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Research Article

Phytochemical and Pharmacological Evaluation along with Antimicrobial Properties of *Gynura Procumbens* Leaves Extract

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ABSTRACT

The accentuated growth inside the use of *Gynura procumbens* go away as suitable for eating leave and herbal medicinal drug to deal with sicknesses. For this it is essential to evaluate the facts available on its like antibacterial, antifungal, phytochemical screening and antioxidant activities. *G. procumbens* leaves methanol and ethyl acetate crude extract were prepared via the cold extraction approach. The methanolic *G. procumbens* extract became used for phytochemical assessments for plant secondary metabolites; carbohydrates, alkaloids, phenolic compounds, flavonoids, tannins, glycosides, steroids, proteins, acidic compounds and saponins using preferred processes techniques. Antibacterial activities of *G. procumbens* extracts was investigated by using agar well diffusion method and antifungal activities by using the disc-diffusion method. *G. procumbens* extracts antioxidant activity was assessed via DPPH assay to measure its free radical scavenging capability. Phytochemical screening revealed that presence of carbohydrate, alkaloids, flavonoids, glycosides, phenolic compounds and tannins but saponins, proteins, acidic compounds and steroids were not found. Antimicrobial investigation showed that only ethyl acetate extracts have the mild sensitivity to almost all the bacteria and fungi (except *Escherichia coli*), whereas methanol extracts did not demonstrate any antimicrobial property. The average zones of inhibition produced by ethyl acetate extracts were found to be 6-7 mm at a concentration of 400µg/disc. Antioxidant study showed that IC_{50} of ethyl acetate extract of *G. procumbens* leaves was 322.244 µg/ml whereas the IC_{50} of standard ascorbic acid was 41.683 µg/ml. From the present experiment it could be concluded that *G. Procumbens* is the source of alkaloids, flavonoids, tannins and it's has also antimicrobial, antioxidant properties. These findings imply that *G. Procumbens* leaves extracts is a good medicinal supply or herbal properties that may have high therapeutic value.

Introduction

Bangladesh has witnessed a variety of troubles caused by natural gathering of different types of pathogenic microorganisms into the topography. While cures for these troubles depend on the specific strategies, the reasons for trouble existence due to reinfection is also an important task to determine. Major challenges in trouble supervision have evolved as specific antimicrobial resistant, posing a significant impact on the productiveness of chemotherapy. The antibacterial exertion has reduced with the emergence of the specific resistant pathogenic bacteria. This has become one of the most severe public health issues in worldwide, which leading to victims from simple microbial infections followed by treatment intervened with higher generation antibiotics (Noor and Munna, 2015).

As a result, there is a lot of research going on to see if active compounds like essential oils and extracts of medicinally important plants, herbs and spices could be used to develop new feedstuffs. Enterococci in

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feedstuffs are of particular concern for public health as these bacteria seem to be likely to cross the food chain and infect humans. As a matter of fact, researchers are refocusing their efforts on using our traditional medicinal system to identify useful herbal plants that can be safely used. Such, *Gynura procumbens* is a plant that has antimicrobial, antioxidant, hepatoprotective and other beneficial therapeutic uses without causing any negative consequences (G. A. Akowuah *et al.*, 2001, 2002). Important in terms of medicine flavonoid, saponin, tannin, glycosides, terpenoid and other secondary metabolites are found in *G. procumbens* (G. A. Akowuah *et al.*, 2002). *G. procumbens* is an important medicinal plant, belongs to the family Asteraceae. The plant is considered to originate from Malaysia, Indonesia and Thailand. Some literature suggested that the leaves of *G. procumbens* has anti-herpes simplex therapy (Nawawi *et al.*, 1999), anti-hyperglycemic and anti-hyperlipidemic (Zhang and Tan, 2000), anti-hyperglycemic (Li *et al.*, 2009), anti-inflammatory (Iskander *et al.*, 2002), anti-carcinogenic (Agustina *et al.*, 2006), blood hypertension reduction skills (Hoe *et al.*, 2007; Kim *et al.*, 2006), anti-proliferative on earthborn mesangial cell (Lee *et al.*, 2007), anti-oxidative (Puangpronp *et al.*, 2010) and anti-ulcerogenic (Mahmood *et al.*, 2010) masses. The leaves of this plant are frequently eaten in the diet, and research has shown that the components of the leaves have no deleterious effects (Rosidah *et al.*, 2008).

G. Procumbens has been used to treat spread of illnesses inside the past. *G. procumbens* was studied in different researches to analyze the pharmacological value for use as an herbal and non-poisonous medication against various diseases. *G. procumbens* has been used for medicinal purposes from many years ego, so it is important to evaluate the phytochemical compositions, antibacterial, antifungal, and antioxidant properties of *G. procumbens* leaves.

Materials and Methods

Ethical approval

The study was conducted in agreement to the research ethics and guidelines followed by the Department of Physiology and Pharmacology of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh. Hence, the approval number is HSTU/VAS/PPH-1300 (Rsolution No: 10).

Method for plant extraction

G. procumbens leaves were collected from Nilphamary. Bangladesh. Any type of adulteration was strictly prohibited during collection. To remove earthy substances, the leaves were thoroughly washed with clean water. The collected leaves were separated from the plants and shade dried for 35-40 days to ensure that the active constituents were free of decomposition and to avoid any photochemical degradation. Using a suitable grinding machine, the leaves were ground into a coarse powder. The powder was kept in an airtight container in a cool, dark, and dry location until the analysis began. Cold extraction was used to extract the leaves. Approximately 250 gm of the coarse powder of *G. procumbens* leaves was extracted for 15 days at room temperature with increasing the polarity to methanol and ethyl acetate. After 15 days, the extract was parted from the plant debris through filtration with a clean, white cotton cloth. The filtrate was transferred to a beaker. Individual extracts were then filtered through a cotton plug and Whatman No.1 filter paper. This filtrate was filtered once more using a cotton plug and whatman filter paper. The filtrate was shifted to a beaker. The opening of the beaker was then wrapped in aluminum foil with perforations for methanol evaporation and stored in a dry and cool place. The filtrate was then evaporated for 3 hours in the chemistry laboratory at HSTU using an evaporator. It produced a deep green concentrate. The extract was allocated as crude methanol (12 gram) and ethyl acetate extract (7.2 gram) of the leaves of *G. procumbens*.

Methods used for phytochemical tests

Chemical tests were carried out to recognize the preparatory phytochemical activity according to the methods of another author (Harborne, 1973). The crude extract was subjectively tried for the nearness of chemical constituents utilizing the particular reagents and chemicals. Unless otherwise specified in the individual test, a 10% (w/v) solution of extract in ethanol was used in each test. The presence of carbohydrate was determined using Benedict's and Fehling's tests. For the evaluation of alkaloids, Hager's test was used. The Ferric Chloride test was used to determine the presence of phenolic compounds. Flavonoids were assessed using General test. Tannins were determined using the Ferric Chloride test. Glycosides were assessed using the Fehling's solution test and the General test. Salkowski's Test was used to determine whether or not steroids were present. Protein levels were measured using the Xantho protein test. Acidic Compound assessments were carried out using general tests. Saponins were measured using froth tests.

Method of determination of antimicrobial activity

Microorganism collection

Two Gram positive (*Bacillus subtilis*, *Bacillus cereus*) and two Gram negative (*Pseudomonas aeruginosa, Escherichia coli*) bacteria and two fungi (*Aspergillus niger*, *Candida albicans*) amassed from the stock cultures of the Microbiology Lab, Hajee Mohammad

Danesh science and technology university, for the antimicrobial investigation.

Antibacterial activity

For screening antibacterial activity, Agar well diffusion method was used (Magaldi *et al.*, 2004). *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa,* and *Escherichia coli* were cultured at 37°C for 18 hours on nutrient agar media plates. The turbidity (OD_{600} nm) of the bacteria was adjusted to match that of standard McFarland No. 0.5. The bacteria suspensions were used to fill 20 mL of nutrient agar media into sterile plates. To prepare a well in the plates, a cork-borer No. 2 was used. On the surface of the inoculated agar well, 100 microliters of each plant solution were placed. At 37°C, the plates were incubated for 18-24 hours. Standard kanamycin (30g/disc) and blank discs were used as positive and negative controls, respectively. The zone of inhibition around the well plates was measured and recorded, indicating the presence of antibacterial activity. Sterile distilled water was used as a negative control.

Antifungal activity

The disc-diffusion method (Bauer *et al.*, 1966) was used to assess antifungal activity. There are two types of fungi that can be tested: *Candida albicans* and *Aspergillus niger* were cultured at 37°C for 24 hours on sabouraud dextrose agar media plates. The crude extracts and the pure compound (GP) were melted distinctly in methanol and allowed to sterile filter paper discs at a rate of 400g/disc before being carefully dried to remove any residual solvent. The test materials were then uniformly placed on sabouraud dextrose agar medium on discs. Kanamycin (30g/disc) standard discs and blank discs served as positive and negative controls, respectively (impregnated with methanol followed by evaporation). The plates were then kept at a low temperature (4 °C) for 24 hours to allow the test samples to diffuse as much as possible. After that, the plates were incubated for 24 hours at 37 °C to allow the organisms to reach their full potential. The antifungal test materials stopped the microorganisms from growing, and there was a clear, distinct zone of inhibition around the disc. The diameter of the zone of inhibition was used to quantity the antifungal property of the test agents in millimeters. The experiment was repetitive three times to ascertain the average zone of inhibition.

Method of determination of antioxidant activity

The antioxidant properties of *G. procumbens* extracts was measured using the DPPH method to determine its ability to scavenge free radicals (Afandi *et al.*, 2014; G. Akowuah *et al.*, 2009). At first 21 test tubes were taken to make aliquots of 11 conc. (1, 2, 4, 8, 16, 32, 64, 128,256 512 and 1024µg/ml) for plant extract (sample) and 10 conc. (1, 2, 4, 8, 16, 32, 64, 128,256 512µg/ml) for ascorbic acid (standard) each. Plant extract and ascorbic acid were weighed three times and dissolved in DMSO to achieve the desired concentrations via dilution. Ascorbic acid was used as a positive control in this study. Weighed DPPH was dissolved in methanol to make a 0.008 percent (w/v) solution. A sonicator was used to dissolve the material uniformly. In each well of the microplate, 10µL of different concentrations of plant extracts and ascorbic acid were added, followed by 190µL of DPPH in methanol. The final DPPH concentration in each well was 200µM. In three wells of a plate, three samples of the extract and standard solution were taken for each concentration. As a control, 190µL of DPPH was used. After mixing for 5 minutes in the microplate, the sample was allowed to stand at room temperature in light for 30 minutes before being measured at 517 nm with a microplate reader % of inhibition was calculated as- % inhibition = [(Blank absorbance - Sample absorbance) / Blank absorbance] X 100.

Statistical analysis

In all the determinations data are express as the Mean±Standard Deviation (SD).

Results

The carbohydrates, alkaloid, phenolic compound, flavonoid, tannins, glycoside, steroid, protein & amino acid, acidic compound and saponins content of leaf extracts of *G. procumbens* was examined qualitatively in this part of the study. Methanolic *G.procumbens* extract was screened the phytochemical compositions such as alkaloids, saponins, steroids, glycosides, tannins and flavonoids using color reaction method as shown in Table 1.

Table 1. Results of phytochemical screening of *G. procumbens* leaves methanolic extract

Sample	Observation	Standard	Results
	Test for Carbohydrate (Reducing sugar)		
Benedict's test	A red colored precipitate was found	Fructose	Present
Fehling's test	Brick red colored precipitate was found		Present
	Test for Alkaloid		
Hager's Test	Yellow color precipitate was formed	Nicotine	Present
	Test for Steroids		
Salkowski's test	Red color was not formed	Norgestrol	Absent
	Test for Saponins		
Froth Test	Persistent frothing was not found	Detergent	Absent
	Test for Phenolic compounds		
Ferric Chloride Test	Dark green color precipitate found	Catechu	Present
	Test for Flavonoids		
General test	A red color was found.	Rose petal	Present
	Test for Tannins		
Ferric chloride test	A blue green colored precipitate was found.	Catechu	Present
	Test for glycosides		
General test	Yellow color was found	Digoxin	Present
Fehling's solution test	Brick-red precipitate was found		Present
	Test for acidic compounds		
General test	Effervescence was not produced	Ascorbic acid	Absent
	Tests for proteins		
Xanthoprotein test	A yellow color was not formed	Egg albumin	Absent

In Benedict's test, red colored precipitation was found which confirmed the presence of carbohydrate. And the presence of carbohydrate was confirmed by brick red colored precipitation in Fehling's test. The presence of yellow color precipitation established that the presence of alkaloid in alkaloid test, Hager's test. No red color was formed, indicating the absence of steroid by Salkowski's test. Saponins were tested using a froth test, and no persistent frothing was found, indicating the absence of saponins. The presence of dark green colored precipitation confirmed the presence of phenolic compounds. The manifestation of flavonoids was checked by red colored precipitation. The existence of tannins was ensured by a ferric chloride test, which yielded a blue green colored precipitation. The presence of glycosides was confirmed by the yellow-colored precipitation in the general test of glycosides. The presence of glycosides was confirmed by brick red colored precipitation in Fehling's solution test. There was no effervescence was observed, indicating the absence of acidic compounds by general test. There was no yellow color was formed, indicating the absence of protein by Xanthprotein test. The results of phytochemical screening revealed the presence of carbohydrate, glycosides, alkaloids, flavonoids, phenolic compounds and tannins but proteins, acidic compounds, saponins and steroids were not found.

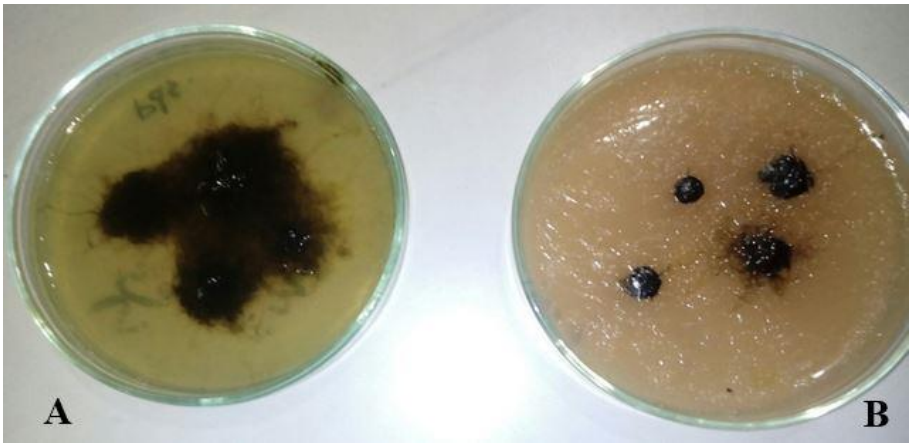

Figure 1. Antibacterial activity of *pseudomonas* (A) *& E. coli* (B) in EA extract respectively.

Figure 2. Antifungal activity of *Aspergillus* (C) *& Candida albicans* (D) in EA extract respectively

The result of the antimicrobial activities of methanol and ethyl acetate extracts of *G. procumbens* has been summarized in Table 2, figure 1 and 2. Present investigation showed that only EA (Ethyl Acetate) extracts had the mild sensitivity to almost all the bacteria and fungi that were tested in our experiment (*Escherichia coli*), whereas ME (Methanol) extracts did not demonstrate any antimicrobial property. The average zones of inhibition produced by EA extracts were found to be 6-7 mm at a concentration of 400µg/disc. Therefore, it can be concluded from the above study that the EA extracts of *G. procumbens* contained mild antimicrobial components and ME extracts had no antimicrobial components.

Table 2. Antimicrobial activity of the leaf extracts of *Gynura procumbens*

Test Microorganisms	Diameter of zone of inhibition (mm) Mean ± Standard Deviation (SD)		
	Methanol	Ethyl acetate	Kanamycin
Gram positive bacteria			
Bacillus subtilis	-	7 ± 0.19	35 ± 0.20
Bacillus cereus	-	7.0 ± 0.09	35 ± 0.25
Gram negative bacteria			
Pseudomonas aeruginosa	-	7.1 ± 0.17	37 ± 0.16
Escherichia coli	-	-	37 ± 0.40
Fungi			
Aspergillus niger	-	6.5 ± 0.19	37 ± 0.23
Candida albicans	-	6 ± 0.16	37 ± 0.10

The result of the determination of quantitative antioxidant activity *G. procumbens* has been summarized in Table 3, Table 4 and figure 3. In the assay of DPPH radical scavenging activity, IC_{50} of ethyl acetate extract of *G. procumbens* leaves 322.244 µg/ml which was comparable to ascorbic acid IC_{50} = 41.683 µg/ml, a very well-known benchmark antioxidant.

Table 3. DPPH scavenging assay of ascorbic acid

Conc. (µg/ml)	Log conc.	Abs. 1	Abs. 2	Average	% Inhibition	IC_{50} (µg/ml)
Blank	0	0.700	0.705	0.702	0.0	
1	0	0.638	0.640	0.639	8.974	
2	0.3	0.618	0.610	0.614	12.535	
4	0.6	0.560	0.558	0.559	20.370	
8	0.9	0.507	0.510	0.508	27.564	41.683
16	1.2	0.481	0.483	0.482	31.339	
32	1.51	0.418	0.421	0.419	40.327	
64	1.81	0.300	0.307	0.303	56.837	
128	2.11	0.212	0.215	0.213	69.658	
256	2.41	0.180	0.174	0.177	74.786	
512	2.71	0.106	0.102	0.104	85.185	

Table 4. DPPH Scavenging Assay of *Gynura procumbens*

Conc. (µg/ml)	Log conc.	Abs. 1	Abs. 2	Average	% Inhibition	IC_{50} (/µg/ml)
Blank	0	0.700	0.705	0.702	0.0	
1	0	0.650	0.658	0.654	6.837	
2	0.3	0.610	0.606	0.608	13.5135	
4	0.6	0.590	0.584	0.587	16.3817	
8	0.9	0.576	0.571	0.5735	18.42105	
16	1.2	0.548	0.542	0.545	22.475	
32	1.51	0.515	0.520	0.517	26.2820	322.244
64	1.81	0.498	0.494	0.496	29.3447	
128	2.11	0.416	0.421	0.4185	40.4694	
256	2.41	0.380	0.376	0.378	46.1538	
512	2.71	0.340	0.344	0.342	51.2820	
1024	3.01	0.235	0.232	0.234	66.714	
2048	3.31	0.210	0.214	0.212	69.800	
4096	3.61	0.145	0.141	0.143	79.629	

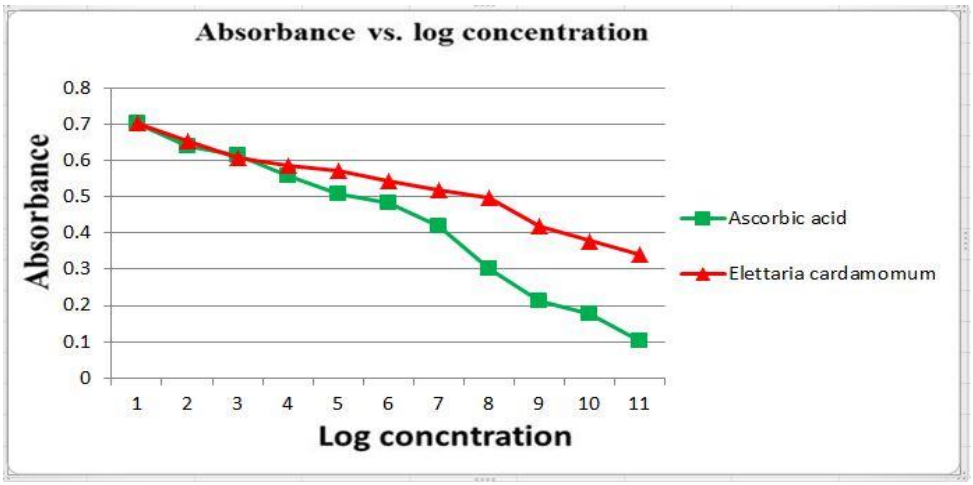

Figure 3. Absorbance vs log concentration for ascorbic acid vs *Gynura procumbens*.

Discussion

Phytochemical screening is necessary to understand the chemical nature of plant extract components. This screening is also used to determine the bioactive molecules for the preparation and synthesis of valuable drugs. In the present study, methanolic *G. procumbens* extract became used to phytochemical assessments for plant secondary metabolites; carbohydrates, alkaloid, phenolic compound, flavonoid, tannins, glycoside, steroid, protein & amino acid, acidic compound and saponins using preferred processes techniques. The methanol extract's percentage yield was found to be 4.8 percent w/w. Phytochemical screening revealed that presence of carbohydrate, alkaloids, flavonoids, glycosides, phenolic compounds and tannins but saponins, proteins, acidic compounds and absence of steroids in *G. procumbens* extract. These phytoconstituents are responsible for healing activities in human body during the use of medicinal plant (Tijjani *et al.*, 2009). Alkaloids were found from *G. procumbens* methanolic extracts through phytochemical screening. These alkaloids are pharmacologically active because they contain nitrogen-bearing molecules. Alkaloids have anti-tubercular, anti-inflammatory, antinociceptive, and anti-pyretic properties and are used in traditional medicine for the treatment diarrhea (Barbosa-Filho *et al.*, 2006; Ivanovska and Philipov, 1996; Kishore *et al.*, 2009; Küpeli *et al.*, 2002). Toxic effects against cells of microbial pathogens are one of the most familiar biochemical activity of alkaloids. Alkaloids have the ability to eliminate and reduce human carcinogenic cells (Nobori *et al.*, 1994). Alkaloids are also used as powerful pain relievers (Kam and Liew, 2002). Glycosides were also found from *G. procumbens* methanolic extracts through phytochemical screening and glycoside also are very crucial classes of secondary metabolites. As they have a few cardio active properties and applied in treatment of heart conditions (Oloyede, 2005). These results indicate that *Gynura Procumbens* extract contains phytocompounds, which could have significant implications for the development of new lifestyle-saving medications. However, specific study

should be needed to use it in medicine. According to the current study, only ethyl acetate extracts of *G. procumbens* leaves have a mild sensitivity against almost all bacteria and fungi that were tested in our experiment (except E. coli), whereas crude methanol extracts of *G. procumbens* leaves have no antimicrobial activity, which is partially consistent with other author (Nazmul *et al.*, 2011) stated that methanol extract of Gynura leaves did not show antifungal activity. Our study is not consistent with other author (Kaewseejan *et al.*, 2015), stated that Gynura extracts did not show antibacterial activity. Our study is also partially consistent with other author (Nasiruddin and Sinha, 2020) who stated that Gynura extracts showed a variable degree of antibacterial activity. For quantitative antioxidant method, the DPPH free radical has been used. The DPPH antioxidant test depend on the ability of a stable free radical, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), to discolor in the existence of antioxidants. An odd electron in the DPPH radical is capable for the absorbance at 517 nm as well as the purple yellow color that can be seen. The extract showed free radical scavenging activity in the DPPH assay (IC_{50} ~ 322.24 µg/ml) comparable to that of ascorbic acid (IC_{50} ~ 41.684 µg/ml), is a very well benchmark antioxidant activity. The antioxidant phenolics and flavonoids in *Gynura procumbens* are moderate (Kaewseejan *et al.*, 2015). It may be the reason of antioxidant activity. When plants are stressed in the photosynthetic electron transport system, which is the significant source of reactive oxygen species in plant tissues, photo-inhibition and oxidation can take place. In our research, we revealed that ROS may be scavenged proficiently by the antioxidant system of the *G. procumbens* leaves. Our study is consistent with other author (Nasiruddin and Sinha, 2020) who stated that Gynura leaves extracts have high antioxidant efficacy. Our research suggested that *G. procumbens* leaves extract can be used as an herbal antioxidant in pharmaceutical industry, however further research should be needed in large scale.

Conclusion

The crude extracts of *G. procumbens* contain important phytochemical constituents, according to the study, and these constituents have medicinal properties. We revealed that *G. procumbens* extracts have antimicrobial properties, which can be used to treat bacterial and fungal infections. We also found that *G. procumbens* extracts have antioxidant properties, which scavenge free radicals from body cells and prevent oxidative damage. The phytochemistry and various pharmacological attributes of the leaf extract and ingredients illustrated in the studies may additionally offer an opportunity for aradical evaluation of the plant's medicinal ability. To summarize, the leaf of *G. procumbens* can be said to have high medicinal benefits and has tremendous potential for use in the advancement of clinical treatments and basic goods (Figure 4). However, there is still a lack of understanding about the underlying mechanisms of action and the precise chemical constituents involved. For the development of standardized drugs or herbal products, research into the mechanisms underlying biological activities is required.

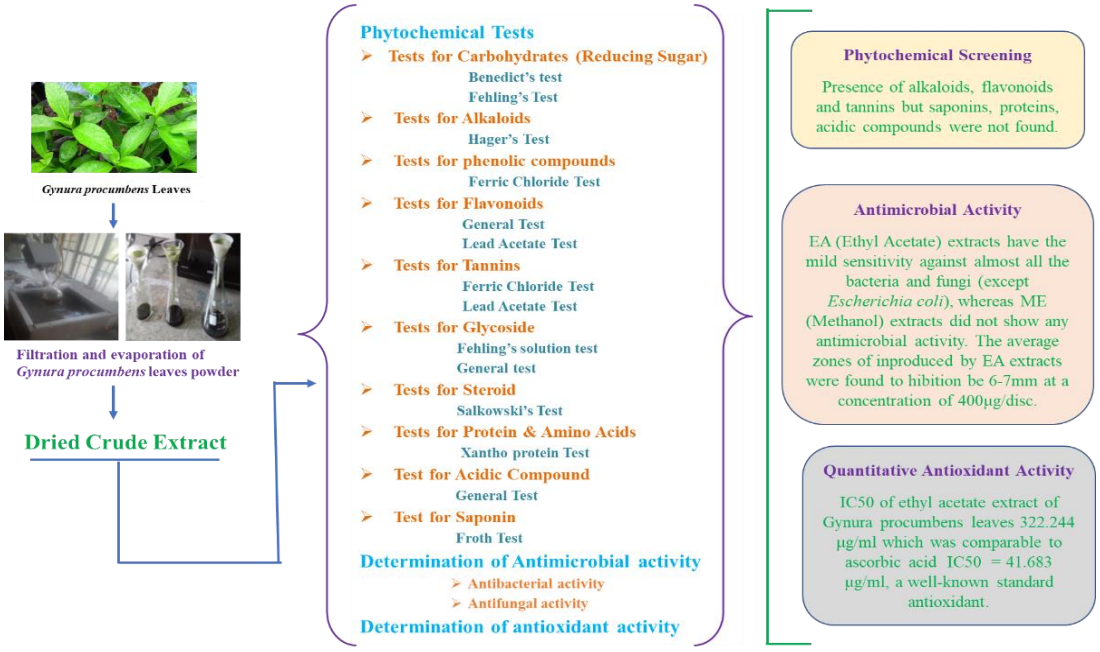

Figure 4. A schematic summary showing the that *Gynura* extract is a good natural source of bioactive compounds

Authors Contribution

SAS carried out the experiment and animal trial, contributed in the sample and data collection. FBA edited and approved the experimental design and methodology, supervised the overall research work. MMH participated in proposing and designing the experiment. RI proposed and approved the methodology. SS and SAS prepared the leaves extract of *G. procumbens*. MMM and MAS wrote and prepared the manuscript. All authors have read and approved the final manuscript submission.

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Competing Interests

The authors declared that they have no conflicts of interest.

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