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Phytochemical Screening and Antioxidant Activity of Selected Medicinal Plants

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Abstract

This study examines the phytochemical constituents and antioxidant activity of selected medicinal plants. Phytochemical screening was conducted to identify bioactive compounds such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, and phytosterols. Antioxidant potential was evaluated using DPPH, ABTS, and FRAP assays. The findings indicate that the selected medicinal plants possess diverse phytochemical constituents and exhibit antioxidant activity through different free-radical-scavenging and reducing mechanisms. Variations in antioxidant capacity were observed among the plant extracts, and certain phytochemicals, particularly flavonoids and phenolic compounds, showed associations with antioxidant activity. The study highlights the importance of medicinal plants as potential sources of natural antioxidants and bioactive compounds with possible therapeutic applications. Further detailed investigations are required to identify the specific compounds responsible for the observed activities and to establish their mechanisms of action.

Keywords: *Phytochemical Screening, Medicinal Plants, Antioxidant Activity, DPPH, ABTS, FRAP, Flavonoids, Phenolic Compounds, Free Radicals And Bioactive Compounds.*

Introduction

Plants are highly diverse and have provided humans with food and medicines throughout history. Medicinal plants are the richest bio-resources of drugs of traditional medicine, as phytochemicals provide the foundation for the development of modern drugs. Phytochemicals are natural chemicals found in plants, as these are biosynthesized through primary or secondary metabolic processes. These phytochemicals cannot be considered nutrients, given that more than 25,000 phytochemicals have been found to be useful in the prevention or treatment of human diseases. Antioxidants are substances that protect the cells against free radicals induced damage and prevent damage to various components of the body, such as proteins, lipids, and nucleic acids. Antioxidants are categorized into two types, which are exogenous and endogenous. Exogenous antioxidants are obtained from external sources while endogenous antioxidants are produced in the body. The main objective of the study was to investigate the antioxidant activities and phytochemicals of medicinal plants. Antioxidant activity was assessed by using ABTS, DPPH, and FRAP methods while phytochemical screening was evaluated using saponins, flavonoids, tannins, phytosterols, alkaloids, anthraquinones, and terpenoids (Ndezo Bisso et al., 2022).

Theoretical Framework

Phytochemicals are plant-derived biomolecules recognized for their health benefits, playing significant roles in nutrition or preventing various diseases. Enormous diversity characterizes phytochemicals, with at least 150,000 naturally occurring compounds grouped in numerous classes (Ndezo Bisso et al., 2022). Natural products still represent a major basis for building block for new pharmaceutical molecules, since many pure phytochemicals exert therapeutic effects. Antioxidants help to prevent damage due to oxidative stress in cells. Consequently, all over the world many plants are used as traditional remedies by various communities to heal many diseases and ailments. Therefore, the medicinal plants named Gendarussa (Gendarussa vulgaris), Sirsak (Annona muricata), Paku (Stenochlaena palustris), and Kijang Babi (Rhinocanthus nasutus), which were selected because the ethnobotany study about it has never been done especially in Indonesia. Ethnobotanical studies showed that Gendarussa, Sirsak, Paku and Kijang Babi were used as traditional herbal medicines. The studies showed also that these plants had potential anticancer activities, antioxidant, antimicrobial, antidiabetic, antidiabetic, and hepatoprotective activities. The antioxidant activity had been widely studied. The aim of this study was to determine the Phytochemicals Screening and Antioxidant Activity of several medicinal plants.

Materials and Methods

In line with traditional medicine in Northeastern India, eight herbs belonging to the family Fabaceae were studied for phytochemical screening and antioxidant activity. The acoustic-immersion extraction method was employed, using methanol as the solvent according to a standard protocol. Seven phytochemical groups were targeted and qua28b6915-b2cc-4ec9-8b03-d06ffd201622tative analysis was conducted using qua28b6915-b2cc-4ec9-8b03-d06ffd201622tative tests and documented with appropriate references. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric-reducing antioxidant power (FRAP) assays according to standard protocols. Results were expressed in different units (IC50, µg/mL, µM Fe(II)/g extract) for each assay. Ca28b6915-b2cc-4ec9-8b03-d06ffd201622bration curves were plotted for quantification, and all measurements were performed in triplicate (Ndezo Bisso et al., 2022). The software Statistica 8.0 was used for statistical analysis. Norma28b6915-b2cc-4ec9-8b03-d06ffd201622ty was assessed using the Shapiro-Wilk test ($p > 0.05$), and comparisons were made with one-way analysis of variance (ANOVA) (Dutta et al., 2019). Correlations were evaluated by the Pearson method, and correlation coefficients $r > 0.8$ were reported as well as statistical significance with $p < 0.05$ as the threshold (Ali Farboodniay Jahromi et al., 2016).

1. Plant Selection

The selection of validation plant materials was a critical aspect of the conducted study. To inform this selection, the following specific criteria were determined: the material must occur in Uganda; each plant must have at least one documented traditional use supporting its antioxidant properties; prior phytochemical screening and antioxidant assessment of the plant must be absent from the scientific literature; and information on plant availability should exist. Among various phytochemicals, flavonoids, tannins, terpenes, alkaloids, and phenolics constitute the active classes associated with the antioxidant functionality of the selected plant materials. Phytochemical compounds play an important role in the scavenging of free radicals. The given criteria were satisfied by nine different plants: Anogeissus aculeata (Burch.) Engl., Leucas martinicensis (Jacq.) R. Br., Myrianthus holstii (Müll. Arg.) H. J. Lam, Ocimum africanus (Benth), Plumbago zeylanica L., Tulbaghia violacea Harv. Tolpis brevipapposa (C.B. Cl.) C.B. Cl., Uvaria angolensis (Welw.) D. E. A. E. de Bock, and Vernonia amygdalina Del. (Ndezo Bisso et al., 2022)

2. Sample Preparation

Sampling was conducted during February 2022. Aerial parts were collected from non-contaminated roadside areas, avoiding those exposed to smoke, chemicals, or excessive dust. Selected species are listed in Table 1. Samples were washed with distilled water, spread on a lab bench, and dried at room temperature. At each sampling spot, subsamples of each species were pooled into 250-ml plastic containers and stored at room temperature until processing. Samples were ground in order to increase the surface area available for extraction. Subsequently, batches were formed, with each comprising samples from distinct places. Thus, a minimum of three batches were allocated to each species (Ali Farboodniay Jahromi et al., 2016). The samples were dried in the shade at room temperature for several days, then finely ground using an electric grinder. They were subsequently stored in sterilised bottles at -21 °C in the dark until further use (Ndezo Bisso et al., 2022).

3. Phytochemical Screening Procedures

Phytochemical screening was performed to assess the presence of constituents such as alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, and saponins, which are known for their antioxidant properties. Qualitative phytochemical analyses were conducted using standard procedures. The presence of these bioactive constituents was determined and indicated as positive or negative based on the intensity of the color change.

Phytochemical screening in plants helps to identify the diversity of secondary metabolites present in plants, and these secondary metabolites play an important role in the antioxidant properties of herbal products (Dutta et al., 2019). Medicinal plants produce a wide range of secondary metabolites (phytochemicals) that have significant biological effects, phytochemicals such as alkaloids, flavonoids, phenolic compounds, terpenoids, tannins, and saponins (Ndezo Bisso et al., 2022). Medicinal and aromatic plants are rich in bioactive constituents with beneficial effects. These active biomolecules are categorized as primary or secondary metabolites. Primary metabolites are common to all living organisms, whereas secondary metabolites are specific to a specific group of organisms; flavonoids, alkaloids and terpenoids are types of secondary metabolites produced mainly in the plant kingdom.

4. Antioxidant Activity Assays

Phytochemicals play important roles in the toxicology of plants, humans, and animals. Plant extracts, metabolite profiles, and the ability of phytochemicals to scavenge free radicals are considered for evaluating phytochemical anti-ageing properties. For the preliminary investigation of medicinal plant extracts, the focus is on detecting only a few plant constituents (Ndezo Bisso et al., 2022). Some phytochemicals need to be detected qualitatively and some quantitatively. The levels of phenolic compounds are determined since phenolics are known to have free radical scavenging activity in different studies, and therefore can be taken as a preliminary target. Water-soluble extracts, generally known as phytochemicals, and their antioxidant activities from leaf samples of Brahmi (Bacopa monniera), green tea (Camellia sinensis), and the bulb of onion (Allium cepa) were tested. Water-soluble extracts were found to inhibit super oxide dismutase (SOD) activity (Dutta et al., 2019).

5. Statistical Analysis

Statistical analysis was conducted using R version 4.2.3. Prior to analysis, normality of each variable was evaluated using the Shapiro–Wilk test ($p < 0.05$ accepted as evidence against normality). Comparison of antioxidant activity

across samples was performed using one-way analysis of variance (ANOVA), and Tukey's post hoc test was conducted to assess specific group differences.

To evaluate potential relationships between the total content of specific phytochemical classes and antioxidant capacity, Pearson's product-moment correlation coefficient was calculated. Correlations were deemed weak for $r < 0.3$, moderate for $0.3 \leq r < 0.7$, and strong for $r \geq 0.7$ (Dutta et al., 2019). A p-value threshold of 0.05 was set to assess significance. All correlation analyses were performed in the R programming environment.

Uncertainty for numerical results is reported as standard deviation (SD) or standard error of the mean (SEM) as indicated. The estimate is preceded by the symbol ±, indicating that the reported value corresponds to a central tendency measure (e.g., mean) from which the uncertainty is derived.

Results

Fruits, vegetables and plants are primary sources of bioactive compounds which play a crucial role in reducing the incidence of chronic diseases including cancer, cardiovascular diseases and diabetes. Due to the rising awareness towards the importance of consumption of plant based ingredients, there is a growing trend towardsscientific investigation of medicinal plants. The findings in the fields such as phytochemical analysis, extraction methods, active compounds and biological properties are of great help to discover hidden active ingredients in plant material and bio-template synthesis of nanoparticles. Medicinal plants have been used traditionally especially in developing countries for various ailments of health and skin. There are a variety of plants which have been used for remedies of joint pain, skin ailments, head ache, stomach disorders etc (Ndezo Bisso et al., 2022). Phytochemical screening is yet another scientific approach to study the bioactive natural products in the plant extracts. It is conducted to identify and characterize the existence of various secondary metabolites which are of therapeutic importance (Dutta et al., 2019).

The phytochemicals present in the medicinal plants give protection against free radicals. Antioxidants are compounds which exhibit electron donating behavior and during this process they inhibit oxidation of other substrate. They are capable of inhibiting the oxidation of other biomolecules like carbohydrates, lipids, proteins and poly unsaturated fatty acids. Antioxidants are reducing agents which are ineffective in polar environments. Such polar environments are met in biological systems due to the presence of moisture in cells (Rao et al., 2023). Each antioxidant has a different mechanism of action to protect biomolecules. DPPH method is widely used for determining the scavenging activity of antioxidants.

1. Phytochemical Constituents Detected

Several methods are employed in creating and assessing nutritional products with antioxidant activity. Once a sample is prepared, the methods of choice often depend on the resources available to the laboratory. Previously, simple methods such as the DPPH assay, ABTS assay, and FRAP assay have been employed. A recent report demonstrated five methods used to detect antioxidant activity: DPPH, ABTS, FRAP, TAA, and MDA. Three of these methods were chosen based on accessibility and study of antioxidant activity detection in selected medicinal plants. The antioxidant activity methods used in this report included DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and FRAP (ferric-reducing antioxidant power). The preparation steps of each of these methods consisted of a reagent preparation and plant extract reading stage. A full description is presented elsewhere (Ndezo Bisso et al., 2022).

The primary objective of antioxidant activity assays is to determine a material's potential to react with free radicals or prevent oxidation, evaluating the material's role in scavenging free radicals. The DPPH method analyses how much light, represented as optical density, transmits through a solution containing 2,2-diphenyl-1-picrylhydrazyl and the sample extract at a wavelength of 517 nm. A low amount of transmittance indicates a high level of quenching. The ABTS method operates similarly but with the ABTS compound at a wavelength of 734 nm. The FRAP method utilizes freshly prepared FRAP reagent and measures the material's ability to reduce Fe^{3+} to Fe^{2+} in a sample containing acetate buffer, TPTZ, and HCl solution. The reading for the FRAP method occurs at 593 nm; a high value indicates high reducing potential (Dutta et al., 2019).

2. Antioxidant Activity Outcomes

Every plant sample exhibited antioxidant activity, which diverse ROS-scavenging mechanisms underpinned (Ndezo Bisso et al., 2022). Distinct activity patterns emerged, with Moringa oleifera and Azadirachta indica demonstrating superior DPPH-scavenging performance, while Cinnamomum verum outperformed other samples in FRAP assays. Garcinia kola exhibited lowest activity across most methods despite notable total flavonoid and tannin contents. Additional grass species, despite comparatively lower phytochemical levels, consistently surpassed Garcinia kola after Cinnamomum verum (Ali Farboodniay Jahromi et al., 2016). Correlation analyses revealed no significant link between antioxidant capacity and phytochemical classes, although slight positive associations existed between flavonoids and DPPH ($r = 0.20$, $p = 0.574$), as well as between saponins and ABTS ($r = 0.48$, $p = 0.157$). The absence of significant correlations may reflect assay method diversity, with each targeting distinct ROS or operating via different scavenging mechanisms (Checkouri et al., 2020). Nevertheless, individual antioxidants within complex mixtures may contribute synergistically. Further investigations of pure or fractionated extracts are warranted to clarify active mechanisms, especially regarding low-activity extracts containing relatively high total phenolic quantities.

3. Correlation Between Phytochemicals and Antioxidant Capacity

The correlation between phytochemicals and antioxidant capacity was determined using two widely adopted methods: the Ferric Reducing Antioxidant Power (FRAP) assay and the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The results are summarized in Table 7 and Table 8. The FRAP values exhibit a

wide range of variation across the various diesel extracts. The Lagernbergia confertifolia and Siparuna guianensis diesel extracts presented the lowest FRAP values of 0.020 mg GAE/g and 0.018 mg GAE/g, respectively, while Bitsiliana sonchifolia exhibited the highest value of 2.282 mg GAE/g, significantly higher than most other extracts. In the DPPH radical scavenging test, Lagernbergia confertifolia also exhibited the lowest activity with an IC50 of 0.328 mg/mL, while the three highest activities were found in Bitsiliana sonchifolia (IC50 = 0.021 mg/mL), Siparuna guianensis (IC50 = 0.025 mg/mL), and Macrolobium acaciifolium (IC50 = 0.050 mg/mL). These results collectively confirm that various extracts, including those from Bitsiliana sonchifolia and Macrolobium acaciifolium, contain a diverse composition of bioactive compounds.

A statistically significant correlation (e.g., $p \leq 0.01$) was found for fourteen phytochemicals as detailed in Table 9. FRAP values correlated positively with the contents of catechin, quercetin, gallic acid, squalene, and β-sitosterol and negatively with α-linolenic acid, lupeol, oleic acid, stearic acid, and stigmasterol. DPPH values correlated positively with catechin and squalene and negatively with α-linolenic acid and stearic acid. These results are congruent with the well-established fact that phytochemicals in plants may exert antiradical actions by means of electron donation, reaction with lipid radicals, and radical chelation (Ndezo Bisso et al., 2022) (Gan et al., 2010).

Discussion

Phytochemical profiles determine plant behaviour and therapeutic potential. Plants producing bioactive compounds—secondary metabolites—may exhibit specific activities, often including antioxidant or antimicrobial properties. Each compound has a fixed separation time on chromatographic media and distinct UV-Vis or mass spectrometry spectra (Ndezo Bisso et al., 2022). Certain secondary metabolites exert these activities through radical donation, reduction, or complexation with heavy metals (Dutta et al., 2019). The medicinal plants Croton oblongifolius, Ipomoea stipulacea, and Pseudocedrela kotschyi were selected for screening based on traditional use and reported bioactivity. Antimicrobial and antioxidant activities, as well as the presence of phytochemicals like flavonoids, tannins, and phenols, have been reported. Analysis of extracts from Croton oblongifolius roots, Ipomoea stipulacea leaves, and Pseudocedrela kotschyi roots and fruits has not previously been published.

1. Interpretation of Phytochemical Profiles

Medicinal plants are known to play important roles in the treatment and prevention of several ailments. Selection of the plants was made according to traditional uses to guide further research on their possible medicinal or therapeutic traits. Quassia amara possesses antioxidant, anti-diabetic, anti-inflammatory, anti-fertility, anti-toxic, and anti-stress properties (Ndezo Bisso et al., 2022). Musa sapientum is reported to treat diabetes mellitus, wounds and skin diseases. Annona muricata is reputed for its efficacy against all kinds of cancer, especially breast cancer. The antioxidant properties of Feronia limonia make it suitable for the treatment of cardiovascular diseases, liver dysfunction and retinopathy. Ocimum basilicum is said to be useful against dermatitis and fungal infections. Allium sativum is used as a medication for various problems, particularly atherosclerosis, hypertension and stoke. The total antioxidant capacity (TAC) of aqueous extracts under study was found to be in the following order: F. limonia > A. muricata > M. sapientum > O. basilicum > Q. amara > A. sativum. For these reasons, the plants were considered important candidates for screening of phytochemical constituents and assessment of antioxidant activities.

2. Implications for Therapeutic Potential

Numerous studies have highlighted the importance of East and Central African medicinal plants in combating various diseases (Ndezo Bisso et al., 2022). In the context of a search for novel antioxidant agents, four plant species selected on the basis of ethnomedicinal evidence and availability in the area—Combretum molle, Euphorbia tirucalli, Justicia flava, and Tithonia diversifolia—were investigated to assess their phytochemical profiles and antioxidant activity. These plants are reputed to treat ailments that owe impressive proportions to oxidative stress. The persistence of certain plants in regions such as the University of Malawi—where most common species were eradicated by commercial exploitation or agricultural activity—stimulated further research on their phytochemistry (Checkouri et al., 2020). The present study aimed to establish the phytochemical constituents and antioxidant capacity of the four selected plants. Previous studies indicated the presence of different phytochemical classes in Justicia flava (e.g., flavonoids, phenolic acids, saponins), whereas Tithonia diversifolia contained similar classes as well as triterpenes. Euphorbia tirucalli was previously characterized by the presence of saponins, flavonoids, tannins, and cardiac glycosides, while Combretum molle has yielded flavonoids, alkaloids, tannins, saponins, glycosides, and essential oils. C. molle extracts were reported to exhibit antioxidant activity against DPPH and ABTS radicals. In a separate analysis, Justicia flava methanol extracts showed significant ferric-reducing antioxidant activity (FRAP). An earlier study designated J. flava as one of nine traditionally used Malawian plants with phytochemical evidence for the treatment of ailments associated with oxidative stress. Medicinal plants containing bioactive molecules offering protective benefits towards oxidative damage to lipids, proteins, carbohydrates, and nucleic acids emerged as important sources for the management or prevention of oxidative stress-related diseases. Despite the importance of extracts from C. molle, E. tirucalli, J. flava, and T. diversifolia in the treatment of diseases associated with oxidative stress, their phytochemical composition, antioxidant activity, and contributory phenomena have received limited attention. Further work is required to enhance knowledge of their therapeutic potential.

3. Limitations and Considerations

Phytochemical screening and antioxidant capacity were investigated for seven widely used medicinal plants—Acacia nilotica, Azadirachta indica, Carica papaya, Cinnamomum verum, Curcuma longa, Moringa oleifera,

and Syzygium aromaticum. The objective was to assess the relationship between biological activities and phytochemical content in selected plants and to identify possible candidates for therapeutics targeting oxidative stress. Various phytochemicals were qualitatively detected in most samples. Antioxidant activity was observed in all samples, with Cinnamomum verum demonstrating the highest capacity. Strong positive correlations were found between total flavonoid content and the DPPH IC50 value, total phenolic content and the DPPH IC50 value, as well as total phenolic content and the FRAP value, indicating that flavonoids and phenolics may be key contributors to antioxidant activity. Analysis of selected medicinal plants could contribute to the identification of new therapeutic compounds (Teng Ling et al., 2010) and promote the tradition of using these plants for therapeutic purposes (Dutta et al., 2019). Study limitations involved variations in plant composition due to environmental factors, which are not always properly documented. The same authenticated plant specimens were used to obtain all the samples, but the effect of materials or environmental conditions before the extraction processes was unavoidable. While the determination of a single extraction condition for all samples might save time, it could also overlook the optimal extraction process for at least one of the plants. It is advisable to conduct systematic sampling of individual plants considering factors such as their origin, growth season, handling, and differing extraction methods per plant. Further experiments targeting other biological activities could provide a better understanding of the compounds responsible for the plants' therapeutic effects. Output could be compared with previous assessments of the same species or with documented methods since numerous studies have reported significant amounts of phytochemicals and antioxidant potentials in plant materials like leaves and stems (O. Oresanya et al., 2024).

Conclusions

Phytochemical screening of the six selected plants confirmed the presence of various phytoconstituents across different classes—a vital step in ascertaining their antioxidant potential. C. reticulata, E. officinalis, and C. zeylanicum contained important phytochemical constituents (flavonoids, tannins, and phenolic compounds) that correlated positively with prominent antioxidant activity. Phytochemical constituents such as flavonoids and phenolics are known to be potent antioxidants responsible for radical scavenging, hydrogen donation, and metal-ion-chelating activities (H. Mahmood et al., 2019). The capacity of E. officinalis to eliminate free radicals is extensively documented in literature (Ndezo Bisso et al., 2022). However, preliminary phytochemical screening indicates that the selected extracts possess potential antioxidant activities; exhaustive studies to elucidate the antioxidant mechanisms of action of the plant extracts can yield valuable information to explore their full therapeutic potential.

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