonn. and *Secamone elliptica* R.Br.

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ABSTRACT

The study investigated the phytochemical profile and the antioxidant and antihypertensive potential of *Phyllanthus amarus* Schumach. & Thonn. and *Secamone elliptica* R.Br. Leaves were collected, air-dried, milled, and successively macerated in n-hexane, ethyl acetate, and methanol. Qualitative phytochemical screening employed standard assays, and antioxidant capacity was evaluated with the DPPH radical scavenging test across 20 to 100 µg/ml. Both species contained carbohydrates, tannins, flavonoids, coumarins, steroids, and alkaloids, while *S. elliptica* additionally showed phenols, saponins, quinones, terpenoids (moderate), cardiac glycosides, anthraquinones, and phlobatannins. DPPH activity increased in a dose-dependent manner for all samples. At 100 µg/ml, percent inhibition reached 58.28 for S. elliptica and 51.95 for *P. amarus*, compared with 89.32 for ascorbic acid. Estimated IC50 values ranked ascorbic acid at about 59.2 µg/ml, S. elliptica at about 83.0 µg/ml, and *P. amarus* at about 94.4 µg/ml, indicating higher antioxidant potency for *S. elliptica* under the assay conditions. Given the role of oxidative stress in endothelial dysfunction and elevated vascular tone, these results support a plausible antihypertensive relevance, particularly for *S. elliptica*. The study concluded that both plants exhibit promising antioxidant properties, with *S. elliptica* emerging as the priority candidate for further work. It recommended quantitative phenolic and flavonoid assays, complementary antioxidant tests (ABTS, FRAP, ORAC), and direct antihypertensive bioassays such as angiotensin-converting enzyme inhibition to clarify mechanisms and translate chemical activity to physiological effect.

***Keywords*:** Antihypertensive, Antioxidant, DPPH, IC50, *Phyllanthus amarus*, Phytochemical screening, *Secamone elliptica*, Solvent extraction

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1. INTRODUCTION

Phyllantus amarus belongs to Euphorbiaceous family which is used worldwide and have well known therapeutic values (Kumar, Chandra, Bajpai, Singh, Srivastava, Mishra & Kumar, 2015). Because of its traditional application in the management of diseases including hepatitis, diabetes and cancer this plant is one that has elicited significant interest in the search and identification of the phytochemical constituents of interest (Nguyen, Thuy Pham, Bowyer, Altenas & Scarlett, 2016). *P. amarus* has been shown to contain a wide range of chemicals including lignans, flavonoids, polyphenols, triterpenes, sterols and hydrolysable tannins (ellagitannins) (Patel, Tripathi, Sharma, Chauhan & Dixit, 2011).

P. amarus is a widely distributed tropical plant in Nigeria that is known by several names in the languages of the indigenous people. It is referred to as "Eyin Olobe" in Yoruba and "Enyikwonwas" in Igbo.

These varied names draw attention to its many applications and cultural significance, *P. amarus* sometimes known as “EyinOlobe” in Yoruba-speaking areas, is still a useful herbal treatment with some health advantage detailed chemical composition revealed that *P. amarus* contains number of chemicals that includes lignans, flavonoids, polyphenols, triterpenoids, steroids and tannins particularly, hydrolysable tannins or ellagitannins (Patel et al in press). *P. amarus*is a tropical plant which is distributed in Nigeria and it has different local names in different Nigeria Indigenous languages. It is also known as “Eyin Olobe” in Yoruba and “Enyikwonwas” in Igbo language. These different local names can assist the reader to remember its many uses and of culture in Yoruba and yet the plant is still capable of being used in the treatment of many conditions as a supplement that results to some health benefits in relation to *P. amarus*. Its conventional use nonetheless continues to be beneficial for carrying forward regional health care traditions and both scholars of medicine and academicians of medicine avow it axiomatically (Aparupa, Anindita & Sharmila, 2022). Its traditional use continues to support regional healthcare practices and both researchers and herbalists acknowledge its significance (Aparupa, Anindita, & Sharmila, 2022).

Secamone elliptica has been widely used in Nigerian traditional medicine, its local names include; arilu (Yoruba), Ewuonkwonegie (Bini) and utunta (Ibo). This climber acquires on fences and trees and it has stems that can reach 12 meters in length. It is well adapted to dense patches of savannah and second growth rainforest, places likely to occur in abandoned fields and fence rows. It is identified in Senegal, south of Nigeria, Cameroon, and Gabon among other regions of the West Tropic Africa (Greenhouse, 2020).

In the traditional medicine, *Secamone elliptica* has been prized, its roots and leaves used in different cultures for treatment of diseases for example; fever, stomachache and skin infections (Bremer, 2010). Earlier chemical analysis of the plant *S. elliptica* identified presence of alkaloids, flavonoids and other secondary products which may be responsible for the medicinal use of this plant. These compounds are antioxidant, anti-inflammatory and antimicrobial and correspond to the folk use of the plant (Raharimampionona, Rakotoarivelo & Randrianasolo, 2002). Also, the finding that *S. elliptica* has analgesic and anti-inflammatory properties to indicate that it may be useful in pain relieving therapies. Despite its prevalence, managing chronic pain and inflammation is challenging, given the side effects of most pharmaceutical drugs (Ajetunmulu & Awe, 2018 Ajithkumar et al., 2015). Through the clarification of the physicochemical and biological characteristics of *P. amarus* extracts and fractions scientists can gain significant understanding of the plant's medicinal effectiveness and

facilitate the creation of innovative treatment approaches. This project has the ability to fully utilize *P. amarus* therapeutic potential and apply its traditional medical knowledge to modern healthcare procedures (Adedapo, Ofuegbe, & Oguntibeju, 2014).

P. amarus as is of interest as a possible source of bioactive compounds because of the diversity of phytochemicals it possesses. Numerous secondary metabolites such as alkaloids, flavonoids, lignans, tannins and phenolic chemicals are present in the plant and many of them have important pharmacological properties. Numerous bioactive substances, including phyllanthin, hypophyllanthin, niranthin, quercetin, rutin and gallic acid, have been discovered by researchers in *P. amarus* (Aparupa, Anindita, & Sharmila, 2022).

There is still much to learn about the precise mechanisms of action and therapeutic potential of these bioactive chemicals even with the corpus of research on the pharmacological characteristics of *P. amarus* and its constituents rising. In order to clarify the precise chemical components in charge of the observed biological activities and to investigate their possible uses in medication development and healthcare, more research through isolation and characterization studies is therefore necessary (Segda, Meda, Bangou, Koama, Ouoba, Kagambega, Kam, & Ouedraogo, 2023). In order to fully utilize the therapeutic potential of natural products, pharmaceutical research must prioritize the extraction and characterization of *P. amarus* bioactive components. Through the identification and comprehension of these compounds chemical structures and features scientists can facilitate the creation of novel medications or therapeutic agents that exhibit enhanced efficacy, safety and specificity. Additionally, by supporting the integration of medicinal plants into contemporary healthcare practices while honoring traditional knowledge and cultural legacy, these research support the conservation and sustainable use of medicinal plants.

2. STATEMENT OF THE RESEARCH PROBLEM

These imply that despite the extensive folk use and promising pharmacological potential of *P. amarus,* we are yet to understand the physicochemical attributes and biological roles of their active compounds. Firstly, as the available studies have detected various materials in extracts from the *P. amarus* plant, more detailed investigations on structure, properties and behavior, as well as functions of each compound in the plant have not been investigated comprehensively yet (Nguyen, Thuy Pham, Bowyer, Altena, & Scarlett, 2016). This lack of understanding hinders efforts to fully harness the potential of *P. amarus* as a therapeutic remedy as well as limit the transformation of the plant into effective pharmacological medications for a myriad of health complications. The need to thoroughly delaminate, isolate and characterize the bioactive constituents derived from *P. amarus* with due accentuation to structural, physicochemical-governing namely and pharmacological profiles of the obtained substances constitutes the main issue assertion.

3. AIM AND OBJECTIVES OF THE STUDY

This study seeks to explore the antioxidant and anti-hypertensive effects of *Phyllanthus amarus* and *Secamone elliptica*, aiming to clarify their potential therapeutic advantages in managing hypertensive and its related complications.

The objectives of this work are to:

i) Maceration of *Phyllanthus amarus* and *Secamone elliptica* using different solvent according to their polarity: N-hexane, Ethyl acetate and methanol.

ii) Succesive extracttion of *Phyllanthus amarus* and *Secamone elliptica.*

iii) Evaluate the antioxidant activity of the extracted compounds using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, while also employing complementary techniques to characterize the phytochemical composition of the extracts

4. LITERATURE REVIEW

4. 1. Introduction to *Phyllanthus amarus* and *Secamone elliptica*

Phyllanthus amarus is a small Euphorbiaceae herb long used against diverse ailments such as cancer, diabetes, hepatitis, and infections, a use profile supported by extensive phytochemical and pharmacological investigations (Kumar, Chandra, Bajpai, Singh, Srivastava, Mishra & Kumar, 2015; Calixto, 2005). It occurs widely across West Africa, including Equatorial Guinea, Sierra Leone, southern Nigeria, and Ghana, and often thrives as a weed in disturbed habitats, which facilitates contact with humans and grazing animals (Bagchi, Srivastava & Singh, 1992; Irvine, 1930; Adedapo et al., 2005; Burkill, 1994; Adedapo, 2002). Traditional indications span jaundice, gonorrhea, dysmenorrhea, urinary infections, and liver disorders, aligning with evidence for antiviral, antibacterial, antidiabetic, and hepatoprotective activities (Calixto et al., 2000; Odetola & Akojenu, 2000; Adeneye et al., 2006; Patel, Tripathi, Sharma, Chauhan & Dixit, 2011).

Secamone elliptica (Apocynaceae), known locally as Ewe Ailu among the Yoruba, is a perennial climber native to tropical Africa and parts of Asia, found near water bodies, savannahs, and woodlands. Ethnomedicinal use highlights antioxidant, antimicrobial, and anti-inflammatory effects attributable to alkaloids, flavonoids, tannins, saponins, and glycosides, which has spurred renewed pharmacological interest and standardization efforts (Adetutu et al., 2022; Akinwunmi et al., 2023; Olowoselu, Adeyemi, Ogunmola & Okpako, 2023; Adebayo, Fawole, Ajayi & Okunola, 2024).

4. 2. Phytochemical Profile

4. 2. 1. Phytochemical Profile of *Phyllanthus amarus*

Comprehensive analyses report phenolics, flavonoids, lignans, alkaloids, and related metabolites in *P. amarus*, with rapid chromatographic screening identifying 52 constituents and quantifying doxophyllanthin, hypophyllanthin, gallic acid, protocatechuic acid, kaempferol 3-O-rutinoside, and quercetin. These map to antibacterial, hepatoprotective, anti inflammatory, and antioxidant activities that underpin the plant's wide therapeutic scope (Patel et al., 2011; Kumar et al., 2015).

4. 2. 2. Phytochemical Profile of *Secamone elliptica*

Key classes reported for *S. elliptica* include alkaloids, flavonoids, tannins, saponins, and glycosides. Alkaloids contribute to antibacterial effects, flavonoids provide free radical

scavenging, while tannins and saponins support anti inflammatory actions. Glycosides add further medicinal relevance, including possible cardiovascular benefits.

These profiles justify continued inquiry into mechanism and therapeutic development (Oluyemi et al., 2021; Ogundipe et al., 2022; Adetoro, Fagbemi, Olowoselu & Ajibade, 2023; Alabi, Olufemi, Ogunyemi & Ajayi, 2024).

4. 3. Extraction Methods and their Influence on Bioactive Compounds

Extraction strategy strongly shapes chemical yield and bioactivity. In *P. amarus*, water and microwave assisted protocols improved recovery of phenolics and saponins of nutraceutical interest (Nguyen, Thuy Pham, Bowyer, Altena & Scarlett, 2016). Advances in analytics, notably HPLC/ESI-QTOF-MS/MS, enabled rapid screening of 52 compounds and quantitation of six bioactives, highlighting both chemical diversity and the potential to target fractions enriched in specific pharmacophores (Kumar et al., 2015).

4. 4. Pharmacological Activities of *P. amarus*

Reported activities encompass antiviral, antibacterial, antiplasmodial, anti inflammatory, antimalarial, anticancer, antidiabetic, hypolipidemic, antioxidant, hepatoprotective, nephroprotective, and diuretic effects, reflecting multi target bioactivity across disease pathways (Patel et al., 2011; Naaz, Javed & Abdin, 2007; Calixto, 2005). The following areas are prominent:

4. 5. Antiviral Activity

Extracts reduced Hepatitis B surface antigen in chronic carriers, with lignans such as phyllanthin and hypophyllanthin implicated through interference with viral polymerase and antigen secretion. Preliminary signals against HIV further support antiviral prospects (Thyagarajan, Subramanian, Thirunalasundari, Venkateswaran & Blumberg, 1988; Patel et al., 2011).

4. 6. Hepatoprotective Activity

Protection against aflatoxin B1 and carbon tetrachloride injuries has been demonstrated, likely via oxidative stress reduction, enhancement of hepatic regeneration, and enzyme modulation. Lignans stabilize membranes and limit toxin entry (Naaz, Javed & Abdin, 2007).

4. 7. Antioxidant Activity

Phenolics and flavonoids in *P. amarus* display strong radical scavenging and reduce oxidative damage to proteins, lipids, and DNA, supporting prevention across oxidative stress related disorders (Nguyen, Thuy Pham, Bowyer, Altena & Scarlett, 2016).

4. 8. Anti-inflammatory Activity

Extracts attenuate inflammatory responses by down regulating pro inflammatory cytokines and inhibiting COX and LOX, with relevance to asthma, gastrointestinal disturbance, and arthritis (Segda, Meda, Bangou, Koama, Ouoba, Kagambega, Kam & Ouedraogo, 2023).

4. 9. Anticancer Activity

Cytotoxicity against diverse cancer cell lines and apoptosis induction through mitochondrial pathways and cell cycle modulation suggest chemopreventive and therapeutic potential (Nguyen, Thuy Pham, Bowyer, Altena & Scarlett, 2016).

4. 10. Hepatoprotective Effects of *P. amarus*

Beyond acute toxin models, hepatoprotection is evidenced by lowered serum liver enzymes and increased antioxidant enzyme activities, indicating mitigation of oxidative injury and reinforcement of endogenous defense systems (Naaz, Javed & Abdin, 2007).

4. 11. Antioxidant Capacity and Its Implications for Health

Antioxidant strength is sensitive to post harvest processing. Infrared drying at 30 °C enhanced phytochemical retention and antioxidant capacity in *P. amarus* extracts, underscoring how processing choices influence bioefficacy and standardization for research and therapeutic use (Nguyen, Van Vuong, Bowyer, Van Altena & Scarlett, 2015).

5. MATERIALS AND METHODS

Leaves of *Phyllanthus amarus* and *Secamone elliptica* were collected from the garden of Federal Polytechnic Ilaro, Ogun State, washed under running water, air-dried at ambient temperature for 30 days with frequent turning to prevent fungal growth, then milled to fine powder.

Reagents were n-hexane, ethyl acetate, and methanol; key apparatus included round-bottom flasks, separating funnels, Whatman filter paper, muslin cloth, reagent bottles, beakers, a rotary evaporator, water bath, and a UV-Visible spectrophotometer.

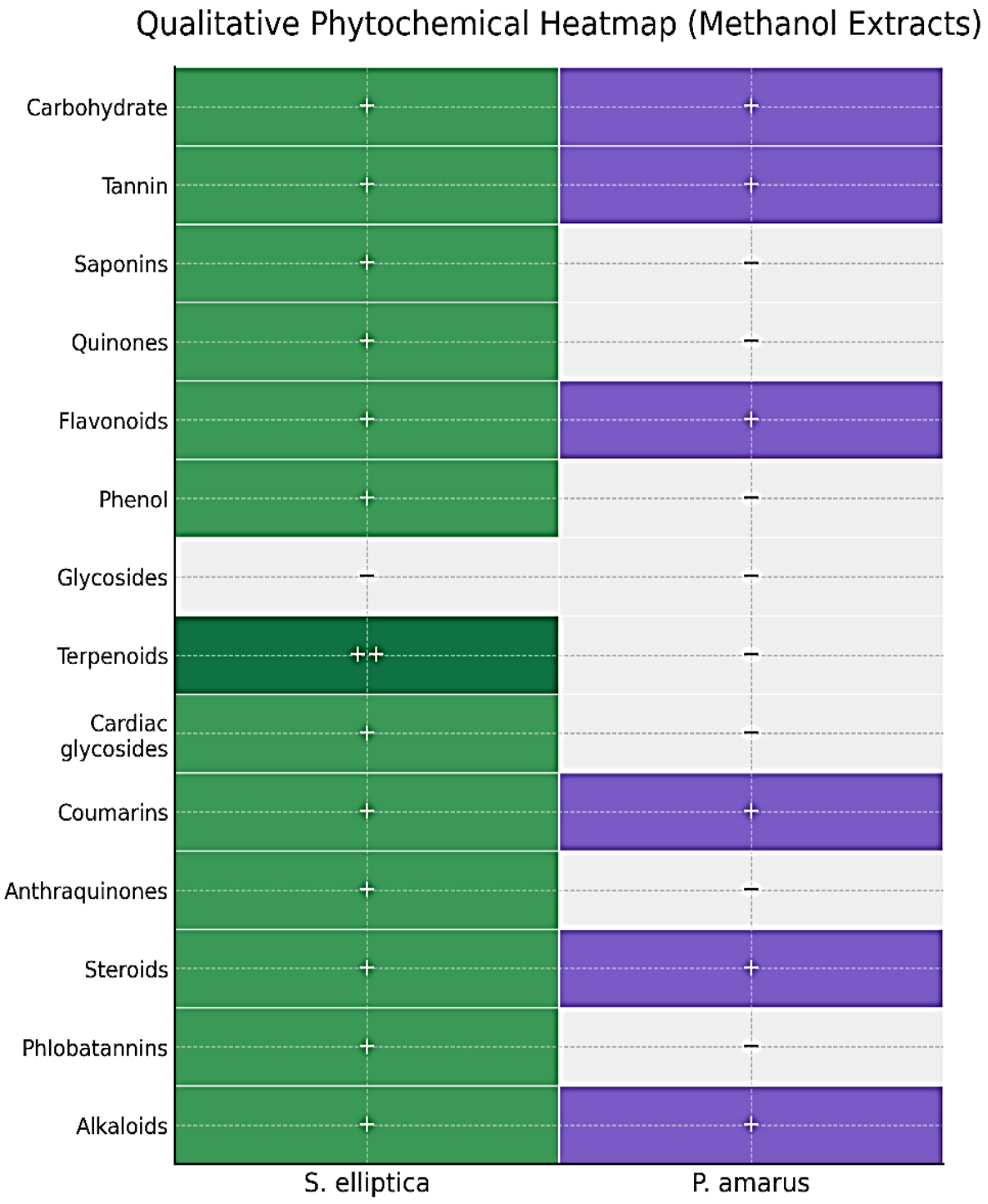
For solvent maceration, 500 g of each powdered sample was soaked separately in 2.5 L of n-hexane for 24 h, and in ethyl acetate or methanol for 72 h; filtrates were passed through muslin and Whatman paper, concentrated on a rotary evaporator to semi-solids, and further solvent removed on a water bath before storage for analysis. Qualitative phytochemical screening followed standard assays (Roghini & Vijayalakshmi, 2018): Molisch's test for carbohydrates; $FeCl_3$ for tannins and phenols; foam test for saponins; alkaline reagent test for flavonoids; concentrated H_2SO_4 for quinones; Salkowski-type interface for terpenoids; Keller-Kiliani-type ring test for cardiac glycosides; NaOH test for coumarins; alkaline reaction for anthraquinones; chloroform - H_2SO_4 ring tests for steroids and phytosteroids; and HCl precipitation for phlobatannins. Antioxidant activity was assessed by DPPH radical scavenging: 0.1 mM DPPH in ethanol (1 ml) was mixed with 1 ml extract at 25-100 µg/ml, incubated 30 min at room temperature, and absorbance read at 517 nm; percent inhibition was calculated as

% inhibition = $[(A_0 - A_1)/A_0] \times 100$, and IC_{50} defined as the concentration giving 50 percent inhibition (Shimada, Fujikawa, Yahara & Nakamura, 1992).

6. RESULTS AND DISCUSSION

6. 1. Results

Table 1. Heat Map of Detected Phytochemical Classes in *P. amarus* and *S. elliptica*.



Keyword: (＋) present,(++) moderately present, (－) absent.

Table 2. DPPH radical scavenging activity (%) of *Phyllanthus amarus* and *Secamone elliptica*.

Sample	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
A3 (*S. elliptica*)	9.64	16.06	36.53	48.55	58.28
B3 (*P. amarus*)	9.09	19.09	33.23	45.06	51.95
Ascorbic acid (standard)	13.99	27.86	50.88	72.25	89.32

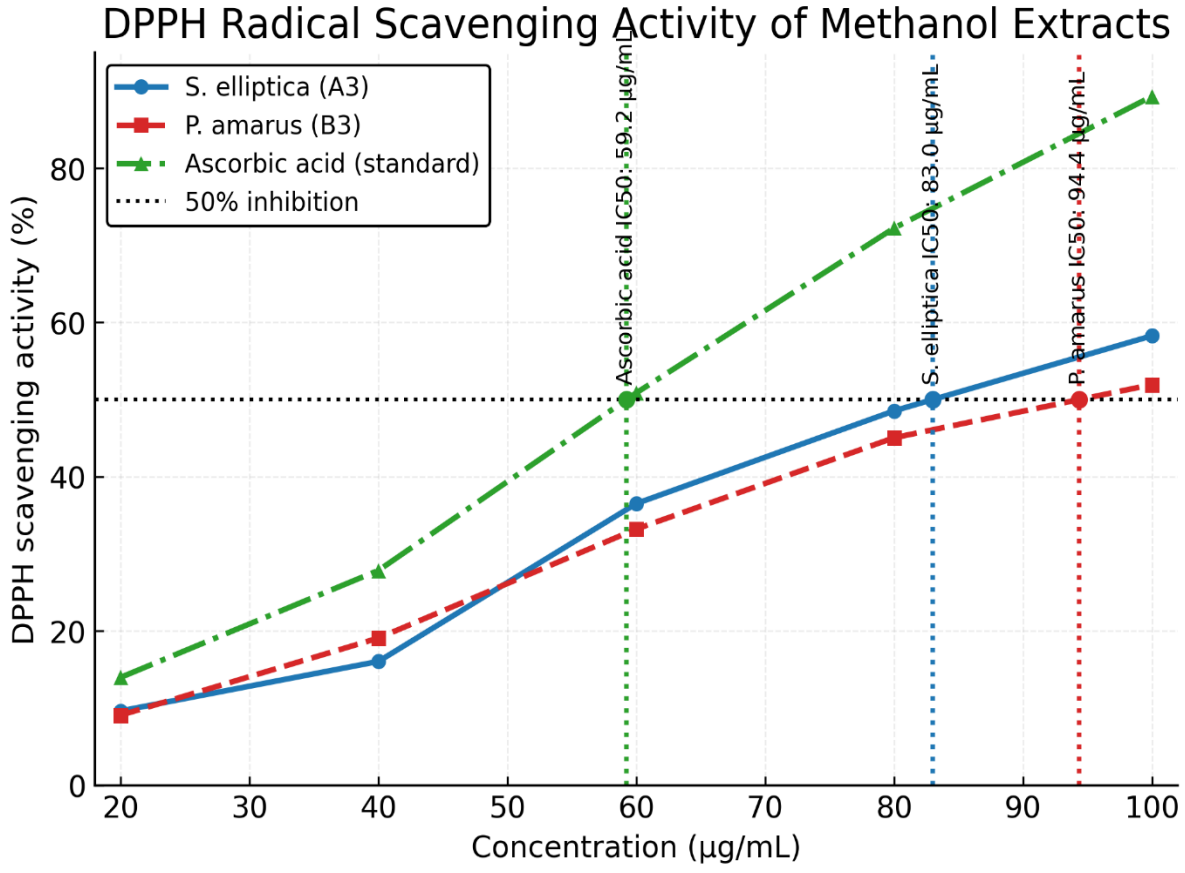


Figure 1. DPPH Radical Scavenging Activity of Methanol Extracts.

6. 2. Discussions

The qualitative screening indicates that the methanol extract of *Secamone elliptica* contains a broader array of phytochemical classes than *Phyllanthus amarus*. Both species tested positive for carbohydrates, tannins, flavonoids, coumarins, steroids, and alkaloids. Only *S. elliptica* showed saponins, quinones, phenols, terpenoids (moderate), cardiac glycosides,

anthraquinones, and phlobatannins, while glycosides were absent in both. This wider profile is consistent with stronger antioxidant behavior, since many of the additional classes in *S. elliptica* are rich sources of hydrogen or electron donors that quench free radicals.

DPPH results showed a clear dose dependent increase in scavenging for all samples. At 100 µg/mL, *S. elliptica* reached 58.28 percent inhibition and *P. amarus* reached 51.95 percent, whereas ascorbic acid achieved 89.32 percent. Across all concentrations, *S. elliptica* outperformed *P. amarus*, and the gap widened at higher doses. The estimated IC50 values further support this ranking. Ascorbic acid exhibited the lowest IC50 at about 59.2 µg/mL, *S. elliptica* followed at about 83.0 µg/mL, and *P. amarus* was least potent at about 94.4 µg/mL.

The relative positions suggest that the methanol extract of *S. elliptica* possesses higher radical scavenging capacity than *P. amarus* under the assay conditions, but neither matched the reference antioxidant.

The phytochemical heat map helps explain these differences. The presence of phenols and a moderate level of terpenoids in *S. elliptica*, coupled with saponins and anthraquinones, likely contributes to synergistic free radical scavenging. Phenolic hydroxyl groups donate hydrogen to DPPH, while flavonoids stabilize the resulting radical through resonance. Terpenoids and saponins can enhance membrane interactions and may facilitate antioxidant effects in complex mixtures. By contrast, the *P. amarus* methanol extract lacked several of these classes in this qualitative screen, which aligns with its weaker DPPH response. It is important to note that *P. amarus* is known to contain potent phenolics and lignin in other contexts, so the present outcome may reflect extractability into methanol under the adopted protocol, plant material variability, or post-harvest handling.

Benchmarking against ascorbic acid underscores two points. First, the plant extracts act as complex mixtures with multiple moderate contributors rather than a single high potency antioxidant, hence their higher IC50 values. Second, the near linear rise of the extract curves between 60 and 100 µg/mL suggests that higher concentrations or enrichment of active fractions could close some of the potency gap. Fractionation guided by the DPPH assay, followed by targeted quantification of total phenolic and total flavonoids, would help isolate the chemical drivers of activity.

These antioxidant results have relevance for antihypertensive potential. Oxidative stress contributes to endothelial dysfunction, nitric oxide depletion, and heightened vascular tone. Extracts that scavenge radicals can, in principle, preserve endothelial function and modulate pathways linked to blood pressure. The presence of coumarins and cardiac glycosides in *S. elliptica* invites further testing of angiotensin converting enzyme inhibition, nitric oxide bioavailability, and vascular relaxation on isolated tissue preparations, to move from chemical antioxidant readouts to physiological mechanisms.

The study has limitations that inform next steps. The phytochemical analysis is qualitative, so the concentrations of active classes remain unknown. Only one solvent system per plant was interpreted for the DPPH comparisons, yet extraction chemistry strongly affects yield and composition. Only a single radical scavenging assay was used. Replicate measurements with error bars and statistical testing would strengthen confidence in the observed differences. Methodologically, four parameter logistic modeling of dose response would provide more robust IC50 estimates than linear interpolation between points.

The methanol extract of *S. elliptica* displayed a richer phytochemical spectrum and stronger DPPH scavenging than *P. amarus*, though both were less potent than ascorbic acid. The data support continued work on *S. elliptica*, prioritizing quantitative phenolic and flavonoid

assays, fraction guided purification, and complementary antioxidant tests such as ABTS, FRAP, and ORAC. Coupling these with anti-hypertensive bioassays will clarify whether the chemical antioxidant advantages observed here translate into vascular benefits relevant to hypertension management.

7. CONCLUSIONS

This study screened the methanolic leaf extracts of *Phyllanthus amarus* and *Secamone elliptica* and evaluated their antioxidant performance as a proxy for antihypertensive relevance. The qualitative profile showed that both plants contain carbohydrates, tannins, flavonoids, coumarins, steroids, and alkaloids, while *S. elliptica* additionally contained phenols, saponins, quinones, terpenoids, cardiac glycosides, anthraquinones, and phlobatannins. In the DPPH assay all samples displayed dose dependent radical scavenging, with activity ranked as ascorbic acid greater than *S. elliptica* greater than *P. amarus*. The lower estimated IC50 for *S. elliptica* relative to *P. amarus* indicates higher antioxidant potency in methanol extracts under the present conditions. Since oxidative stress contributes to endothelial dysfunction and elevated vascular tone, the stronger antioxidant response of *S. elliptica* supports a higher likelihood of antihypertensive benefit, although direct vascular or enzyme based evidence is still required. Overall, both species show promising antioxidant properties, with *S. elliptica* emerging as the more compelling candidate for follow up antihypertensive investigations.

Recommendations

Building on the phytochemical screening and DPPH results, the following focused steps will strengthen the evidence for antioxidant and antihypertensive relevance of *Phyllanthus amarus* and *Secamone elliptica*, while improving rigor, comparability, and reproducibility.

1) **Direct antihypertensive biochemical assays**

   Screen for angiotensin converting enzyme and renin inhibition, nitric oxide related chemistry, and xanthine oxidase inhibition to link constituent classes to blood pressure pathways.

2) **Potency modeling and statistics**

   Generate replicated dose - response data, fit a four parameter logistic model, and report IC50 with confidence intervals, including analysis of variance and appropriate multiple comparison controls.

3) **Assay triangulation**

   Complement DPPH with ABTS, FRAP, ORAC, and lipid peroxidation suppression assays, and then correlate outcomes with total phenolic and total flavonoids to identify drivers of activity.

4) **Bioactivity guided fractionation**

   Partition the extracts and track activity during fractionation to localize potent pools, prioritizing phenolic and terpenoid enriched fractions in *S. elliptica* and lignin rich fractions in *P. amarus*.

5) **Quantitative standardization**

Profile marker compounds by HPLC or LC–MS, report extraction yield and drying conditions, and propose specification ranges that link marker levels to antioxidant and antihypertensive assay outcomes.

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