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Evaluation of phytochemical, trace metals content, antioxidant and antimicrobial potential of *Bonnaya Ruellioides* (Colsm.) Spreng: An ethnomedicinal plant of North Eastern India

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Abstract: *Bonnaya ruellioides* (Colsm.) Spreng is an ethnomedicinal plant widely found in Manipur, a northeastern state of India and it belongs to the Linderniaceae family. The study aimed to evaluate the phytochemical content, trace metals, antioxidant and antimicrobial activity of this ethnomedicinal plant in various extracts including chloroform, petroleum ether, ethyl acetate, methanol, and water extracts. Phytochemical analysis was done using standard protocols, trace metals content by using Atomic Absorption Spectrophotometer (AAS), antioxidant by DPPH, Reducing Power and ABTS assay. Antimicrobial activity of the plant was also determined by Zone of Inhibition (ZOI) assay. Phytochemicals such as phenol, saponin, flavonoid, alkaloid, and terpenoid were detected in the water extract, whereas tannin, glycoside, flavonoid, steroid, alkaloid, and terpenoid were found in the plant extracts of petroleum ether, chloroform, and ethyl acetate. Trace elements detection using AAS revealed the presence of Fe (0.298 ± 0.011 mg/g), Zn (0.083 ± 0.003 mg/g), Cu (0.024 ± 0.002 mg/g) and Mn (0.014 ± 0.002 mg/g) in the plant. Further, evaluation of antioxidant activity using DPPH scavenging assay in chloroform and methanol extracts recorded 411.59 ±2.62 IC_{50} (µg/mL ± SD) and 80.96 ± 0.68 IC_{50} (µg/mL ± SD) respectively. Reducing power activity of the chloroform and methanol extracts showed 249.27 ± 1.48 µM FeSO4/mg sample and 44.55 ± 0.47 µM FeSO4/mg sample, respectively, and for ABTS assay, 281.82 ± 2.19 TEAC/g dw and 168.65 ± 2.06 TEAC/g dw are recorded respectively. The antimicrobial study of the chloroform and petroleum ether extracts showed negative results for all the selected pathogens of bacterial and fungal strains. At the same time, the methanol extract showed positive for bacterial strains including *Micrococcus luteus* (17.50 ±0.54) mm and *Escherichia coli* (15.0 ± 2.0) mm, while negative *for all the fungal strains.* The results of the study demonstrated for the first time that *B. ruellioides* plant has notable phytochemical, essential trace metals, promising antioxidant and antimicrobial capacity. Thus, the findings of our study will contribute a scientific insight into the therapeutic applications of this ethnomedicinal plant in folklore medicines and enable the prediction of further applications in drug development.

Keywords: *Folklore medicine, Herbal drugs, Medicinal plants, Northeast India, Phytocompounds.*

1. Introduction

Bonnaya ruellioides (Colsm.) Spreng is an ethnomedicinal plant of the Linderniaceae family which is widely found in Manipur, North East India, and used in the folklore medicine by the natives for the

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treatment of various health problems including bruises, boils, jaundice, snakebite, dysentery, urinary troubles, healing of wounds, skin disease, dysentery, intestinal problems and as postpartum tonic [1]. The scientific interest in the study of plant's secondary bioactive compounds is increasing all over the world, especially in countries where the use of plants in folklore medicines is common and popular for their primary health needs [2]. Since time immemorial, medicinal plants have been used all over the world to cure a wide range of illnesses, such as kidney stones, asthma, stomach issues, skin conditions, respiratory and urinary issues, liver and cardiovascular problems [3-4]. The source of this traditional knowledge is the plant defense system, which produces a wide variety of phytocompounds with unique molecular structures that are far safer and more effective than those made from synthetic materials [5]. For this reason, there is a great deal of interest in the characterization and development of plant-derived novel active drugs. Various phytochemicals with strong antioxidant potential can be found in certain medicinal plants. Plants may contain compounds that can be isolated and used to generate medications that will improve the management of a variety of metabolic disorders by lowering oxidative stress [6-7]. Since the last few decades, researches focused on plant-based compounds of high antioxidant activities have increased, and evidence has indicated that cellular damage caused by free radicals has been regarded as a significant etiological factor in aging and the pathophysiology of several human diseases, including autoimmune diseases, inflammatory diseases, degenerative and neurodegenerative diseases. Thus, the importance of exploring ethnomedicinal plants with antioxidant effects is emphasized, as they can neutralize free radicals before they attack DNA, proteins, and lipids [8-9].

In the human body, trace metals are required for their normal functioning, and when found in high amounts or in imbalance concentrations, they can lead to many health disorders [10]. The human body needs several essential trace elements in order to support various biological processes such as metabolism, proper functioning of enzymes, and enhancement of the immune system, and their insufficiency linked to elevated or decreased concentrations results in a range of illnesses [11]. In recent years, it has become clear how important these micronutrients are for physiological stability and biological cellular signaling [12-13]. The long-term buildup of high amounts of potentially hazardous metals in the human body is subsequently linked to various disorders [14]. Furthermore, one of the biggest issues with global public health is resistance to antimicrobial agents. It has been reported that despite advancements in the knowledge of microorganisms and their management in developed countries, drug-resistant microorganism occurrences and the emergence of unidentified disease-causing germs posed severe public health concerns [15]. Microorganisms that cause respiratory and cutaneous infections, such as *Streptococcus pyogenes*, *Stephylococcus aureus*, and *Streptococcus pneumonia*, as well as Enterobacteriaceae members and *Pseudomonas* that cause diarrhea, urinary tract infections, and sepsis, have been found to become resistant to almost all known antibiotics. The widespread use of antimicrobial medications for the treatment of infectious diseases has contributed significantly to this resistance [16-17]. Furthermore, as certain antibiotics have serious adverse side effects that restrict their applicability, there is a critical need to develop new antimicrobial medicines that are very effective with minimum undesirable side effects. To our knowledge, no pharmacological studies have been done on this plant for therapeutic uses. Therefore, the study aims to evaluate the phytochemicals, trace metals, antioxidant and antimicrobial activity of this important ethnomedicinal plant of North East India.

2. Materials and Methods

2.1. Chemicals

All the chemicals and reagents used in this research work are in analytical grade. Folin–Ciocalteu reagent, $AlCl_3$, HCl, Na_2CO_3, H_2SO4, CH_3COOK, and Dimethyl Sulphoxide (DMSO) were purchased from Thermo Fisher Scientific India, Pvt. Ltd. Muller Hinton Broth (MHB), Ascorbic acid, Quercetin, Gallic acid, and Muller Hinton Agar (MHA) of Himedia Laboratories Company Ltd., India, were used. The reagents n-hexane, methanol, chloroform, dichloromethane, ethyl acetate, and ethanol were

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obtained from Merck Life Science. Neomycin and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were acquired from Sigma-Aldrich and Tokyo Chemical Industries Co. Ltd, respectively.

2.2. Plant Sample Collection and Processing

The plant sample was collected from its natural habitat village known as Awang Sekmai, Imphal West, Manipur State, and is identified at Botanical Survey of India (BSI), Shillong Branch, Meghalaya (identification no BSI/ERC/Tech/19-20/725) (**Figure 1**). The plant samples were washed with clean water first, then again with distilled water, and lastly with deionized water to remove impurities present in the plant sample. The sample was shade-dried to prevent the loss of volatile components present in the plant sample for about two weeks. The dried plant sample was made powdered with the help of a mortar and pestle. The powdered sample was kept in a glass container until further analysis.

A B C

Figure 1.
B. ruellioides (Colsm.) (A- Single plant; B- group of plants; C- Dry & powder form).

2.3. Preparation of Plant Extracts

The dried plant samples were soaked in chloroform, petroleum ether, ethyl acetate, methanol, and water for three days and filtered. The filtrates were collected, concentrated through a rotary evaporator, and dried to solid powder. These plant extract samples were used for photochemical, antioxidant, and antimicrobial activities. For elemental analysis, powdered plant sample was used.

2.4. Phytochemical Screening

Using standard protocols, the presence of several phytochemicals in various *B. ruellioides* (Colsm.) Spreng extracts were evaluated [18-20]. Tests were carried out to determine the presence of alkaloids, carbohydrates, amino acids, proteins, steroids, flavonoids, glycosides, phenols, saponins, tannins, and terpenoids.

2.5. Traces Elements Assay

The trace elements content in the powdered plant sample was determined using flame atomic absorption methods (HHPN-FF-AAS).

2.6. Antioxidant Assay

DPPH radical scavenging assay Briefly 0.1mM solution of DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution in methanol was prepared. In a transparent 96-well flat bottom plate, 100µL of samples (or ascorbic acid in standard) in various concentrations and 200 µL of DPPH solution were added. The reaction mixture was kept at room temperature for 30 minutes in the dark. The absorbance of each well was measured at 517 nm in a multiscan spectrophotometer. Ascorbic acid was added as a positive control. The mixture was incubated at room temperature for thirty minutes in the dark, and the optical density was measured at 517 nm in comparison to a blank. Using the following formula, the free radical

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scavenging activity of various extracts and standard ascorbic acid were determined [21]. The half-maximal inhibitory concentration (IC_{50}) of the various extracts and the positive control were evaluated from the values of % Scavenging. % Scavenging = (The absorbance of control − absorbance of sample)/ absorbance of control × 100

2.7. Reducing Power Assay

100 µL of extract at different concentrations were mixed with 250 µL of Potassium ferricyanide and 250 µL of Sodium phosphate buffer (0.2 M, Ph 6.6). The mixture was then incubated for 20 mins at 50ºC. After the incubation, 250 µL of 10% Trichloroacetic acid (TCA) was added to the mixture, which was then centrifuged at 3000 rpm for 10 mins. Finally, 250µL of the supernatant was mixed with 250µL of distilled water, followed by 50 µL ferric chloride (0.1%). The absorbance was measured at 700 nm subsequently. An increased in reducing power is denoted by the increased absorbance of the reaction. A standard curve of ferrous sulphate at concentrations of 1000 mmol/L to 62.5 mmol/L was used to perform the calculations [22].

2.8. ABTS Assay

ABTS ((2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical is produced by reacting aqueous 7 mM ABTS stock solution with 2.45 mM potassium persulfate and keeping the mixture in the dark temperature for 12-16 hours at room temperature before use. ABTS solution was diluted with methanol, pH 7.4, till we got an absorbance (0.70 ± 0.2) at 734 nm and equilibrated at 30ºC. 50 µL of plant extract solution in methanol and the standard taken in different concentration ranges were mixed with 300 µl of ABTS solution in a 96-well plate. After 10 mins of incubation in the dark, the absorbance was measured at 734 nm. The degree of discoloration is proportional to the antioxidant activity of plant extract or the ABTS cation inhibition. The percentage inhibition of absorbance at 734 nm is calculated and plotted as a function of the concentration of antioxidants and Trolox for the standard reference data [23]. IC_{50} of the antioxidant activity is expressed in terms of Trolox equivalent antioxidant capacity (TEAC).

2.9. Anti-Microbial Assay

The antimicrobial activities were tested against selected pathogens of the bacterial and fungal strains. Pathogens for bacterial tests included Gram-positive bacteria *M. luteus* (MTCC 106), *B. subtilis* (MTCC 121), and Gram-negative bacteria *E. coli* (MTCC 739), which were sub-cultured in nutrient broth at 37°C for 24 hr. Fungal test pathogens included *F. oxysporum* (MTCC 287), *R. solani* (MTCC 4633), and *C. oryzae* (MTCC 2605), which were sub-cultured in potato dextrose broth at 37°C for 24 hours. Ampicillin (25 µg/ml) and Fluconazole (25 µg/ml) were used as the positive controls for antibacterial and antifungal screening, respectively. The methanolic extract (MeOH) showed antibacterial activities against *M. luteus* and *E. coli*. However, none of the extracts showed activity against any fungal test pathogens [24].

2.10. Statistical Analysis

All data are represented as mean ± SD from at least three independent replicas. Significant differences were determined by one-way analysis of variance (ANOVA) with IBM SPSS 23.0 statistical software. Differences at $p \leq 0.05$ were taken as significant using the least significant difference (LSD test).

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3. Results

Table 1.
Result of Phytochemical screening of the plant extracts.

Name of test	Water extract	Petroleum extract	Chloroform extract	Ethyl acetate extract
Test for phenol	+	-	-	-
Test for tannin	-	+	+	+
Test for Saponin	+	-	-	-
Test for glycoside	-	+	+	+
Test for flavonoid	+	+	+	+
Test for steroid	-	+	+	+
Test for alkaloid	+	+	+	+
Test for protein	-	-	-	-
Test for amino acid	-	-	-	-
Test for terpenoid	+	+	+	+
Test for carbohydrate	-	-	-	-

Table 2.
Trace metals content in *B.ruellioides*. Values are expressed as mean ± SD (n=3).

Name of trace metals	Concentration (mg/g)
Fe	0.298 ± 0.011
Zn	0.083 ± 0.003
Cu	0.024 ± 0.002
Mn	0.014 ± 0.002

Table 3.
Result of Antioxidant activity assays in methanol and chloroform plant extracts. Values are denoted as mean ± SD (n=3). DPPH = 2, 2-diphenyl-1-picrylhydrazyl; ABTS = ((2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TEAC = Trolox equivalent antioxidant capacity.

Name of extracts	IC_{50} for ABTS (TEAC/g dw)	IC_{50} for reducing power (µM $FeSO_4$/mg)	IC_{50} for DPPH (µg/mL)
Ascorbic acid (Standard)	12.54 ± 0.03	13.03 ± 0.52	11.88 ± 0.053
Methanol	168.65 ± 2.06	44.55 ± 0.47	80.96 ± 0.68
Chloroform	281.82 ± 2.19	249.27 ± 1.48	411.59 ± 2.62

Table 4.
Results showing Antibacterial activity of the Petroleum ether (PE), Methanol (MeOH) and Chloroform ($CHCl_3$) extracts. Values are given as mean ± SD (n=3).

Extract/sample	Concentration (µg/mL)	Diameter of zone of inhibition (in mm)		
		Bacillus subtilis	Micrococcus luteus	Escherichia coli
PE	25.0	-	-	-
MeOH	25.0	-	17.50 ± 0.54	15.0 ± 2.0
$CHCl_3$	25.0	-	-	-
Ampicillin	25.0	25.0 ± 0.62	30.50 ± 0.58	28.20 ± 1.20

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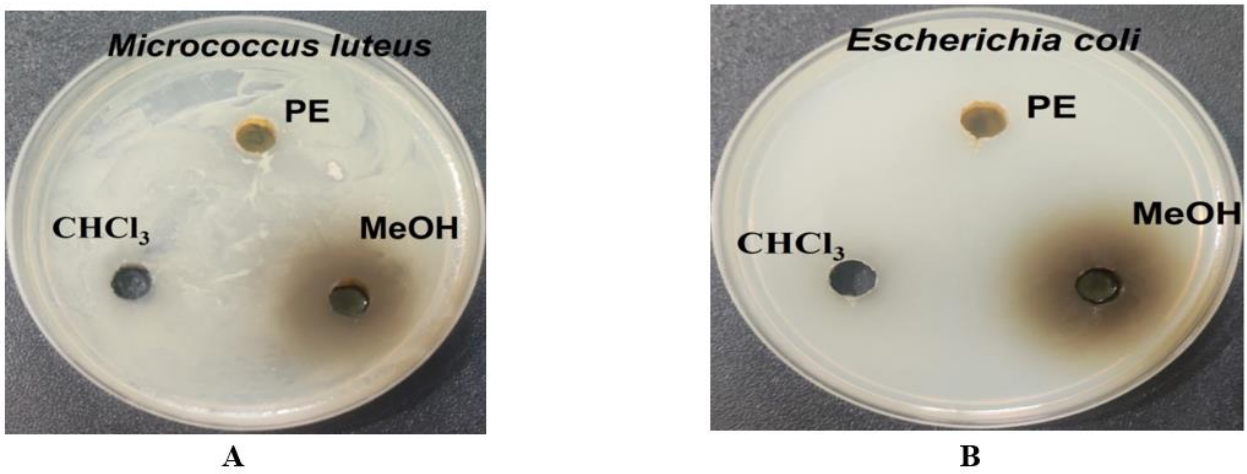

Figure 2.
Zone of Inhibition (ZOI) antibacterial assay of the Petroleum ether (PE), Methanol (MeOH) and Chloroform (CHCl3) extracts. A- Against *Micrococcus luteus*; B- Against *Escherichia coli*.

Table 5.
Antifungal activity profile of the Petroleum ether (PE), Methanol (MeOH) and Chloroform ($CHCl_3$) extracts. Values are represented as mean ± SD (n=3).

Extract/sample	Concentration (µg/ml)	Diameter of zone of inhibition (In mm)		
		Rhizoctonia solani	Aspergillus niger	Curvularia oryzae
PE	25.0	-	-	-
MeOH	25.0	-	-	-
$CHCl_3$	25.0	-	-	-
Fluconazole	25.0	20.5 ± 0.5	17.5 ± 0.5	18 ± 0.5

4. Discussion

The qualitative phytochemical analysis of *B.ruellioides* exhibited the presence of phenol, saponin, flavonoids, alkaloids, and terpenoid in water extract, whereas tannin, alkaloid, terpenoid, glycoside, flavonoid, and steroid in the chloroform, petroleum ether, and ethyl acetate extracts (**Table 1**). The presence of these potent phytoconstituents made it abundantly evident that *B. ruellioides* possesses strong antioxidant qualities and that its pharmacological potential needs to be further investigated. Plant extracts have been reported to have a wide range of therapeutic applications due to the presence of various phytochemical active constituents, which can contribute significantly to their antioxidant and antimicrobial activities [25]. Phytochemicals like phenol, saponin, flavonoids, alkaloids, and terpenoids are major phytocompounds that are widely studied because of their various therapeutic potentials in reducing oxidative stress-related degenerative diseases, such as antibacterial, anticancer, anti-inflammatory, anti-hypertensive, and cardio-protective [26-27]. The results obtained for tannins, flavonoids, and polyphenols in *B.ruellioides* may support their widespread usage as therapeutic herbs for a variety of conditions because of its pharmacological potential. The presence of various important phytochemicals in *B. ruellioides* may support its widespread usage as a therapeutic herb for a variety of conditions, as these secondary metabolites are frequently linked to the plant's therapeutic benefits.

Analysis using an Atomic Absorption Spectrophotometer (AAS) in *B.ruellioides* revealed the presence of various trace metals in appreciable amounts and wide variation in their concentrations (**Table 2**). Fe was found in *B.ruellioides* at the greatest concentration of all the trace elements examined. The concentration of the trace elements found in the medicinal plants is arranged as follows: Fe>Zn>Cu>Mn. The content of the Fe, Zn, Cu, and Mn recorded in the *B.ruellioides* were 0.298 ±

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0.011mg/g, 0.083 ± 0.003 mg/g, 0.024 ± 0.002 mg/g and 0.014 ± 0.002 mg/g respectively. The human body needs iron (Fe) to produce hemoglobin and oxygenate red blood cells. It is also necessary to generate energy and maintain a healthy immune system. [28]. Several studies have demonstrated the significance of Fe availability in determining the bactericidal action of lysozyme and lactoferrin, which can destroy gram-negative bacteria [29]. Zinc is another essential element needed for the metabolism of many biological processes. It is present in almost all plant and animal tissues, and by regulating the enzymes that function and regenerate our body's cells, it is essential for maintaining healthy skin [30]. Zinc-containing metalloenzymes contribute to tissue metabolism, development, and repair, as well as the stabilization of cell membranes. They also enhance immunological response, particularly T-cell-mediated immunity [31]. The body's third most plentiful trace element, after zinc and iron, is copper (Cu), which helps boost immunity against infection, restore damaged tissues, and promote healing. Furthermore, Cu is necessary for the synthesis of connective tissues, including the cross-linking of elastin and collagen. [32- 33]. Like Fe, Mn plays a crucial role in regulating our body's immune responses by the breakdown of amino acids, energy production through controlling the metabolism of vitamins B1, C, and E, as well as activation of several enzymes necessary for healthy food digestion and utilization. [34]. Additionally, Mn is a part of the mitochondrial antioxidant defense system and the metalloenzyme manganese superoxide dismutase, which work together to protect the skin from free radicals produced by injured cells [35]. Considering the several beneficial effects of Fe, Zn, Cu, and Mn on the immune system, it is possible that the traditional practitioners of northeastern India used this medicinal plant to treat various health problems because of the significant amounts of the previously mentioned metals present in it.

DPPH, reducing power activity, and ABTS showed considerable antioxidant activity in the chloroform and methanol extracts. The highest DPPH radical inhibitory action was demonstrated by methanol extract; however, it was less than the standard. The extracts demonstrated promising antioxidant activity when compared to the standard (ascorbic acid) in terms of their antioxidant-reducing capacity and scavenging activity against DPPH and ABTS radicals. The inhibitory capacity of the standards and extracts, as determined by the IC50 value for all the three assays, are as follows: ascorbic acid > methanol> chloroform. Evaluation of antioxidant activity using DPPH scavenging assay in chloroform and methanol extracts recorded 411.59 ± 2.62 IC50 (µg/mL ± SD) and 80.96 ± 0.68 IC50 (µg/mL ± SD) respectively. Reducing power activity of the chloroform and methanol extracts showed 249.27 ± 1.48 µM FeSO4/mg sample and 44.55 ± 0.47 µM FeSO4/mg sample, respectively, and for ABTS assay, 281.82 ± 2.19 TEAC/g dw and 168.65 ± 2.06 TEAC/g dw are recorded respectively for the two extracts (**Table 3**). These days, there is a lot of interest in researching the antioxidant activity of plant extracts to look into potential therapeutic benefits [36]. Many studies have shown a significant relationship between antioxidant activity and polyphenolic substances present in plants [19]. This study showed that the methanol extract of *B.ruellioides* has a high level of polyphenolic chemicals, which may be the reason for its maximum antioxidant activity. The results support the idea that the plant extract with high levels of flavonoids and phenolics has the most significant bioactive potentials. Thus, the findings of our study offer some empirical support for the biological activities of *B.ruellioides*.

Recently, there has been a greater focus on creating therapeutic plant-based antibacterial drugs rather than synthetic ones. The antibacterial capabilities of plant extracts are often investigated against a range of Gram-positive and Gram-negative microorganisms [16-17]. The antimicrobial result of chloroform and petroleum ether extract shows negative to *B. subtilis*, *M. luteus*, *E. coli*, *R. solani*, *A. niger*, and *C. oryzae*. The methanol extract shows positive to *M. luteus* (17.50 ± 0.54 mm) and *E. coli* (15.0± 2.0 mm) while negative to *B. subtilis*, *R. solani*, *A. niger*, *and C. oryzae* (**Table 4 and Table 5; Figure 2**). This study revealed that out of the various extracts of *B.ruellioides*, antimicrobial activity can be seen in methanol extract. The findings of our study correspond with those of McGaw et al. [36], who reported that only ethanol extract showed antibacterial activity when *B. subtilis*, *S. aureus*, *E. coli*, and *K. pneumoniae* were tested against hexane, ethanol, and aqueous leaf extracts of *H. arborescens*. The results are similarly consistent with those by Wigmore et al. [37], who found that plant aqueous extracts

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exhibited lower levels of antibacterial activity in comparison to other solvents. The phenolic and flavonoid contents of this plant may be responsible for the notable antibacterial activity of the methanolic extract obtained in the current investigation [38]. Compared to gram-positive bacteria, most extracts demonstrated weaker antibacterial activity against gram-negative bacteria, which might be caused by their complex cell wall structure. Therefore, the plant may provide the food industry with natural sources of antibiotics and antioxidants to replace synthetic ones. Finding and isolating the active ingredients in *B.ruellioides* that give it its significant biological characteristics will require an extensive amount of investigation.

5. Conclusion

This is the first study to chemically profile the various phytometabolites of *B. ruellioides*, a herb used in ethnomedicine to treat a variety of ailments. Moreover, the presence of a significant amount of essential trace metals, antioxidant activities, and antibacterial potential provides scientific validation of the use of *B. ruellioides* as a therapeutic herb for the treatment of several health problems, including urolithiasis, skin ailments, and other kind of diseases. Therefore, the study suggests that this plant can be considered as a good source of phytochemicals, trace metals, antibacterial, and natural antioxidants, which may be essential for human health. However, further investigations are required to separate, refine, and characterize the several active phytocompounds found in this study.

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