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Preliminary Phytochemical Screening Of *Gynura Procumbans*

Pragya Shrivastava1,Ranjan Bhowmick 2 and Abhishek Gupta 3

1,2 Department of Life Science, RNTU, Bhopal (M.P.) India .

3. CMBT Research and Training Institute, Bhopal (M.P.) India .

ABSTRACT

This study delves into the phytochemical composition of *Gynura procumbens*, a medicinal plant with reported therapeutic efficacy. Through rigorous extraction and analysis, we aimed to identify and quantify the bioactive compounds present in its hydroalcoholic extract, focusing particularly on phenolic compounds and flavonoids. Qualitative screening revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, and phenolic compounds, affirming its rich chemical diversity. Subsequent quantitative analysis using spectrophotometry and chromatography provided insights into the concentration of these phytochemicals. Biological assays were conducted to evaluate the antioxidant potential and cytotoxic effects of the extract, shedding light on its possible therapeutic applications. Advanced analytical techniques, including mass spectrometry, facilitated the identification of active compounds, thus elucidating the extract's chemical profile. These findings contribute to a deeper understanding of *Gynura procumbens'* medicinal properties and its potential role in disease management. Harnessing the bioactive constituents of this plant could lead to the development of novel pharmaceuticals or nutraceuticals with significant health benefits. This research underscores the importance of exploring natural sources for new therapeutic agents to address current healthcare challenges.

Key words – *Gynura procumbens*, Phytochemical screening, potent, Hydroalcoholic extract, pharmaceuticals, therapeutic.

INTRODUCTION - Plants are universally recognized as vital component of the world's bio-diversity and very essential resources for the planet. The art of healing has its origin in the ancient past of human civilization. The medicinal value of the plant lies in some of its chemical substances that produce a definite physiological action on human body. (Sisodiya, and Shrivastava,2018). There are thousands of herbal plants have already been recognized as blessing of Nature to mankind for the leading source of traditional medicines. They are huge reservoir of various effective chemical substances with potential therapeutic

properties which are very much necessary and effective in present medical science. Regarding these medicinal properties of these plants, our modern scientists are very much interested and excited and globally admitted and accepted that the herbal elements are essential for World health prevention and protection without creating any side effect if justified with scientific approach and compared to current synthetic drugs. For this reason, all types of biologist are working on the searching of safe and effective natural remedies. Though these herbs have been used by all cultures throughout history and thus herbal medicine is the oldest form of health care known to mankind, it was noted that all medicinal plants are not safe to consumption or to use in medicine due to it's toxicity. Worldwide, especially in developing countries there is a general old age myth that herbal drugs are safe and non-toxic but according to Zhang et al. (2000)the traditional uses of plants may cause adverse effects in humans or animals.

My subjected medicinal plant named *Gynura procumbens* belongs to family Asteraceae is a fast growing evergreen herb which is commonly found in tropical Asia countries such as China, Thailand, Indonesia, Malaysia, and Vietnam. Traditionally,and presently in India, Bangladesh, Myanmar etc. In Malay, G. procumbens is called Sambung nyawa which means "prolongation of life" whereas, in Chinese, it is called Bai Bing Cao which means "100 ailments". It is a small plant that can grow up to a height of approximately ~1–3 m, with a fleshy stem and purple tint. The leaves of *G. procumbens* are ovate-elliptic or lanceolate, with 3.5–8 cm long and 0.8–3.5 cm wide and the flowering heads are panicled, narrow, yellow, and 1–1.5 cm long.

It is widely used in many different countries for the treatment of a wide variety of health ailments such as diabetes mellitus, Antiinflammatory, Anticancer, Wound healing, Antiglycemic, and hypertension as *G. procumbens* is a valuable plant that contains various chemical constituents that show excellent therapeutic effects. The leaves contain important active chemical constituents such as flavonoids, saponins, tannins, terpenoids, and sterol glycosides.It contains several constituents including kaempferol, quercetin, kaempferol-3-O-β-D-glucopyranoside, kaempferol-3-O-rutinoside, rutin, chlorogenic acid and 3,5-dicaffeoilquinate methyl ester, terpenoid, tannin, alkaloid, saponin, and astragalinin . Several studies reported that ethanolic leaves extract of *Gynura procumbens* extract is good natural source of bioactive compounds include chlorophylls, carotenoids, alkaloids and volatile oils with huge Antioxident property used in various treatment . This plant is also found with huge nutritional and commercial benefits in food and cosmetic industry practically which is proven beneficiary to mankind and are using in many countries like Chaina, Korea and many other countries.

MATERIALS AND METHODS –

Collection of samples – Identified, fresh and matured leaves of *Gynura Procumbens* are collected from a Herbal garden named " Nature's Spot", addressed - Kawgachi, Shyamnagar, North 24pgs,West Bengal, In huge amount. These leaves are cleaned twice in purified water to remove all kind dust and microbes. These were then dried for 8 to 10 days at room temperature on a clean cotton cloth. Second, dried leaves were coarsely used with a mortar and pestle and then a mechanical blender was used to ground them further to ready powder form for extraction.

Preparation of plant extracts-

The plant materials (*Gynura procumbens)* leaves & Roots were washed under running water, cut into pieces, air shade dried and pulverized into fine powder in a grinding machine then keep into storage box for further procedure.

A quantity of 100g of the dried powder of *Gynura procumbens* powder extracted individually with individual solvent (Hydroalcohol 1:1). The soxhletion with Hydroalcohol solvent were due for a week to obtained extract. After that, the Extract was evaporated in water bath at 50ºC to obtained crude for antioxidant assay, phytochemical analysis, Determination of Bioactive compound, Antimicrobial Susceptibility (Gupta *et al.,* 2011).

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACT

The existence of numerous phytoconstituents such as carbohydrates, proteins, flavonoids, glycosides, hormones, alkaloids, tannin and phenolic compounds was qualitatively studied in the Hydro-Alcoholic extracts. The qualitative phytochemical screening tests (Kokate et al. ,1993), (Trease & Evans,1989). They performed the following qualitative tests:

Test for carbohydrate: -

Molisch's reagent was added to 2-3 ml of aqueous plant extract. For 2-3 ml of aqueous plant extract, Molisch's reagent was added. Naphthol solution alcohol was added to a limited volume and the mixture was shook well, then added cons. On the sides of the sloping test tube, sulphuric acid (H2SO4) is applied to allow it to stand for 3-5 minutes. Violet ring is located at the junction of 2 solution suggests the presence of carbohydrate

Naphthol solution alcohol was added to a limited volume and the mixture was shook well, then added cons. On the sides of the sloping test tube, sulphuric acid (H2SO4) is applied to allow it to stand for 3-5 minutes. At the intersection of 2 solutions, the violet ring shows the presence of carbohydrate.

Test for on reducing poly saccharides

(a) Iodine test: 2-3 ml of assay solution and a few drops of iodine solution is dissolved. The presence of iodine is suggested by the blue precipitate pigment.

(b) Tannic acid test for starch: Slowly apply 20% tannic acid to the 2-3ml test solution and a few drops combined. If the PPT in the tube is shown, it means that the test is positive.

Test for proteins:

(a) Biuret Test: The test solution was treated with 4% NaoH solution and 2-3 drops of 1% Cuso4 and observed violet or pink color formation.

(b) Million's test: Add 1 to 2 ml of concentrate, and a few drops of reagents from Million. The existence of protein is suggested by White ppt.

Test for amino acid

Cysteine test: 5ml test solution, a few drops of 40 percent NaoH and 10 percent lead acetate solution are placed in the test tube and then further heated for 10-15 minutes in boiling water in 40-45 C if the presence of amino acid is shown after 10-15 minutes when black ppt is seen.

Test for steroid

(a) Salkowski test: TO1-2 ml of extract, 2 ml of chloroform and 2 ml of Contras. Then shake well with H2SO4. If the chloroform coating is red and the layered sulfuric acid displays greenish yellow fluorescence, the presence of steroids is shown.

Test for glycosides:

The compound formed in the sugar molecule by removing the hydroxyl group. -glycosides (a)Killer killani test: Added 3 ml of test solution extract in a test tube and added 1.5 ml of glacial acetic acid, then mixed 5 percent fecl3 and kept for some time after adding 1 ml of Cons. From H2So4. If the reddish brown colour appears at the junction of the 2 liquid layers and the bluish green rings appear at the top layer, the presence of de-oxy sugar is indicated.

Test for anthraquinone glycosides

Borntrager test: Add 5 ml of test solution and dilute H2SO4 to boil for 10-12 minutes in a water bath and filter. Add equal amount of chloroform shake after filtrate well then separate added ammonia. If the ammonia layer turns pink and red, this means the presence of anthraquinone glycosides.

Test for coumarins glycosides:

2 ml of 10 percent sodium hydroxide was added to the 1-2 ml test solution. The presence of coumarins is shown by the formation of a yellow color that fluoresces under ultra violet light (Sangeeta et al., 2015)

Test for flavonoids:

Shinoda test: Add 2-3 ml of test solution to the test tube and 1 ml of CONS. A few HCL drops. Add a 4 to 5 Mg ribbon after 5 minutes. Orange, red pink, or purple color formation indicates the presence of flavonoids.

Alkaloids test:

Wagner's test: Wagner's reagents added to 1-2 ml filtrate solution and a few drops if seen radish brown

Precipitate confirms the test as positive.

Test for tannin compound:

Acetic acid test: 2 ml of test solution with the addition of acetic acid solution. The red color formation indicated the presence of tannins. (b) Lead acetate test: 2 ml evaluation solution and applied a solution of lead acetate. White ppt formation suggests the presence of tannins.

Test for phenolic compound:

Iodine test: 2 ml of test solution and iodine solution in the test tube is applied. The presence of phenol was suggested after 2-4 minutes of red colour formation.

Test for organic acid

Confirmatory Oxalic Acid Test: Lead acetate test: A 2 ml test solution and a few drops of 5 percent lead acetate were added. The presence of oxalic acid was indicated in the formation of white ppt.

The Malic Acid Confirmatory Exam: Ferric chloride test: 2 ml of test solution and a few drops of 40 percent Fecl3 solution were added. The yellowish colour formation indicated the presence of malic acid.

Test for inorganic acid

(a)Test for sulphate: Add 10 percent white lead acetate reagents to 2 ml test solution, then add 0.50 ml NaoH solution to white ppt indicated sulphate presence formation after 5 to 7 minutes.

Test for chloride Add 3 ml of lead acetate solution to 5 ml of filtrate test solution in the test tube and heat for 5 minutes at 60 C in the water bath. White ppt indicated the presence of chloride after refrigerated formation.

Test for carbonate:

(a) With dilute acid: 3ml test solution and added dilution acid to the test tube (HCL). CO2 bubbles are provided as soon as we mix dilute acids. That is shown by the presence of carbonate.

Test for nitrate:

The brown ring test: With test solution of ferrous sulphate yield no brown colour but if we are slowly added sulphuric acid. A brown ring will form at the junction of the two layers after 5 -10 minutes, indicating the presence of nitrate ions.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACT

Total phenolic content assay:

Total phenolic content determination (TPC) The total phenolic content was determined using the Folin Ciocalteu reagent method, based on colorimetric reduction (Singleton et al.1965). Test samples are 0.05 mg in 50 ml D/W, 0.05 mg tannic acid in 50 ml, 1 ml FCR in 9 ml D/w or 1:9 and 7 gm 35 percent sodium carbonate in 20 ml D/W. Then prepare 3 different types of solutions in 7 different test tubes (5 Normal, 1 blank and 1 sample) with the first Standard solution, 0.2 ml of tannic acid, 0.8 ml of D/W and 1 ml of FCR, 1 ml of sodium carbonate added after a few minutes. 1 ml FCR for 1 ml D/W null, and 1 ml Na2co3 added after 3-4 minutes. 0.6 ml tannic acid solution, 0.4 ml D/W, 1 ml FCR and 1 ml sodium carbonate were applied to the last sample after a few minutes. The entire mixture was permitted to stand for 90 minutes in the dark. The blue color solution absorbance was read 765 nm against the blank on a UV visible spectrophotometer. In triplicates, the overall phenolic concentration (mg/ml) of the sample was measured and the normal curve extrapolated.

• **Reagents**

1. Sodium carbonate solution (35%): 35 gm of sodium carbonate was dissolved in 100 ml of water and then purified after allowing it to stand overnight at room temperature.

2. Tannic acid stock solution 0.05 mg Dissolve tannic acid in 50 ml of distilled water.

Total flavonoid content assay :

The cumulative flavonoid content of all extracts estimated in this process is as follows, based on the aluminium chloride method. For the calculation of the overall flavonoid content of sample samples, the aluminium chloride process (Change et al., 2002) is used. Aliquots of extract solutions are taken and up to 3 ml of D/W are made up of the volume. Then 0.1 ml of aluminum chloride (10 percent), 0.1 ml of sodium hydroxide (1 M) and 2.8 ml of purified water were sequentially added. Then prepare diff (Hajimahmoodi, et al., 2013)erent solutions of 3 forms in various 7 test tubes (5 Standard, 1 blank and 1 sample). Prepare a distinct catecholine concentration norm curve (20ul, 40ul, 60ul, 80ul, 100ul). Following 40-50 minutes of dark room temperature incubation, the test solution is vigorously shook. Absorbance at 510 nm is reported by UV spectrophotometer triplicate tests for each sample. The flavonoid concentrations determined from the calibration plot in the test samples are expressed as mg rutin equivalent/g of the sample. (In 2013, Hajimahmoodi M, et al.). By using the following formula,

TFC was computed: TFC = (R × D.F× V×100) /W

D.F-dilution factor, V-volume of stock solution, 100-for 100 gm dry product, W-weight of plants used in the procedure, when- R- outcome obtained from the standard curve.

Reagents

1. 10g Chloride aluminium was dissolved in 100 ml of D/W.

2. Dissolve 0.4gm with 10 ml of distilled water.

3. Standard rutin solution (1mg/ml): Rutin 10 mg dissolved in 10 ml of D/W

RESULTS AND DISCUSSION - Hydro-Alcoholic extract has revealed Citrus lemon peels include proteins, carbohydrates, amino acid, glycosides, flavonoids, phenolics, etc.

Table No. 1: Preliminary phytochemical analysis of *Gynura procumbens* extract -

S.NO.	Phytochemicals	Test performed	Results
1	Carbohydrates	Molisch's test	-Ve
2	Reducing polysaccharides (Starch)	Iodine test Tannic acid	+Ve +Ve
3	Proteins test	Biuret test Million's test	-Ve -Ve
4	Amino Acids test	Cysteine test	-Ve
5	Steroids test	Salkowski test	-Ve
6	Glycosides	Killer Killani test	-Ve
lp7	Anthraquinone Glycosides	Borntrager's test	-Ve
8	Coumarins Glycosides	With NaoH	
9	Flavonoids	Shinoda test	+Ve
10	Alkaloids	Wagner test	-Ve
11	Tannins compounds	With Lead acetate Acetic Acid	-Ve -Ve

12	Phenolic compounds	Iodine test	+Vr
13	Organic Acid	Oxalic Acid test Malic Acid test	-Ve
14	Inorganic Acid	Sulphate test	+Ve
15	Chloride	Chloride test	-Ve
16	Carbonate Acid	liberate test Mercuric chloride test	-Ve
17	Nitrate	Brown ring test	-Ve
18	Cobalt	Cobalt chioride test	-Ve
19	Ferric chloride	FeCl3 test	-Ve
20	Gelatin	Gelatin test	+Ve

Total Phenolic Contents (TPC):

Table No. 2: Total Phenolic Contents of *Gynura procumbens* extract

S.No.	Concentration	Absorbance
1.	20	0.908
2.	40	0.984
3.	60	1.106
4.	80	1.196
5.	100	1.301

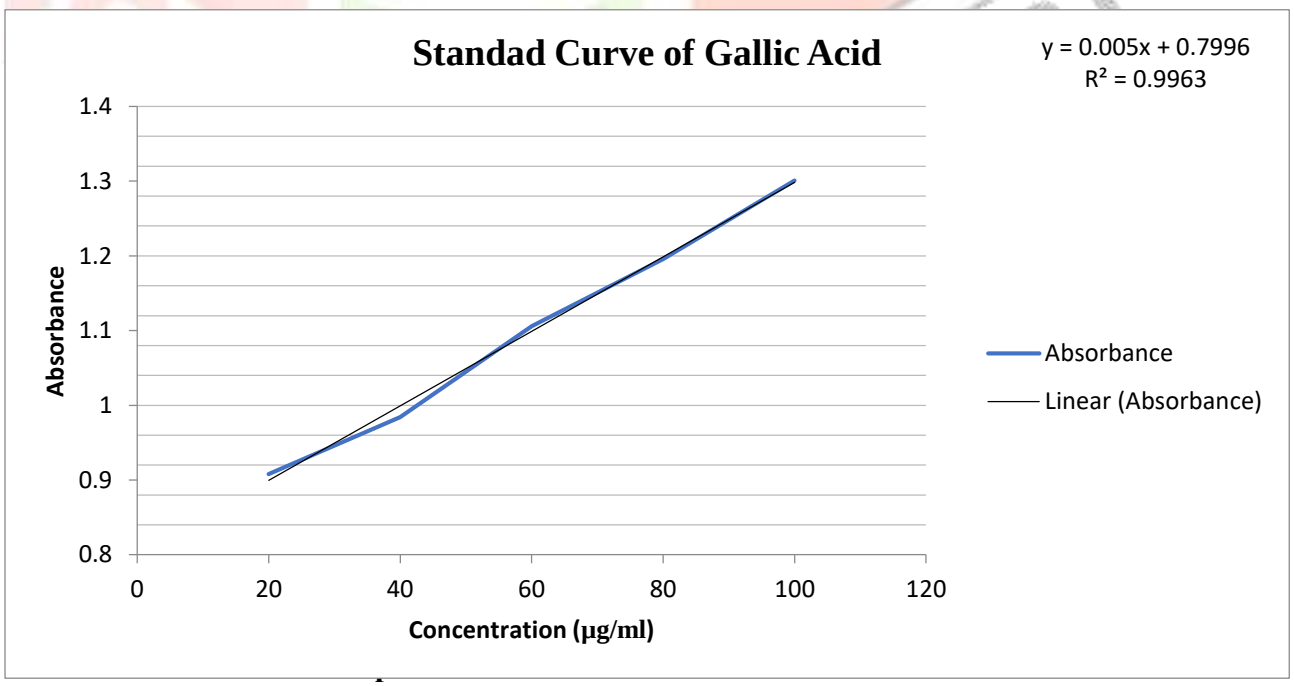


Table No. 3: Total Phenolic Content in *Gynura procumbens Plant*

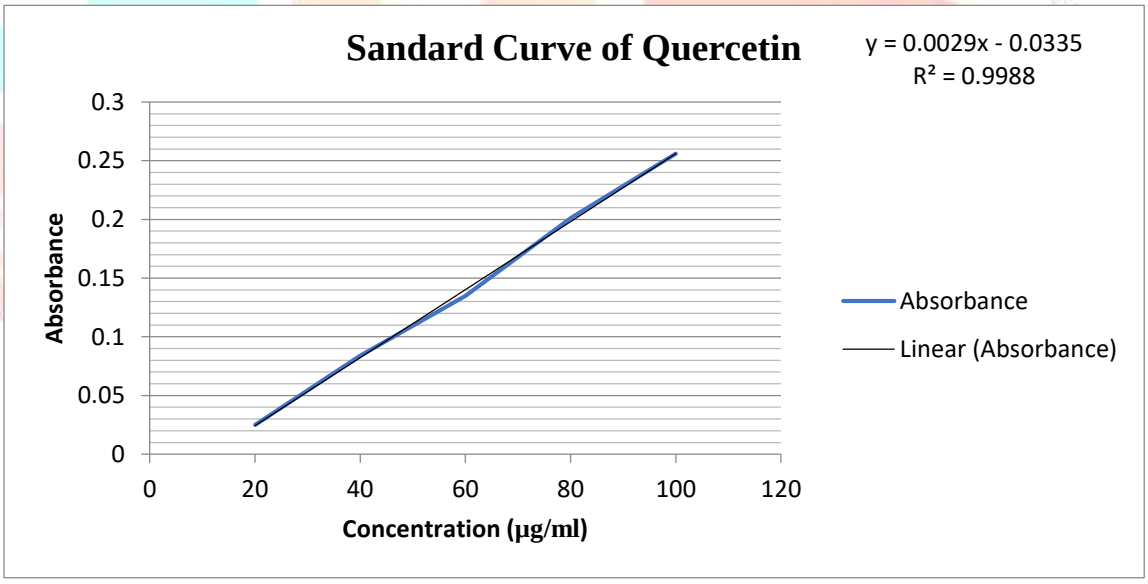
Graph No. 1: Standard Curve of Gallic Acid

QUANTITATIVE ANALYSIS	*Gynura procumbens Plant*
TOTAL PHENOLS (µg of GAE/serving)	37.637±28.59

Total Flavonoid Contents (TFC):

Table No. 2: Total Phenolic Contents of *Gynura procumbens* extract

S.No.	Concentration	Absorbance
1.	20	0.055
2.	40	0.064
3.	60	0.121
4.	80	0.201
5.	100	0.302



Graph No. 2: Standard Curve of Quercetin Acid

TABLE 4: Total Flavonoid Content in *Gynura procumbens Plant*

QUANTITATIVE ANALYSIS	***Gynura procumbens Plant***
TOTAL PHENOLS (µg of QUE/serving)	45.784±17.38

CONCLUSION Herbal plants are widely used for medicinal purposes in these days, and they have various phytochemical entity, so they have been widely used for research investigations. The objective of this study was to test the effective dosage response and minimal side effects of *Gynura procumbens* used for base compounds compared to synthetic compounds. It is seen as a beneficial medicinal plant with various medicinal properties by the robust linear associations found between the phenolic, flavonoid and antioxidant potential phytochemical screening of *Gynura procumbens* extract. Since there are more constituents in the Hydro Alcoholic extract of *Gynura procumbens*, it may be considered beneficial for further investigation. For its better therapeutic and commercial utilization, a typical research and developmental job needs to be carried out.

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