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# INVESTIGATING PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT POTENTIAL, AND ANTIDIABETIC EFFECTS OF GROUND APPLE AMONG MEDICINAL PLANTS

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## ABSTRACT

Context. There are several medicinal chemicals found in plants that have wide-ranging uses in the pharmaceutical sector.

This study sought to determine which phytochemicals were found in the seven chosen medicinal plants, as well as the antibacterial and antioxidant properties of these compounds. Techniques. Phytochemical screening, total phenolic content, and favonoid levels were measured by means of conventional techniques. Using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (OH), and nitric oxide (NO) radical scavenging tests, the antioxidant activity of plant extracts was assessed. The broth microdilution method was used to assess the plant extracts' antibacterial properties. Conclusions. The phytochemical investigation revealed that all plant extracts contained phenols, favonoids, and steroids. The highest total phenolic and flavonoid concentrations were found in the extract of Psychotria peduncularis, which had 5.57 ± 0.22 mg GAE/g and 1.38 ± 0.06 mg QE/g, respectively. The DPPH and NO radical scavenging activities of all plant extracts demonstrated extremely significant antioxidant activity, with IC50 values ranging from 0.55 to 49.43 µg/mL and 0.65 to 13.7 µg/mL, respectively. The antibacterial activity of P. peduncularis and Tristemma mauritianum extracts was significant, with MIC values ranging from 16 to 1024 µg/mL. All investigated species were resistant to the bactericidal effects of T. mauritianum extract. The antifungal activity of P. peduncularis and Alsophila manianna extracts against the Candida albicans strain was significant (MIC 64 µg/mL). In conclusion. The screened extracts of medicinal plants utilized in our investigation may be employed as resources for the creation of novel medications as well as possible antioxidant and antibacterial agents.

## I. INTRODUCTION

The emergence and spread of drug-resistant pathogens that have acquired new resistance mechanisms, leading to antimicrobial resistance, continues to threaten our ability to treat common infections [1]. Especially alarming is the rapid global spread of multi- and pan-resistant bacteria (also known as “superbugs”) that cause infections that are not treatable with existing antimicrobial medicines such as antibiotics or antifungals [2]. Te clinical pipeline of new antimicrobials is dry. In 2019, the World Health Organization (WHO) identifed 32 antibiotics in clinical development that address the WHO list of priority pathogens, of which only six were classifed as innovative. Furthermore, a lack of access to quality antimicrobials remains a major issue. Antibiotic and antifungal shortages afect countries of all levels of development, especially in health-care systems [3].

In addition, the overproduction of reactive oxygen species (ROS) has been implicated in the development of various chronic and degenerative diseases such as cancer, respiratory, neurodegenerative, and digestive diseases [4]. Under physiological conditions, the concentrations of ROS are subtlety regulated by antioxidants, which can be either generated endogenously or externally supplemented. A combination of antioxidant-defciency and malnutrition may render

individuals more vulnerable to oxidative stress, thereby increasing the risk of cancer occurrence [4]. In addition, antioxidant defense can be overwhelmed during sustained infammation such as in chronic obstructive pulmonary diseases, infammatory bowel disease, neurodegenerative disorders, cardiovascular diseases, and aging [5]. Certain antioxidant vitamins, such as vitamin D, are essential in regulating biochemical pathways that lead to the proper functioning of organs. Antioxidant supplementation has been shown to attenuate endogenous antioxidant depletion thus alleviating associated oxidative damage in some clinical research [6]. Increasing trends of microbial resistance to antibiotics and various chronic and degenerative pathologies of humans caused by reactive oxygen species (ROS) have triggered the search for bioactive compounds from plants with alternative mechanisms of action to counteract pathogenic microbes and natural antioxidants capable of protecting the body against oxidative stress and free radical-induced damage [7, 8]. Te proper use of medicinal plants requires accurate scientifc information and an understanding of their chemical constituents. Te therapeutic efects in plants are due to the chemical compounds therein [9]. Medicinal plants play a very important role in the development of alternative drugs without the adverse efects of synthetic drugs [10, 11]. Plants and natural products form the basis of both modern and traditional medicines and are currently widely used in the production of commercially produced drugs. Scientifc and reliable reports indicated that about 25% of prescribed medicines worldwide are taken from herbs [12, 13].

Heterotis decumbens, Lavigeria macrocarpa, Tristemma mauritianum, Cyanthillium stelluliferum, Alsophila manianna, Crassocephalum bougheyanum, and Psychotria peduncularis are promising underinvestigated medicinal plants from Cameroon (Table 1). Although not indicated in the literature, they are used in Tombel locality in Cameroon for the treatment of microbial infections. H. decumbens of the Mecastomataceae family, it is largely used in traditional medicine for eye infection sprain, female infertility, trypanosomiasis, hernia, beriberi, and gastralgia [14]. L. macrocarpa is a traditional medicinal plant belonging to the Icacinaceae family and is used as a genital stimulant, depressant, and aphrodisiac [15]. T. mauritianum is a specie of fowering plants in the Mecastomataceae family. Previous studies on T. mauritianum reported its antioxidant and antisalmonellal activities [17]. Phytochemical investigation of T. mauritianum has resulted in the isolation of 2, 4-ditert-butylphenol, 2 ((octyloxy) carbonyl) benzoic acid and sitosterol with antibacterial activity [18]. C. stelluliferum, also called Triplotaxis stellulifera, belongs to the Asteraceae family. Traditionally, it has been used for the treatment of polyhydramnios and amnionitis afecting newborns. It is also known to have immunomodulatory properties [19, 20]. A. manianna synomyn Cyathae manianna is a species of tree fern belonging to the Cyatheaceae family. Its leaves and seeds have been used to treat flariasis, while its stembark has been used for the treatment of backache [22, 23]. In addition, the antioxidant activity of A. manianna has been reported [24]. C. bougheyanum is a species of herb in the family Asteraceae. A previous study showed that C. bougheyanum did not produce any toxicity efect on Swiss albino mice [25]. P. peduncularis is a plant in the Rubiaceae family. It has been traditionally used in several countries to treat toothache, convulsion, yellow jaundice, stomachache, earache, backache, and skin infection [27].

Despite the traditional use of these medicinal plants, very little work has been done to investigate their phytochemical constituents. In addition, there are few studies on the antioxidant and antimicrobial activities of these medicinal plants. Terefore, in the present study, we evaluated the phytochemical constituents of extracts of these medicinal plants, and determined their antioxidant and antimicrobial activities against microbial pathogens.

## II. MATERIALS AND METHODS

**2.1. Chemicals**. DPPH (2, 2-diphenyl-1-picrylhydrazyl), (±)-α-tocopherol, Folin-Ciocalteu's reagent, dimethyl sulfoxide (DMSO), p-iodonitrotetrazolium chloride (INT), quercetin, gallic acid, ascorbic acid, butylated hydroxytoluene (BHT), ciprofoxacin, and ketoconazole were purchased from Sigma-Aldrich. Te solvent and all reagents used in the analysis were of analytical grade.

**2.2.Microorganisms andMedia.** Four fungal strains: Candida albicans (ATCC 90029), Candida parapsilosis (ATCC 22019), Candida krusei (ATCC 6258), and Candida tropicalis (ATCC 750) were used. Te bacterial spp. used were Escherichia coli (ATCC 10536), Staphylococcus aureus (ATCC 25923), and Enterobacter aerogenesis (ATCC 13048), and three clinical isolates, namely, Providencia stuartii, P. aeruginosa, and Vibrio cholerae C06. Fungal and bacterial strains were obtained from the American Type Culture Collection (ATCC) while the clinical bacterial isolates were obtained from the Pasteur Institute Yaounde´ (Cameroon). Mueller Hinton agar (MHA, Dominique Dutscher SAS) and Mueller Hinton broth (MHB, Dominique Dutscher SAS) were used for the activation of bacteria and antimicrobial assays, respectively. Sabouraud Dextrose agar (SDA, Lioflchem) and Sabouraud Dextrose broth (SDB, Lioflchem) were used for the activation of yeasts and antimicrobial assays, respectively.

**2.3. Plant Sample Collection.** Seven fresh plants (H. decumbens, L. macrocarpa, T. mauritianum, C. stelluliferum, A. manianna, C. bougheyanum, and P. peduncularis) (Table 1) were collected from various areas in the Tombel subdivision in southwest region of Cameroon in September 2016. Te plants were authenticated at the Cameroon National Herbarium. Te voucher number given for each plant is listed in Table 1.

**2.4. Preparation of Plant Extracts.** Te collected plants were washed with water and dried in the shade at room temperature. Dried plant samples were powdered and 100 g of each plant sample powder was macerated with 800 mL of methanol. Ten, each sample was fltered using Whatman

Table 1: Characteristics of the medicinal plants investigated in this study

| Scientific names (Family) voucher number | Part used | Traditional use | Previous pharmacological studies | Isolated phytochemical compounds |
|---|---|---|---|---|
| H. decumbens (Mecostemataceae) 18026/ SRF/Cam | Leaves | Eye infection spread, female infertility, trypanosomiasis, hernia, furuncle, and gonorrhea [14] | Not reported | Not reported |
| L. macrocarpa (Icacinaceae) 17876/ SRF/cam | Fruit | Genital stimulants/depressants, aphrodisiac [15] | Not reported | Not reported |
| T. mauritianum (Mecostemataceae) 8992/ SRF/Cam | Leaves | Wounds, cough, and pneumonia tonsils [16] | Antiplasmodial and antioxidant [17] | 2,4-di-tert-butylphenol 2 [(octyloxy) carbonyl] benzoic acid and sitosterol [18] |
| C. stelluliferum (Asteraceae) 20891/SRC | Whole plant | Anemonia affecting the newborn, polyhydramnios [19] | Immunomodulatory [20] | Taraxer [21] |
| A. manianna (Cynthiaceae) 23494/ HNC | Leaves, seeds, fruit/bark | Filariasis [22] Sickacks [23] | Antioxidant [24] | Flavonoids, quinones, tannins, terpenoids, and steroids [24] |
| C. bougheyanum (Asteraceae) 7653/HNC | Whole plant | Not reported | Acute and sub-chronic toxicity [25] | Not reported |
| P. peduncularis (Rubiaceae) 35638/HNC | Leaves | Heart conditions [26] toothache, convulsion, yellow jaundice, stomachache, earache, backache, and skin infection [27] | Not reported | Not reported |

No. 1 flter paper and from each fltrate the methanol was removed using a rotary evaporator (Buchi R-200) under reduced pressure. Te extracts were stored at 4°C for further studies.

2.5. Preliminary Phytochemical Screening. Te presence or absence of diferent constituents, such as alkaloids, steroids, glycosides, favonoids, tannins, saponins, and terpenoids in each plant extract was determined using the method of Harbone (1984) [28]. Determination of the total phenolic content (TPC) and total favonoid content (TFC) were performed using the method of Dzoyem and Elof [29].

**2.6. Antioxidant Assay**

**2.6.1. DPPH Radical Scavenging Assay.**

The DPPH assay was performed using the method described by Dzoyem and Elof [29]. Briefy, 900 µL of DPPH solution (0.2 mM) prepared in methanol was mixed with 100 µL of each plant extract sample at various concentrations (12.5 to 200 µg/mL). After incubation in the dark at room temperature for 30 min, the absorbance of the mixture was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as a positive control, methanol as a negative control, and extract without DPPH as a blank. Te percent of inhibition of DPPH radical scavenging (%I) was calculated using the formula: %I = ((AbsorbanceControl − AbsorbanceSample)/ AbsorbanceControl)) × 100. Te concentration of each plant extract necessary to scavenge 50% of radicals (IC50) was calculated by plotting inhibition

percentages against concentrations of each sample.

## III. RESULTS

### 3.1. Phytochemical Analysis.

The results of qualitative analysis of phytochemicals of the methanolic extracts of seven medicinal plants are shown in Table 2. It was observed that all plant extracts contained phenols, favonoids, and steroids. The L. macrocarpa extract had all phytochemical constituents except anthraquinone. Additionally, saponins were present in all plants except A. manniana and P. peduncularis.

### 3.2. Total Phenolic and Flavonoid Contents.

The quantities of phenolic and favonoid contents in the different medicinal plants are presented in Figure 1. The extracts of P. pedunculagins and T. Mauritian presented the highest TPC (5.57 ± 0.22 mg GAE/g and 4.92 ± 0.55 mg GAE/g, respectively). However, the extracts of C. Boughey Anum and H. decumbent presented the lowest TPC (0.79 ± 0.06 mg GAE/g and 0.48 ± 0.05 mg GAE/g, respectively). The plant extract of P. pedicularliids (1.38 ± 0.06 mg QE/g) presented the highest TFC while the plant extract of L. macrocarpa (0.11 ± 0.01 mg QE/g) showed the lowest TFC. The TFC of the C. stelluliferum (0.36 ± 0.02 mg QE/g) extract was similar to that of the A. manniana extract (0.39 ± 0.04 mg QE/g).

### 3.3. Antioxidant Activity.

The antioxidant activities of medicinal plant extracts as determined by the DPPH, OH, and NO radical scavenging assays are shown in Table 3. The IC50

Table 2: Qualitative analysis of phytochemicals of the methanolic extracts of seven medicinal plants.

| Phytochemical groups | Plant extracts | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Hd* | *Lm* | *Tm* | *Cs* | *Am* | *Cb* | *Pp* |
| Alkaloids | − | + | − | + | − | + | − |
| Phenols | + | + | + | + | + | + | + |
| Flavonoids | + | + | + | + | + | + | + |
| Saponins | + | + | + | + | − | + | − |
| Triterpenes | + | + | − | − | + | − | + |
| Steroids | + | + | + | + | + | + | + |
| Anthraquinone | − | − | + | − | + | − | − |
| Tannins | + | + | + | + | + | − | + |

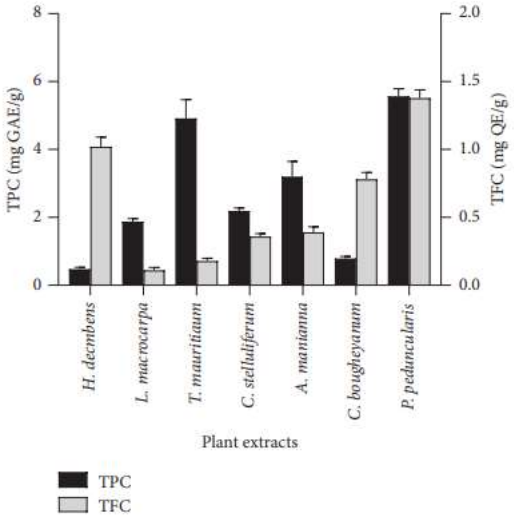
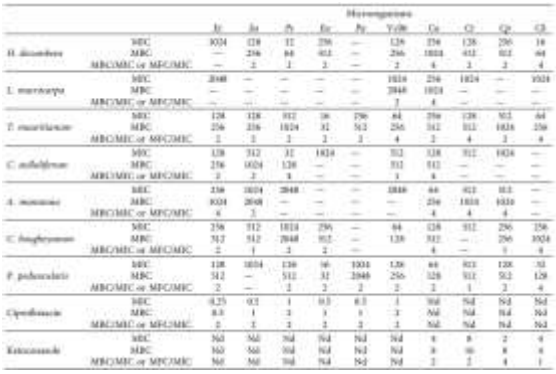



Figure 1: TPC and TFC of seven medicinal plant extracts.

values of the plant extracts ranged from 0.55 to 49.43 µg/mL and 0.65 to 13.7 µg/mL in the DPPH and NO methods, respectively. Compared to ascorbic acid, the IC50 values of the P. peduncularis extract in the DPPH and NO methods were similar.

Table 3: IC50 (µg/mL) values of seven medicinal plant extracts against DPPH, OH, and NO radical scavenging

Table 4: Minimum inhibitory concentration (MIC in µg/mL), minimum bactericidal or fungicidal concentration (MBC or MFC in µg/mL), and MBC or MFC/MIC ratio of the seven selected medicinal plants.



was used as a control drug, and its MIC and MBC values ranged from 0.25 to 32 µg/mL and 0.5 to 64 µg/mL, respectively.

Concerning antifungal activity, the extract of H. decumbens displayed the best activity (MIC values ranging from 16 to 256 µg/mL) followed by the extracts of P. peduncularis and T. mauritianum with MIC values ranging from 32 to 512 µg/mL and 64 to 512 µg/mL respectively. In addition, the extracts of H. decumbens, T. mauritianum, and P. peduncularis showed fungicidal activity against all fungal strains. However, the lowest antifungal activity was obtained for L. macrocarpa, with MIC values ranging from 256 to ≤2048 µg/mL. Ketoconazole exhibited fungicidal activity against all tested fungal strains.

## IV. DISCUSSION

The use of medicinal plants for their pharmacological properties is being increasingly reported in the diferent countries. Te World Health Organization estimates that more than 25% of prescription drugs derived from plants [12, 35]. In the present study, the phytochemical analysis revealed the presence of phenols, favonoids, and steroids in all extracts of medicinal plants. Due to their various biological properties, phenolic and favonoid compounds are considered the most important classes of phytochemicals [36]. In fact, some efects of phenolic and favonoid compounds include anti-infammatory, antispasmodic, antiulcer, antidepressant, antidiabetic, cytotoxicity and antitumor, antimicrobial, and antioxidant properties. Additionally, steroids derived from medicinal plants are known to possess antibacterial and insecticidal properties [37]. Tese results are in agreement with those obtained by Ngbolua et al., who found that A. manniana contained favonoids, quinones, tannins, terpenoids, and steroids [24]. In addition, similar funding was obtained by Wickens and Burkill, who showed the presence of tannins in the extract of C. stelluliferum [21]. Our results showed that saponins were present in all plants except C. stelluliferum and P. peduncularis. Plant extracts containing saponins have been used to treat infammation, cerebrovascular and cardiovascular diseases, gastric ulcers, and ultraviolet damage [38]. In addition, saponins have been used as adjuvants to enhance the absorption of bioactive molecules and drugs [39]. Te presence of these phytochemical compounds in the plant extracts of this study could be the reason for their use as a traditional medicine by the population of Tombel subdivision .

Te total phenolic and favonoid contents in selected medicinal plants were also investigated. Te extracts of P. penduncularis presented the highest TPC and TFC. Te high amounts of phenolic and favonoid compounds in this plant could increase its biological properties compared to other studied medicinal plants. Te antioxidant activity should not be concluded on the basis of a single method [40]. In order to determine the antioxidant activity of studied medicinal plants, DPPH, OH, and NO radical scavenging assays were used. Antioxidant activity is considered as follows: very strong (IC50150 µg/mL) [41]. On this basis, all plant extracts showed very strong antioxidant activity DPPH and NO radical scavenging activity. Additionally, C. stelluliferum and C. bougheyanum extracts exhibited strong OH scavenging activity with IC50 values of 79.06 µg/mL and 67.29 µg/mL, respectively. Tis antioxidant activity observed in the studied medicinal plants could be attributed to the presence of phenolic compounds such as phenolic acids and favonoids. Tese phenolic compounds act as antioxidants by hydrogendonating properties of their phenolic group hydroxyls [42]. Additionally, phenolic compounds can chelate the metal ions involved in the production of

ROS [43]. Our results are similar to those obtained by Ngbolua et al., who reported the antioxidant activity of A. manniana [24]. Additionally, Tsafack et al. reported the antioxidant activity of T. mauritianum [17].

Plants are a good source of new medicine. In our study, we also tested the antimicrobial activity of seven medicinal plants against bacterial and fungal pathogens. Te antibacterial or antifungal activity is considered signifcant (MIC 625 µg/mL) [11]. On this basis, the H. decumbens extract showed signifcant antibacterial activity (MIC$\leq$ 32 µg/mL) against P. stuartii isolates. In addition, the extracts of T. mauritianum and P. peduncularis displayed signifcant antibacterial activity (MIC$\leq$ 16 µg/mL) against S. aureus strain. Concerning antifungal activity, the extracts H. decumbens, T. mauritianum, and P. peduncularis exhibited signifcant activity against C. krusei strain. Additionally, A. manianna and P. peduncularis showed signifcant antifungal activity (MIC $\leq$ 64 µg/mL) against C. albicans strain. However, the majority of plant extracts exhibited moderate antibacterial and antifungal activities. Te diferent antimicrobial activities of plant extracts could be attributed to the presence of phytochemical compounds such as phenolics, favonoids, alkaloids, tannins, saponins, steroids, and triterpenes, which have antimicrobial properties and cause damage of the cell membrane, leading to cell death through its disruption [9]. In addition, these phytochemical compounds can inhibit of cell wall formation, mitochondrial dysfunction, DNA replication, protein synthesis, bioflm formation, and efux pumps [44–46]. Several studies have demonstrated that medicinal plants containing phenolics, favonoids, alkaloids, tannins, saponins, steroids, and triterpenes have the antimicrobial potential as bactericidal, bacteriostatic, fungicidal, or fungistatic agents against microbial pathogens [47–49]. Limited information exists on the antibacterial activity of these medicinal plants. However, Tsafack et al. reported the antibacterial activity of T. mauritianum against Salmonella [17].

## V. CONCLUSION

The study's findings demonstrated the medicinal plants' ability to combat diseases that are resistant to drugs by acting as antibacterial and antifungal agents. These therapeutic plants may also be utilized as an organic antioxidant source.

Additional refinement and separation of the bioactive elements present in these plant extracts could potentially yield the identification of the mechanism of action and potential lead compounds for the creation of novel pharmaceuticals.

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