SCREENING OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF WHOLE PLANT AND PARTS EXTRACTS OF *HYGROPHILA SPINOSA* T. ANDERSON (ACANTHACEAE).

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Abstract
The herbal medicines are considered as healthier substitute for conventional and therapeutic drugs. Medicinal plants can be potential source of antimicrobial activity as well as they exhibit antioxidant properties. The present study deals with assessment of antimicrobial and antioxidant activity of Hygrophila spinosa T. Anderson (Family Acanthaceae). Hexane, ethyl acetate, methanol, and water were used to extract the entire plant and its parts. Antimicrobial activity was assessed against eight MTCC Gram-positive and Gram-negative bacteria and their corresponding clinical isolates by agar well diffusion method. Ferric reducing power assay, DPPH inhibition, and DMPD inhibition assays were used to assess antioxidant activity. Qualitative and quantitative phytochemical analysis was done. The highest percentage yield was observed in the leaf aqueous extract. Plant extracts from the whole plant and its parts had no significant antibacterial action against the tested bacteria. The assays for DPPH and DMPD were carried out in a time-dependent manner. The inflorescence aqueous extract has recorded highest reducing power capacity. Methanol and aqueous inflorescence extracts had the highest scavenging effect against DMPD radical in the shortest amount of time. The H. spinose whole-plant aqueous extract scavenged the DPPH radical more effectively in less time. The content of terpenoids were highest in the ethyl acetate extract of the inflorescence; phenol in the aqueous extract of the leaf; and flavonoid in the aqueous extract of the inflorescence.

Keywords: *Hygophila spinosa* T. Anderson, Solvent extraction, Antimicrobial activity, Antioxidant activity.

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1. INTRODUCTION

Infectious agents are a major issue in today's living culture. Different forms of infectious agents have emerged in recent years, and this trend is rising continuously year after year. Indiscriminate use of the antibiotics, on the other hand, is causing antimicrobial resistance in the pathogenic microbial community (Pandey et al., 2018). Antimicrobial resistance (AMR) is a worldwide danger to human health and development. To attain the Sustainable Development Goals, urgent multi-sectoral action is required (SDGs). AMR has been named as one of the top ten worldwide public health hazards facing humanity by the World Health Organization. Antimicrobial resistance (AMR) is a global health and development threat. The drugs with single site action are usually susceptible to resistance. So the multiple site action is required to achieve for the prevention. The World Health Organization has designated AMR as one of the top 10 global public health threats facing humanity (WHO, 2021). Because a single treatment is always prone to resistance, medicine's combinatorial effect is required to reduce the danger of pathogen infection (Efferth and Koch, 2011).

Alternatively, oxidative stress has been hypothesized as a regulating factor in the aging process and various brain diseases. Excess oxidants cause antioxidants to be depleted, resulting in an oxidation-reduction imbalance in organisms (Kroemer and Reed, 2000). Oxidative stress is caused by a deficiency in the antioxidant system, which is characterized by high quantities of reactive species (oxygen, hydroxyl free radical, and so on). Mitochondria are involved in the creation of important biological compounds as well as the delivery of ATP to cells via oxidative phosphorylation. In

the oxidative phosphorylation process, enzymes catalyze a variety of redox reactions. Reactive oxygen species (ROS) are produced when oxidative phosphorylation is inefficient, which can lead to mitochondrial malfunction. The principal and possible sources of free radicals are determined to be mitochondrial redox metabolism, phospholipid metabolism, and proteolytic processes. Higher concentrations and long-term exposure of ROS induce damage to cellular macromolecules such as DNA, lipids, and proteins, eventually leading to necrosis and apoptotic cell death (Singh et al., 2019).

Various antioxidant systems are formed against the radicals, including glutathione, taurine, creatine, zinc, vitamin E, vitamin A, vitamin C, and polyphenols, phenol, and flavonoid from plant extract. Antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and others increase and maintain the antioxidant effect (Fang et al., 2002).

Medicinal plants have been used to treat human diseases for thousands of years because they contain a wide range of chemical components that have a specific physiological effect on the human body. Alkaloids, tannins, flavonoids, terpenoids, saponins, and phenolic compounds are the most important of these chemicals. These chemicals are appealing to pharmacists because of their therapeutic efficacy and low toxicity (Inayatullah et al., 2012). Several such compounds have been identified from plants, and they could be utilized to produce new medications to prevent the growth of bacterial and fungal infections, as well as to quench ROS, with low toxicity to the host cell (Ahmad and Aqil et al. 2007).

Hygrophila spinose A. (Acanthaceae Family) is known in Ayurved as Ikshugandha and locally as gonkhul kanta. With synonyms such as, *Hygrophilla auriculata* (Schumach), Heine; *Astercantha longifolia* (L.) Nees, is extensively found throughout India, from the Himalayas to Ceylon, Sri Lanka, Burma, Malaysia, and Nepal. *H. spinose* is used as a vegetable in some states like Odisha, Chhattisgarh, and West Bengal (Kshirsagar et al., 2010). It's an annual, robust, and erect herb with subquadrangular thickened nodes, oblanceolate leaves with yellow spines in the axils, light to purple-blue flowers densely crowded in the axils, and oblong, glabrous capsules with 4 to 8 seeds (Nadkarni, 2007).

Phytosterols, polyphenols, proanthocyanins, mucilage, alkaloids, flavonoids, terpenoids, glycosides, and other phytoconstituents are found in *H. spinosa*. Cancer, arthritis, hepatotoxicity, inflammation, blood disorders, diabetes, fever, constipation, and bacterial infection are all treated with *H. spinosa*. It can be used to treat anasarca, urinogenital tract disorders, chronic Blight's disease dropsy, hyperdipsia, vesical calculi, flatulence, diarrhea, dysentery, leucorrhoea, gonorrhea, asthma, blood diseases, gastric diseases, painful micturition, menorrhagia, and other conditions. Anti-inflammatory, analgesic, and antipyretic properties have been seen in chloroform and alcoholic extracts. Methanol extract have antibacterial action, petroleum ether, chloroform, and an alcoholic aqueous extract of leaves have anthelmintic activity. Methanol extract of leaves have hepatocarcinogenic activity, and hydroalcoholic extract of aerial parts exhibits breast cancer therapy potential (Dash et al., 2012).

The purpose of this study was to assess the antibacterial and antioxidant activities of H. spinose T. Anderson of the Chhattisgarh region and to compare the quantities of these bioactive chemicals to other findings. The state of Chhattisgarh has a diverse range of medicinal plants. It is anticipated that the percentage of bioactive chemicals in plants from the Chhattisgarh region is higher and that it varies depending on geographic location (Maurya et al., 2014).

2. MATERIALS AND METHODS

Plant Material

Hygrophila spinose T. Anderson was collected in Raipur, Chhattisgarh, during the flowering stage (September – November 2018). A plant

taxonomist who is currently a consultant at the National Center for Natural Resources (NCNR), Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India, identified the plant. They were cleaned with distilled water, sterilized for 5 minutes with 0.1 percent mercuric chloride, rinsed with water, wiped, and air-dried at room temperature until they reached a constant weight. The dried parts, including the complete plant, leaf, inflorescence, stem, and root, were ground into a fine powder using an electric blender.

Preparation of extract

The extraction was done with the plant's nature in mind. Because it is an aromatic plant, cold extraction was used as previously reported (Harborne, 1998). For 48 hours at room temperature, dried powders of inflorescence, leaf, stem, and root were macerated in a 1:10 ratio with hexane, ethyl acetate, and methanol. The solvent was evaporated in rotator evaporator at 40°C. To separate polar chemicals from non-polar molecules, the methanol extract was fractionated with chloroform and water in a separating funnel. The non-polar portion contained mostly chlorophyll and was not used for further investigation. The dried extract yield was calculated. After solubility standardization, a 10% stock solution of extracts was prepared in the appropriate solvent. The hexane extract was dissolved in 50% hexane with ethyl acetate, the ethyl acetate extract in 30% DMSO with ethyl acetate, and the polar component of the methanol extract in 40% DMSO with methanol.

Antibacterial activity test

IMTECH Chandigarh on request sourced the bacterial cultures for the antibacterial assay. Gram-positive bacteria, *Bacillus cereus* MTCC-430, *Bacillus subtilis* MTCC-441, *Staphylococcus aureus* MTCC-96, *Staphylococcus epidermidis* MTCC-435, and Gram-negative bacteria, *Escherichia coli* MTCC-1687, *Klebsiella pneumoniae* MTCC-3384, *Pseudomonas aeruginosa* MTCC-741 and *Proteus vulgaris* MTCC-744. Their corresponding clinical isolates were obtained from Department of Microbiology, Pt. Jawaharlal Nehru Memorial Medical College, Raipur (Chhattisgarh), India. The agar well diffusion method following the method of CLSI (2012) was used to test antibacterial activity with certain modifications. Using a UV-VIS spectrophotometer (Model 108, Systronics, India), the six-hour-old bacterial cultures were adjusted to 0.08 OD to achieve $1x10^8$ CFU/ ml of bacteria at 620 nm. Using a sterile cotton swab, the inoculum was streaked on sterile Mueller-Hinton agar petri plates. To allow excess surface moisture to be absorbed, the plate was left for 10-15 minutes. A 6 mm cork borer was used to bore the well. In the test well, 50 µl (5 mg/ 50 µl) extract was poured. The positive control was streptomycin (10g/ ml), and the negative control was the solvent. Petri plates were incubated in triplicate at 37°C for 18 hours. The antibacterial activity was recorded as zone of inhibition of bacterial growth around the well.

In-vitro antioxidant activity assay

Reducing power assay

Antioxidant activity was determined using the Ferric reducing power test following the procedure of Oyaizu (1986). 1 ml methanol polar extract of whole plant, leaf, inflorescence, stem, and root (200 µg/ ml) was combined with 2.5 ml phosphate buffer (0.2M; pH 6.6) and 2.5 ml 1% $K_3[Fe(CN)_6]$ and incubated for 30 minutes at 50°C. After the incubation period, 2.5 ml of 10% TCA was added to the reaction mixture, which was then allowed to cool to room temperature. 2.5 ml of the aforementioned solution was combined with 2.5 ml deionized water (Milli Q, Merk) and 0.5 ml 0.1% $FeCl_3$. A spectrophotometer was used to measure the absorbance at 700 nm against a blank. The standard was ascorbic acid (30-70 µg/ml). The assay was done three times and the average values were calculated. The results were represented in µg of ascorbic acid equivalent (AAE)/ ml of plant extract.

DMPD cation free radical scavenging assay

As previously stated by Asghar et al., (2007) the radical scavenging experiment was performed utilizing the DMPD free radical

scavenging assay. Briefly, the DMPD free radical was created by dissolving 0.1 ml of DMPD aqueous solution (100 mM) in 0.05 ml of aqueous $K_2S_2O_8$ (0.4 mM) and diluting with sodium acetate buffer to a final volume of 10 ml (pH 5.6). Before use, the solution was incubated in the dark for 5 hours, until it reached an O.D. of 0.7-0.8 at 517.4 nm. The scavenging effect of extracts was determined by mixing 50-200 µl of extract into 3.5 ml DMPD radical and incubating for various periods of time. Using the equation: the radical scavenging activity was expressed as a percentage of DMPD discoloration:

$$DMPD^{\cdot} radical\ scavenging\ (\%) = [(A_{control} - A_{sample})/ A_{control}] \times 100,$$

where $A_{control}$ is the absorbance of the DMPD solution without the addition of extract, and A_{sample} is the absorption of the extract/ reference component. In the concentration range of 40-80 µg/ ml, ascorbic acid was employed as the reference compound. The assay was done three times and the average values were calculated. The results were represented in µg of ascorbic acid equivalent (AAE)/ ml of plant extract. Regression analysis was used to calculate the IC_{50}. The radical absorbance decreases following increment of inhibition in this experiment, which is an end point measuring assay. As a result, the test was performed at one-minute intervals to verify reaction completion time and kinetics.

DPPH scavenging assay

The DPPH scavenging assay was carried out according to the instructions by Brand-Williams et al., (1995). In a nutshell, the 50 M DPPH solution was made in methanol. The scavenging effect of extracts was determined by mixing 50-200 µl extracts with 3 ml DPPH solution and incubating for various periods of time. The radical scavenging activity was expressed as a percentage of DPPH discoloration using the equation:

$$DPPH\ scavenging\ (\%) = [(A_{control} - A_{sample})/ A_{control}] \times 100,$$

where $A_{control}$ is the absorbance of the DPPH solution without the addition of extract, and A_{sample} is the absorption of the extract/ reference component. In the concentration range of 40-80 µg/ml, ascorbic acid was employed as the reference compound. The assay was done three times and the average values were calculated. The results were represented in µg of ascorbic acid equivalent (AAE)/ ml of plant extract. Regression analysis was used to calculate the IC_{50}. To assess reaction completion time and kinetics, the assay was performed at one minute intervals.

Preliminary phytochemical analysis

Screening of various phytoconstituents was performed as described earlier by Harborne (1998).

Total terpenoid assay

With few modifications, the total terpenoid assay was carried out as previously described by Ghorai et al. (2012). As an unknown sample, 200 µl of extract was extracted. A standard curve was created using linalool (40 to 100 mg). Each tube received 1.5 ml chloroform, which was properly mixed and left to rest for 3 minutes. Each tube received 100 µl of concentrated H_2SO_4 and was incubated for 10 minutes. Terpenoids sank to the bottom as a dark brown precipitate. The precipitate was dissolved in 1.5 ml methanol after the supernatant was properly decanted. In a spectrophotometer, the absorbance was measured at 538 nm against methanol. The assay was carried out in triplicate, and the concentration was calculated as mg linalool/ g of extract.

Total phenol assay

The total phenol content of the extracts was determined using the Folin–Ciocalteu reagent (Singleton & Rossi, 1965), with gallic acid as a reference. 0.2 ml of two-fold diluted Folin–Ciocalteu reagent was combined with 0.1 ml of polar fraction of methanol extracts (2.5 %). After 3 minutes of room temperature incubation, 0.6 ml of a 2% Na_2CO_3 aqueous solution was added. With distilled water, the complete reaction mixture was prepared up to 6 ml and then incubated for 1 minute in boiling water. After cooling the tubes for 10 minutes, absorbance was measured at 760 nm against a blank. Gallic acid in various concentrations

(20-60 µg/ml) was utilized to create the standard curve. Triplicates of the test were run. The concentration of phenol was expressed as mg gallic acid equivalent (GAE)/ g of extract.

Total flavonoid assay

As previously published (Zhishen et al. 1999), the total flavonoid content was calculated. 1 ml extracts were combined with 4 ml deionized water and 0.3 ml $NaNO_2$ at a concentration of 5% (w/v). After a 5-minute incubation period, 0.3 ml (10% w/v) $AlCl_3$ was added. 2 ml of 1M NaOH was added after 6 minutes. To produce a final amount of 10 ml, around 2.4 ml of deionized water was added immediately. A UV–visible spectrophotometer was used to evaluate the absorbance of the mixture at 510 nm after it was vigorously shaken. A standard solution with varied concentrations (20-100 g/ ml) of quercitin was prepared. Results were indicated as mg quercitin equivalents (QE)/ g on a dry weight of extract.

Statistical analysis

The data was evaluated using one-way ANOVA. Tukey's test was employed to find significant differences between mean values (SPSS Statistics 20.0, IBM, Armonk, New York, USA). The difference in means was considered statistically significant at the 5% level ($p < 0.05$).

3. RESULTS AND DISCUSSION

The entire plant, as well as other sections of *Hygrophila spinosa*, such as the leaf, inflorescence, stem, and root, was utilized to extract phytochemicals and test antibacterial and antioxidant properties. To guarantee that a wide spectrum of bioactive chemicals could be recovered, the plant samples were cold macerated with four solvents based on their polarity index: hexane, ethyl acetate, methanol, and water. The polar fraction was employed after the non-polar material was removed from the methanol extract.

Plant extracts

The leaf had the highest overall percentage production of all the plant components, followed by the whole plant, inflorescence, root, and stem. The highest yield (15.36%) was obtained in leaf aqueous extract; whereas the lowest yield was found in root hexane extract (0.55%). The extracts had a sticky to viscous texture and a yellowish to radish-brown color. Table 1 shows the yield, texture, and color.

Table 1: The yield of different part of *Hygrophila spinosa* extracted in various solvents.

Solvents	% Yield	Color	Consistency
Whole plant			
Hexane	2.80%	Yellowish	Sticky
Ethyl acetate	3.26%	Dark green	Sticky
Methanol-PF	8.52%	Reddish brown	Viscous
Aqueous	13.66%	Reddish brown	Highly viscous
Leaf			
Hexane	4.44%	Yellowish	Sticky
Ethyl acetate	6.95%	Dark green	Sticky
Methanol-PF	10.55%	Reddish brown	Viscous
Aqueous	15.36%	Reddish brown	Highly viscous
Inflorescence			
Hexane	3.35%	Yellowish	Sticky
Ethyl acetate	6.26%	Light green	Sticky
Methanol-PF	7.85%	Reddish brown	Viscous
Aqueous	10.98%	Reddish brown	Viscous
Stem			
Hexane	0.78%	Yellowish	Sticky
Ethyl acetate	2.36%	Dark green	Sticky
Methanol-PF	5.88%	Reddish brown	Viscous
Aqueous	9.02%	Reddish brown	Viscous
Root			
Hexane	0.55%	Brown	Sticky
Ethyl acetate	0.75%	Dark brown	Sticky
Methanol-PF	8.06%	Reddish brown	Viscous
Aqueous	12.62%	Reddish brown	Viscous

Antimicrobial activity

With repeated attempts against all of the examined pathogens, cold extracts of different sections of *H. spinosa* with various solvents showed no significant action. However, only a few studies found antibacterial efficacy in hot extracts of *H. spinosa* against harmful bacteria (Jani et al., 2011; Pawar et al., 2015).

Antioxidant activity

Due to the limitations of reactivity with hydrophilic phytoconstituents, the reducing power ability was assessed in a polar fraction of methanol extracts and aqueous extracts. Inflorescence aqueous extract (352.13±0.88 g/ ml) had the maximum reducing capacity, while a polar fraction of root methanol extract (57.48±0.45 g/ ml) had the lowest, as shown in figure 1.

DMPD scavenging capacity was assessed in the polar fraction of methanol and aqueous extracts for the same reason as in the reducing power test, while DPPH scavenging activity was measured for all *H. spinosa* extracts. To further understand the behavior of active chemicals, the scavenging activity of *H. spinosa* was measured over time against DMPD and DPPH radicals at one-minute intervals. At the lowest volume (50 µl) of the extracts tested, the inhibitions in terms of time varied depending on parts, extracts, and phytochemical composition of *H. spinosa*.

When polar fractions of methanol extracted from the whole plant, leaf, inflorescence, stem, and root were compared to their aqueous extracts to block the DMPD radical, the degree of inhibition differed depending on the phytochemical components (Figures 2A & B). Within 32 minutes, the polar fraction of methanol from the total plant extract showed inhibition of 19.34%. Inflorescence (43.75%) had the strongest inhibition, followed by leaf (41.33%), stem (39.83%), and root (17.49%). The inhibition took 16, 21, 15, and 14 minutes to complete, correspondingly. In 25 minutes, the entire plant aqueous extract showed 17.65% inhibition. The inflorescence showed the greatest inhibition (46.40%) against the DMPD radical, followed by the leaf (42.66%), stem (17.12%), and root (11.55%). The inhibition took 19, 20, 21, and 17 minutes to complete, respectively. Overall, the methanol and aqueous flower extracts scavenged more DMPD radical in less time.

As demonstrated in figure 3, the polar fraction of methanol extract and the aqueous extract of inflorescence had stronger DMPD radical scavenging capacity, with IC50 values of 29.01±1.07 µg/ ml and 28.99±3.08 µg/ ml, respectively.

The ability of different components and extracts to inhibit the DPPH radical differed as well (Figures 4 A-E). The inhibition of whole plant extracts ranged from 29.80% to 89.36%. The maximum inhibition was attained by aqueous extract (89.36%), followed by methanol polar part (86.65%), hexane (29.80%), and ethyl acetate extract (19.09%). They took 13, 17, 28, and 21 minutes to complete the reaction, correspondingly.

The extracts of various portions of *H. spinosa* have the scavenging activity in following order: water > methanol polar portion > ethyl acetate > hexane extract. The radical was suppressed by the leaf extracts in a range of 36.18% to 83.92%. Aqueous extracts had the strongest inhibition (83.92%), followed by methanol polar part (80.38%), ethyl acetate (51.23%), and hexane extract (36.18%). In that order, the reaction took 12, 15, 26, and 27 minutes to complete. The flower extracts inhibited between 31.76% and 88.67% of the radical. The aqueous extract was the best DPPH radical inhibitor (88.67%), followed by the polar fraction of methanol (80.58%), ethyl acetate (54.56%), and hexane (31.76%) extracts. Within 15, 18, 23, and 22 minutes, the DPPH was suppressed, respectively.

The root extract had a potency of inhibition ranging from 17.64% to 66.60%. The aqueous extract inhibited the most (66.60%), followed by the polar portion of methanol (63.27%), ethyl acetate (35.35%), and hexane extracts (17.64%). The inhibition took 22, 18, 21, and 20 minutes to complete, accordingly. Inhibition was found in the range of 27.92% to 81.00% in stem extract. The aqueous extract had the highest scavenging potential (81.00%), followed by the methanol polar part (79.88%), ethyl acetate (74.24%), and hexane extract (27.92%). To scavenge the DPPH

radical, the extracts took 17, 18, 19, and 29 minutes, respectively.

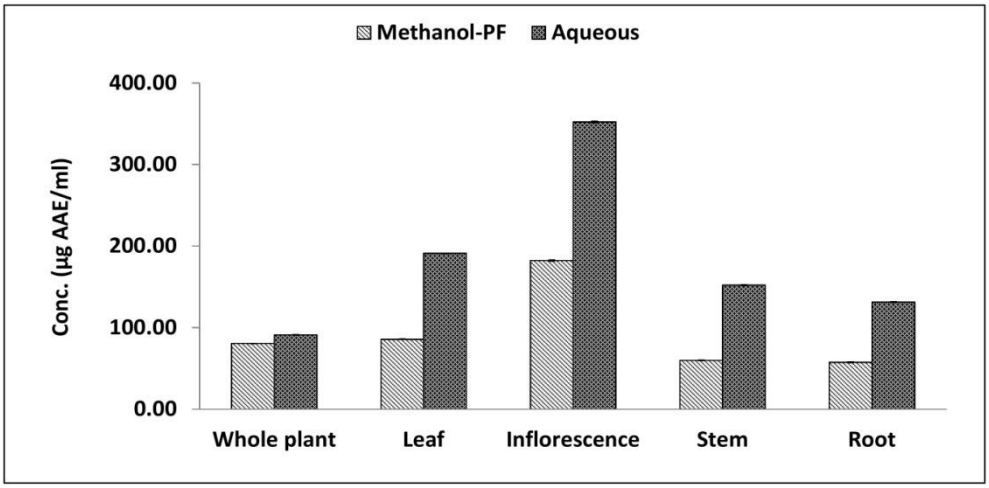


Figure - 1: Reducing power capacity of different parts and their extracts in *Hygrophila spinosa*.

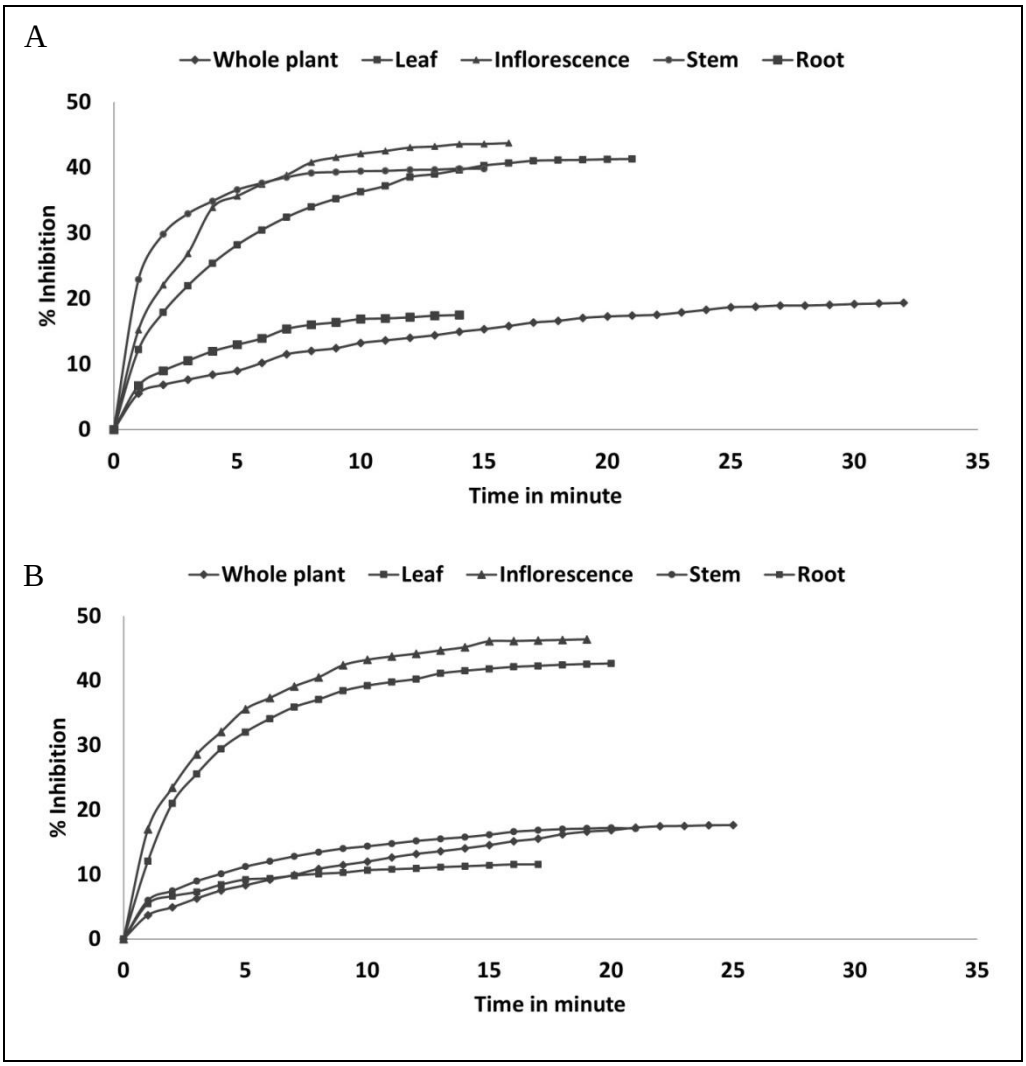


Figure - 2: Antioxidant activity of polar fraction of methanol (A) and aqueous extracts (B) of *Hygrophila spinosa* by the DMPD assay over time and comparison with whole plant and parts.

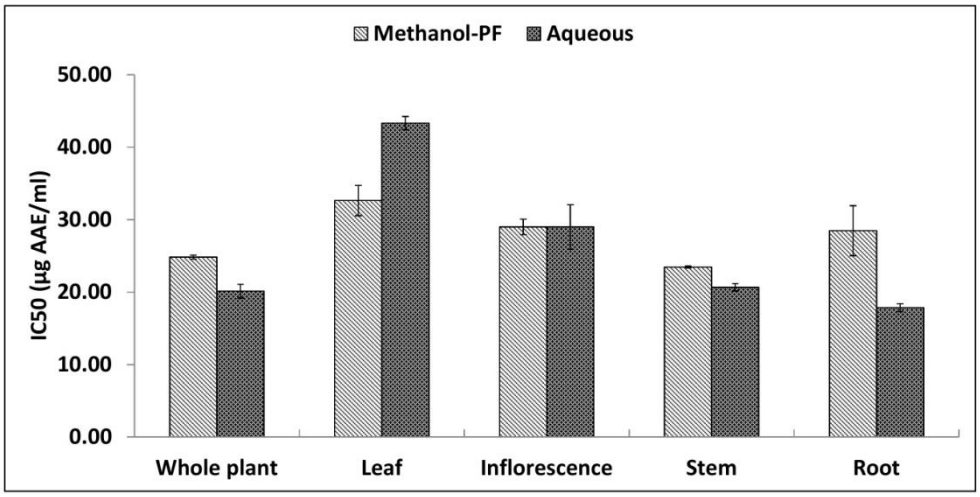
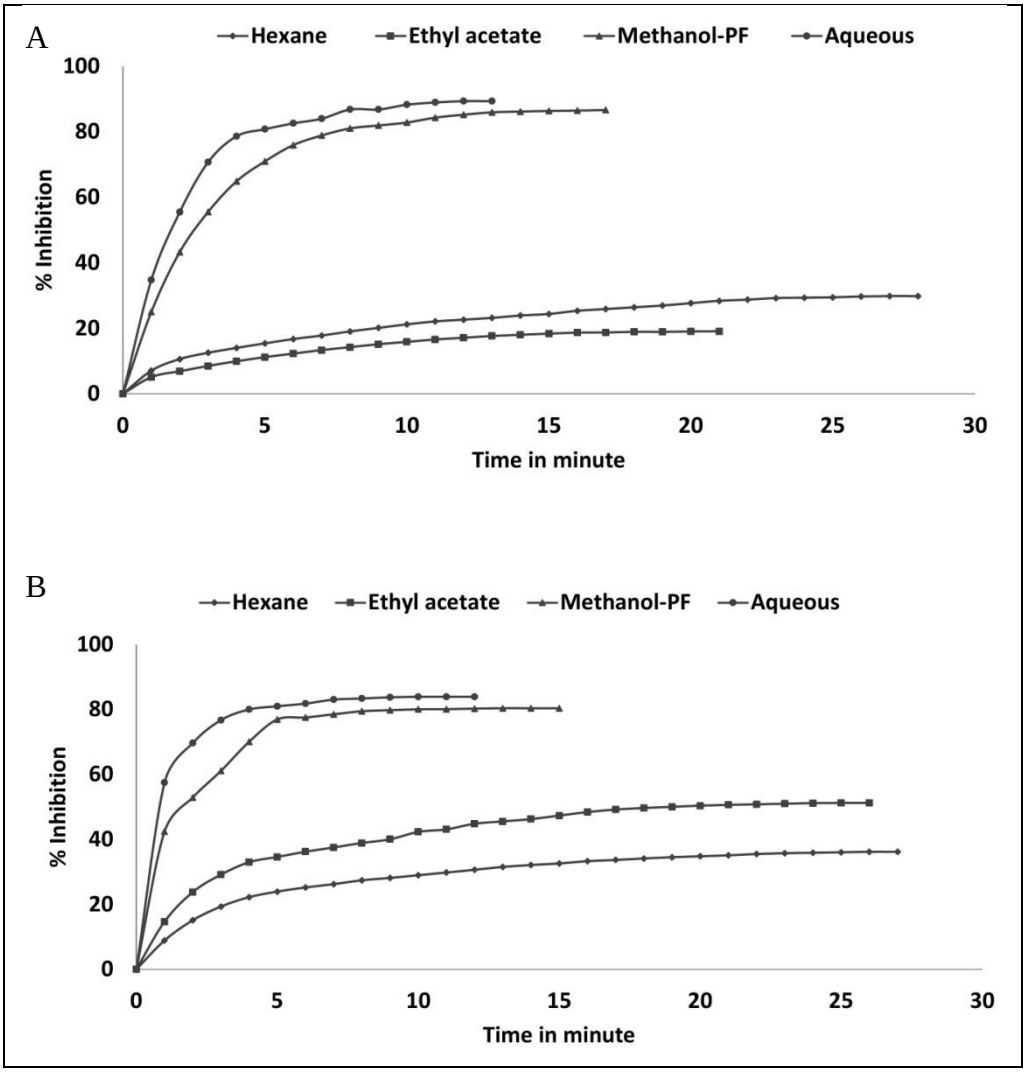


Figure - 3: DMPD radical inhibition by different parts and their extracts in *Hygrophila spinosa*.



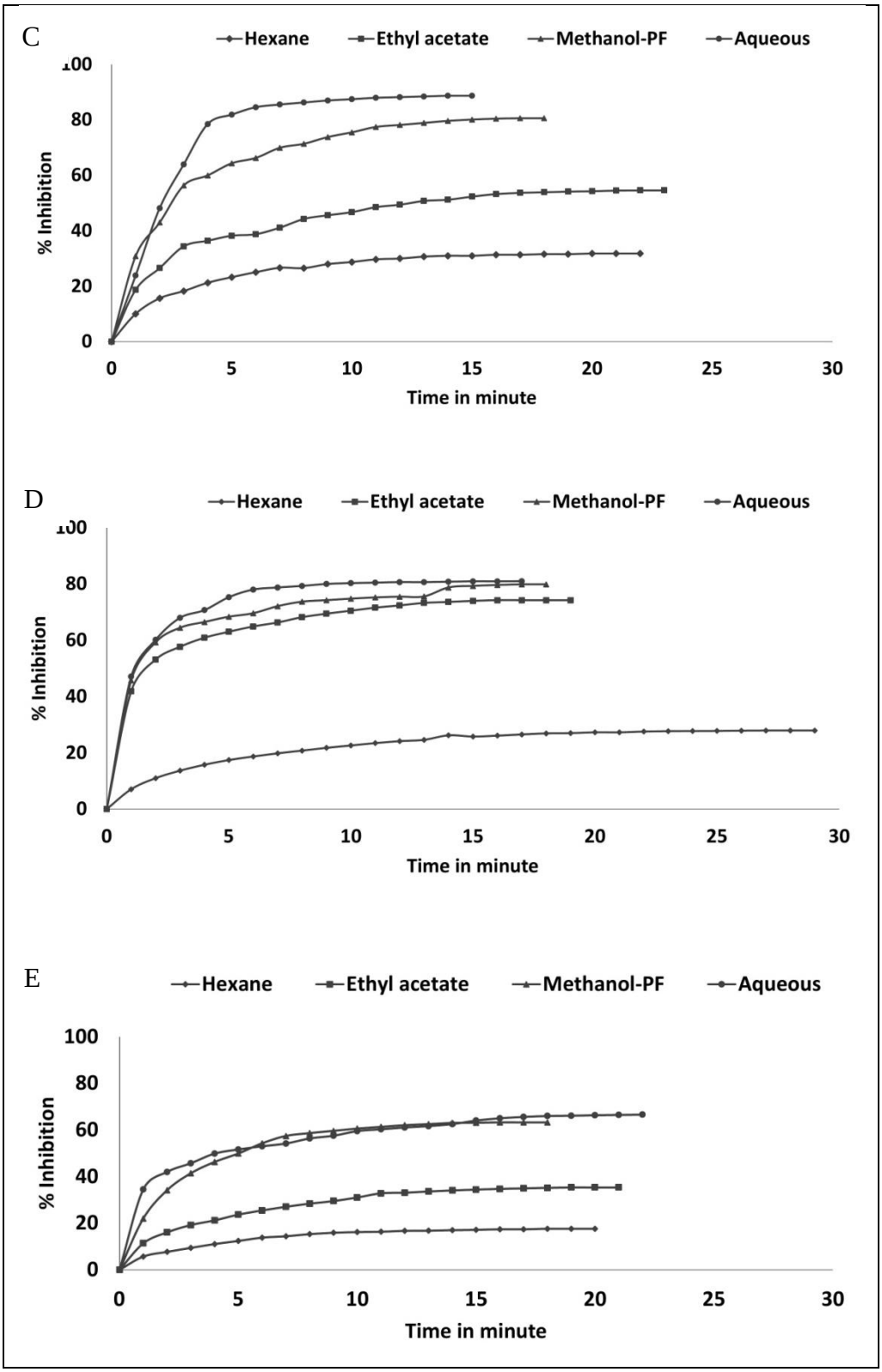


Figure - 4: Antioxidant activity of (**A**) whole plant, (**B**) leaf, (**C**) inflorescence, (**D**) stem and (**E**) root extract of *Hygrophila spinosa* using DPPH assay over time and comparison between parts and extracts.

Figure 5 describes the IC50 value for DPPH scavenging capacity. The whole-plant aqueous extract was shown to be more powerful (38.12±0.02 g/ ml) than other extracts.

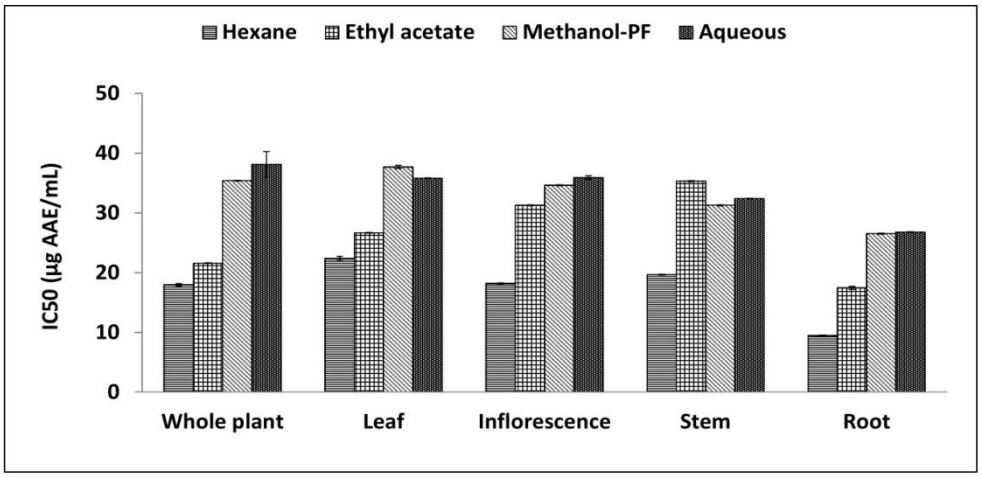


Figure - 5: DPPH radical inhibition by different parts and their extracts in *Hygrophila spinosa*.

So, the whole plant aqueous extract of *H. spinosa* suppressed the radical more effectively and in less time. The findings support the DPPH scavenging and lowering power activity of the *Allium sativum* bulb, in which the aqueous extract outperformed the methanol extract (Meriga et al., 2012). The diverse ways of scavenging, the chemical structure of antioxidants, the amount of active groups, and the hydro/lipophilicity of the secondary metabolite based antioxidants present in them all contribute to the different activity behavior of plant parts and extracts (Bruni et al., 2004).

In this plant extracts, the antioxidant reaction reached a steady-state against both radicals (DMPD and DPPH), resulting in a plateau condition. Mishra et al. (2012) provided the following equation to explain the mechanism:

$$[R^{\cdot}] + [AH] \leftrightarrow R\text{-}H + [A^{\cdot}] \quad (1)$$

$$[R^{\cdot}][A^{\cdot}] \rightarrow R\text{-}A \quad (2)$$

$$[A^{\cdot}] + [A^{\cdot}] \rightarrow A\text{-}A \quad (3)$$

The equation explains how antioxidants transfer hydrogen ions to scavenge radicals at a faster rate (Eq. 1), which leads to antioxidants being converted into the radical form [A] (Eq. 2), which then reacts with available radicals to form a radical-antioxidant complex. There was a gradual reaction at this moment, leading to the steady-state condition. The remaining [A] interacted with themselves, and the reaction was eventually halted (Eq. 3). The hydrogen atom transfer (HAT) ability of antioxidant-based techniques is demonstrated in the DMPD and DPPH scavenging experiments. These are kinetic assays that use a competitive reaction scheme in which antioxidants and substrate compete for radicals, as shown in the equations above. Reducing power and the Folin-Ciocalteu assay are electron transfer (ET) based methods for measuring antioxidant reduction potential through colour changes. Chromogenic redox reagents are used in these tests (López-Alarcón and Denicola, 2013).

The antioxidant agents are categorized into two categories based on their hydrogen atom transfer (HAT) or electron transfer ability (ET). The current analysis discovered that all of the extracts have both mechanisms. HAT ability was best in *H. spinosa* whole plant and leaf aqueous extract, while ET ability was highest in *H. spinosa* aqueous extract. On the antioxidant activity of *H. spinosa*, similar results have been observed. 77.02 percent of the DPPH radical was scavenged by the hydroalcoholic extract of *H. auriculata*. The inhibition of DPPH was time-dependent and followed a linear trend (Vijayakumar et al., 2005). *Hygrophilla auriculata* was found to have antioxidant activity by Hussain et al. (2009). Aqueous, alcoholic, petroleum ether, chloroform, ethyl acetate, and n-butanol extracts yielded the terpenoid-rich fraction, which had free radical scavenging activity against DPPH.

Preliminary phytochemical analysis

The biological activities were used to screen phytochemicals (Table 2). Except for the aqueous extract, terpenoids were found in all of the extracts. In the methanol polar fraction and aqueous extracts of all the components, phenol, flavonoids, tannin, and cardiac glycosides were found, which were in agreement with other studies (Hussain & Kumaresan, 2013).

Quantitative estimation of phytochemicals

Table 3 shows the quantitative assessment of phytochemicals. There was a statistically significant difference between plant parts and extracts ($p < 0.05$). The floral methanol polar fraction had the most terpenoids (237.97±0.79 mg LE/ g of extract) while the root hexane extract had the least (10.47±0.06 mg LE/ g of extract).

The phenol concentration in the polar component of the whole plant methanol extract was 14.99±0.07 mg GAE/ g of extract. The stem had the highest concentration of phenols (18.60±0.08 mg GAE/ g extract), followed by the flower, leaf, and root. The aqueous extract of the complete plant, on the other hand, contained 17.76±0.08 mg GAE/ g of extract, with the leaf containing the most phenolics (21.34±0.03 mg GAE/ g of extract), and followed by the inflorescence, stem, and root.

The flavonoid content of the methanol polar part of the total plant was 102.01±1.33 QE/ g of extract. The flower has the highest flavonoid content (198.72±1.34 QE/ g of extract), followed by the stem, leaf, and root. The complete plant's aqueous extract contained 161.42±1.10 QE/ g of extract, with the inflorescence containing the most flavonoid (257.95±0.81 QE/ g of extract), followed by the stem, leaf, and root.

Cold extraction of this plant was shown to be ineffective in extracting the antibacterial active component; nonetheless, the extracts showed strong antioxidant activity. The secondary metabolite compositions of the plants are diverse. As a result, the structure of isolated chemicals influences their biological action. Structure-Activity Relationship is the term for this phenomenon (SAR). Phenols are a well-known antioxidant that can be separated with polar solvents like methanol and water. Most metabolites, from lipophilic to hydrophilic, can be extracted using methanol (Saravanan and Parimelazhagan, 2014).

4. CONCLUSION

The present study revealed that *H. spinosa* extracts from various sections had little antibacterial action but did have antioxidant activity. The aqueous extract of the inflorescence displayed the highest reducing power capacity when antioxidant properties of the plant were evaluated. Methanol and aqueous inflorescence extracts had the best inhibition capacity against the DMPD radical. The whole plant aqueous extract inhibited the DPPH radical more effectively and in less time. The phytochemical research indicated a wide range of secondary metabolites in the plant. Terpenoids were found in the highest concentration in the ethyl acetate extract of the inflorescence, phenol in the aqueous extract of the leaf, and flavonoid in the inflorescence aqueous extract of *H. spinosa*. As a result, these portions and their extracts may be a source of antioxidants. Therefore, further research is needed for the isolation and identification of individual bioactive compound(s) from these parts and their extracts.

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Table - 2: Phytochemical analysis of the different parts of *Hygrophila spinosa* extracted in various solvents.

Phytochemical	Whole plant				Leaf				Inflorescence				Stem				Root			
	H	EA	Met-PF	Aq	H	EA	Met-PF	Aq	H	EA	Met-PF	Aq	H	EA	Met-PF	Aq	H	EA	Met-PF	Aq
Alkaloid																				
Mayer's test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hager's test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wagner's test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tannic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Marquis test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenol																				
Ferric chloride test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	+	+	+
Lead acetate test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Gelatin test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids																				
Alkaline reagent test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Lead acetate test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Terpenoids																				
Salkowski test	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
Tannin																				
Ferric chloride test	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Gelatin test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycoside																				
Killer killiyani test	+	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-
Salkowski test	+	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-

Table - 3: Total terpenoid, total phenol and total flavonoids in different parts and extracts of *Hygrophila spinosa*.

Parts	Extract	Total terpenoids (mg LE/ 1g dry weight)	Total phenol (mg GAE/ 1g of dry weight)	Total flavonoid (mg QE/ 1g of dry weight)
Whole plant	Hexane	60.16 ± 2.73^{n}	0.00	0.00
	Ethyl acetate	115.68 ± 3.22^{i}	0.00	0.00
	Methanol-PF	136.30 ± 1.23^{g}	$14.99\pm0.07^{t,u,v}$	102.01 ± 1.33^{k}
	Aqueous	0.00	$17.76\pm0.08^{r,s,t,u}$	$161.42\pm1.10^{d,e}$
Leaf	Hexane	128.51 ± 0.90^{h}	0.00	0.00
	Ethylacetate	147.16 ± 1.18^{f}	0.00	0.00
	Methanol-PF	158.40 ± 1.54^{e}	$13.64\pm0.03^{u.vw}$	55.16 ± 1.40^{o}
	Aqueous	0.00	21.34 ± 0.03^{r}	114.74 ± 1.10^{i}
Inflorescence	Hexane	74.77 ± 0.59^{m}	0.00	0.00
	Ethyl acetate	237.97 ± 0.79^{b}	0.00	0.00
	Methanol-PF	143.07 ± 2.38^{f}	$15.56\pm0.13^{t,u,v}$	198.72 ± 1.34^{c}
	Aqueous	0.00	$18.49\pm0.06^{r,s,t}$	257.95 ± 0.81^{a}
Stem	Hexane	$19.94\pm1.25^{r,s}$	0.00	0.00
	Ethyl acetate	37.92 ± 0.79^{p}	0.00	0.00
	Methanol-PF	163.38 ± 1.37^{d}	$18.60\pm0.08^{r,s,t}$	97.59 ± 0.53^{l}
	Aqueous	0.00	$17.00\pm0.08^{s,t,u}$	157.17 ± 0.81^{e}
Root	Hexane	10.47 ± 0.06^{w}	0.00	0.00
	Ethyl acetate	29.10 ± 0.70^{q}	0.00	0.00
	Methanol-PF	74.03 ± 5.41^{m}	$12.26\pm0.04^{v,w}$	52.16 ± 0.81^{o}
	Aqueous	0.00	$15.18\pm0.06^{t,u,v}$	109.61 ± 1.10^{j}

The results are Mean ± SE (n=3). The different superscript letters (column wise) denote the significance at $p < 0.05$ (Tukey's test). Methanol-PF: Polar fraction of methanol extract.

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