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Original Article

Phytochemical, Antioxidant and Trace Element Analysis of *Hypericum japonicum* Thunb.

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ABSTRACT

Background: Plants possess various bioactive compounds with many biological activities. Antioxidant plays vital roles in initiating deleterious free scavenging radical reactions. **Objectives:** The present study was done to determine the presence of phytochemical properties of the plant along with its antioxidant analysis of four solvent fractions (hexane, diethyl ether, ethyl acetate and methanol) from *Hypericum japonicum*. Furthermore, five heavy metals- Zn, Cu, Cd, Pb and Cr were analyzed. **Materials and Methods:** All the experiments for phytochemical and antioxidants of different solvent extracts were investigated following standard protocols. The heavy metal content was analyzed using spectroscopy method. **Results:** Qualitative analysis revealed that almost all the phytochemicals were present on one or other solvents. The carbohydrate and protein content was highest in hexane and ethyl acetate extract. Ethyl acetate showed highest phenol content while diethyl ether showed highest in flavonoid content. Similarly, ethyl acetate extract showed strongest antioxidant activity. Heavy metals were found in very negligible amounts. **Conclusion:** The present study suggests that *H. japonicum* might have the potential to be a good source of phytochemicals and antioxidants. Further studies are required to analyze the pharmacological properties of the plant.

Keywords: Antioxidant, Heavy metal, *Hypericum japonicum*, Medicinal plants, Phytochemical.

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INTRODUCTION

Plants are a good source of medicine and have been used in well-established medicinal form since primitive days. Medicinal plants are used worldwide to treat different diseases.[1] Plants possess numerous bioactive compounds having several biological activities such as anthelmintic, antifungal, anti-inflammatory, antiviral, antioxidant, antimicrobial, etc.[2-6] Today, an estimated 3,91,000 species of vascular plants are known to science, of which about 3,69,000 are flowering plants, and at least 31,128 plant species have a documented use.[7] Alkaloids, terpenoids, tannins, saponins and phenolic compounds are the most promising bioactive compounds in plants. These chemical compounds are called secondary metabolites. They work as a medicine against various conditions like diseases and stress.[8] The various phytochemicals are known to exhibit physiological activity along with medicinal properties.[9] Antioxidant has the property which can act against toxic as well as molecules which can cause damage to living organism. These compounds are generated during the different metabolic reactions in the body.[10,11] The phenolic and flavonoid compounds are known as sources of antioxidants and can clear reactive oxygen species (ROS).[12]

Heavy metals are one of the major environmental contaminants. Plants exposed to high metal concentrations illustrate down-regulated growth and development.[13] Soil contamination due to heavy metals can occur naturally or through various artificial activities like agriculture, burning fossil fuel, mining, etc. The plants are highly affected due to heavy metals present in the soil, which can cause various complications like oxidative stress resulting in damage to RNA and DNA, degradation of several proteins, and inhibits the functions of several enzymes.[14] Mostly in developing and developed countries, human activities have caused the pollution of water, air, and soil with toxic heavy metals. Therefore, these polluted environment results in declining the phytochemical content of the secondary metabolites of the medicinal plants.[15] The food quality of the plants is also affected by the presence of heavy metals in the soil.[16]

Assam is a place which is among the eight North-eastern states of India and has been known for its richness in vegetation as well as wildlife. Many studies have documented several plants for their phytochemical, antioxidant and medicinal properties.[17-19] *Hypericum japonicum* Thunb. of the family Hypericaceae is an important plant of western Assam known for its medicinal value, commonly used to treat helminth infections by

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local people.[17] *H. japonicum* is an annual herb growing 5-35 cm in height and having small diffused branching. The plant is mainly distributed throughout the South East Asian countries, including India (India Biodiversity Portal https://indiabiodiversity.org/species/show/249777). With a rich source of phytochemicals, the plant has been reported to contain high antioxidant properties.[20] It has also been experimentally reported by some of the authors to contain anti-tumour, antiproliferative, anthelmintic, antibacterial, and antiviral activities.[17,21-23] The current study analyzed the phytocompounds and free radical scavenging activities from different solvents (hexane, diethyl ether, ethyl acetate and methanol) extracts of *Hypericum japonicum* and trace element content of the plant.

MATERIALS AND METHODS

Collection and identification of plant

The collection area for the sample plant was from Kokrajhar district, Assam. The taxonomic identification of the plant was made by the Department of Botany, Bodoland University, where herbarium sheet was supplied for identification purpose. The plant was identified as *Hypericum japonicum* Thunb. (Identification no. BUBH2000129).

Preparation of plant crude extract and solvent fractions

The plant was collected from Kokrajhar locality with the help of native people. The method used by Seidel,[24] was followed for plant crude extract preparation. Firstly, the leaves were washed thoroughly with distilled water and then were kept for drying at 45±2°C in a hot-air oven. After proper drying, with the help of a mixer grinder, the leaves were powdered. The powdered form of the plant was soaked with 80% methanol (1:5, w/v) and kept for three days. After 72 hr, the solvents were filtrated with Whatman filter paper no. 1. The fluid obtained after filtrate was dried with the help of a rotary evaporator. The remaining solid part after evaporation was collected as methanolic crude extract and kept at -20°C. Next, solvent fractionization of the crude extract of the plant was done following the method of liquid-liquid partitioning. The solvents taken were n-hexane (hex), diethyl ether (DE), and ethyl acetate (EA) based on their polarity. The solvents were then again dried in a rotary evaporator, and the remaining solid part after evaporation was collected and stored at -20°C for further study.

Qualitative phytochemical analysis

For qualitative phytochemical screening, methods of Trease and Evans,[25] and Sofowara,[26] were followed.

Alkaloids test

Dragondroff's test

In this method, 1 ml extract of the plant was taken where 0.5 ml of dragondroff's reagent was put in the test tube. The presence of alkaloids can be confirmed with the appearance of a reddish-brown colour.

Tannins test

Ferric chloride test

In this method, 1 ml extract of the plant was taken where 2 ml 5% ferric chloride was put in the test tube. The presence of tannins can be confirmed with the appearance of dark blue or greenish colour.

Phenols test

In this method, 1 ml extract of the plant was taken. Firstly, 2 ml of distilled water was added to the solution and then 0.5 ml of ferric chloride was put in the test tube. The presence of phenols can be confirmed with bluish or green colour.

Quinone test

In this method, 1 ml extract of the plant was taken where 1 ml H_2SO_4 was put in the test tube. The presence of quinones can be confirmed with the appearance of red colour.

Terpenoids test

In this method, 0.5 ml extract of the plant was taken where 2 ml chloroform and 1 ml conc. sulphuric acid was put in the test tube. The presence of terpenoids can be confirmed with the appearance red-brown colour at the interface.

Flavonoids test

In this method, 2 ml extract of the plant was taken where 1 ml of 2N sodium hydroxide was put in the test tube. The presence of flavonoids can be confirmed with the appearance of yellow colour.

Coumarins test

In this method, 1 ml extract of the plant was taken where firstly 2 ml distilled water was added and then 0.5 ml ferric chloride was put in the test tube. The presence of coumarins can be confirmed with the appearance of bluish or green colour.

Anthracyanine test

In this method, 1 ml extract of the plant was taken where 1 ml of 2N sodium hydroxide was put and heated for 5 min. The presence of anthracyanine can be confirmed with the appearance of bluish-green colour.

Glycosides test

In this method, 2 ml extract of the plant was taken where firstly 2 ml chloroform was added and then 10% ammonia solution was put in the test tube. The presence of glycosides can be confirmed with the appearance of pink colour at the interface.

Anthraquinones test

In this method, 1 ml extract of the plant was taken where 1 ml of ammonia solution was put in the test tube. The presence of anthraquinones can be confirmed with the appearance of pink colour.

Steroids test

Salkowski's test

In this method, 1 ml extract of the plant was taken where 0.5 ml concentrated sulphuric acid was put in the test tube. The presence of steroids can be confirmed with the appearance of red colour at the lower level, and the presence of triterpenoids can be confirmed with the development of yellow colour.

Carbohydrates test

In this method, 1 ml extract of the plant was taken where firstly, Molisch's reagent was added and then 0.5 ml concentrated sulphuric acid was put in the test tube. The presence of carbohydrates can be confirmed with the appearance of a purple or reddish colour.

Saponins test

In this method, 0.5 ml extract of the plant was taken where 0.5 ml of olive oil was put in, and shaken vigorously for some time. The presence of saponins can be confirmed by forming a fairly stable emulsion.

Protein test (Ninhydrin)

In this method, 2 ml extract of the plant was taken where 0.5 ml of ninhydrin reagent was put in and heated for five minutes. The presence of amino acids can be confirmed with the appearance of blue colour.

Phlobatannins test

In this method, 1 ml extract of the plant was taken where 0.5 ml of 2% HCl was put in the test tube. The presence of phlobatannins can be confirmed with the appearance of a red precipitate.

Quantitative phytochemical analysis

Carbohydrate (Glucose) Assay

The carbohydrate contents for the different solvent extracts of the plant, Anthrone method,[27] was followed.

Protein Assay

By following the Lowry method,[28] the protein content of all the plant solvent extracts was estimated.

Total Phenolic Content

By following the method of FolinCiocalteu reagent test, the total phenolic content of different solvent of the plant was estimated.[29] The total phenolic content (TPC) was calculated from a gallic acid calibration curve. The results were expressed as microgram (µg) gallic acid equivalent (GAE)/milligram plant extract.

Total Flavonoid Content

By following Ordonez *et al.*[30] the total flavonoid content was estimated. The calculation of the total flavonoid content (TFC) was done from the standard curve of quercetin (1–25 µg/mL), and the values obtained were shown as microgram quercetin equivalent (QE)/milligram of plant extract.

Antioxidant study

Total antioxidant capacity (TAC) assay

Phosphomolybdate method,[31] was followed to carry out the total antioxidant capacity assay. Firstly, 1 mL (5 mg/mL) extract of the plant was taken, and distilled water was added, and then 1 mL of reagent solution (600 mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added. For 30 min the solution mixture was incubated at 95°C. The absorbance to be taken for this assay is 645 nm which is measured against a blank solution. The expression of the TAC was done as µg ascorbic acid equivalent (AAE)/mg plant extract.

Ferric reducing antioxidant power assay (FRAP)

The procedure of Benzie and Strain was followed for FRAP assay estimation as described by Iloki Assanga *et al.*[32] Firstly, 1 mL (5 - 100 µg/mL) of different solvent extracts of the plant was taken, and 2 mL of FRAP reagent (10 mL of acetate buffer (pH 3.6), 1 mL of 10 mM TPTZ solution in 40 mM HCl and 1 mL of 20 mM iron (III) chloride solution) was mixed. The solution mixture was then incubated for 30 min at 50°C. The standard taken was ascorbic acid. The absorbance was measured at 593 nm using a spectrophotometer.

1,1 Diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH)

By following Mamta *et al.*[33] the DPPH scavenging activity was estimated. Firstly, 2 mL of DPPH (0.135 mM) was prepared in methanol and was added to 1 mL of ascorbic acid and different solvent extracts (100-1000 µg/mL) of the plant. The incubation of the mixture solution was done for 30 min at 37°C. The absorbance was measured at 517 nm.

Table 1: Qualitative Analysis of Different Solvent Extracts of *Hypericum japonicum*.

Test	Hex	DE	EA	Met
Alkaloids	+	++	+++	+
Tannins	-	+++	+++	++
Phenol	-	+++	+++	++
Quinones	-	+	++	+++
Terpenoids	+	++	++	++
Flavonoids	+	+++	++	++
Coumarins	++	+++	+++	+++
Anthracyanine	-	-	-	-
Glycosides	-	-	-	-
Anthraquinones	-	+	+	+
Steriods – Steroids	-	++	+	+++
Triterpenoids	+	-	-	-
Carbohydrates	+	++	++	++
Saponins	+	+	+	+++
Proteins	+	+	+	+++
Phlobatannins	_	_	+	+

'–' indicates absence; '+' indicates presence; Hex- Hexane; DE- Diethyl ether; EA- Ethyl acetate; Met- Methanol

The calculation for the DPPH assay was done following:

$$\text{DPPH inhibition (\%)} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100 \quad \text{.....(1)}$$

Here, Abs control = assay mixture excluding sample/standard

And Abs sample = assay mixture including sample/standard

2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) Assay

Following the method of Re *et al.* [34] the ABTS activity was estimated. Here, in this method, stock solutions of ABTS (7 mM) and potassium persulphate (2.5 mM) were added and kept for 12–16 hr in the dark at room temperature to generate the free radical. Then the dilution of ABTS solution is required, which is done with methanol (60%). The working solution of ABTS has adjusted at 0.70 ± 0.02 at an absorbance of 734 nm. Next, 1 mL (5 - 100 µg/mL) of different solvent extracts of the plant was taken, and 2 ml of the working ABTS solution was mixed. For the standard of ABTS, gallic acid was taken. The absorbance was measured at 734 nm. Using formula (1), the inhibition of the assay was calculated.

Lipid peroxidation inhibition activity assay (Thiobarbituric acid reactive species assay, TBARS)

By following the method of thiobarbituric acid reactive species (TBARS) assay with little modification, the lipid peroxidation inhibitory activity was estimated.[35] The lipid peroxide formation was measured using egg yolk homogenates as lipid-rich media. 1 mL (0.05-1.0 mg/mL) of different solvent extracts of the plant was taken where 0.1 mL of egg homogenate (10%v/v) was added along with it 0.05 mL of 75 mM $FeSO_4$ was mixed. Incubation was done at 37°C for 30 min. Then, 1 mL of 10% TCA and 1 mL of 0.8% (w/v) TBA in 1.1% SDS was mixed. The solution mixture was then vortexed, and heating was required for one hour at 95°C. The mixture was then allowed to cool down, and then it was centrifuged for 10 min at 3,000 rpm. After centrifugation, only the organic upper layer was taken and the absorbance was measured at 532 nm. Using formula (1) the inhibition of the assay was calculated.

Trace elements

For trace elements, study for heavy metals – chromium (Cr), lead (Pb), copper (Cu), zinc (Zn) and cadmium (Cd) was done using AAS. Raw plant powder was sent to NEHU SAIF to detect the heavy metals in *H. japoniucm.*

By following the method reported by Welz and Sperling,[36] the heavy metal analysis was done. Firstly, digestion of 1 gram of plant power was done by using concentrated HNO_3:HCl (3:1 ratio) for three hours at 85°C. Then, 1 ml of concentrated $HClO_4$ was added. Filtration of the solution was done, and it was diluted with distilled water to 50 ml. For all of the absorption measurements of metal contents of the plant, an Analytik Jena AAS Vario-6 Graphite furnace spectrometer furnished with a PC-controlled 6-piece lamp turret and argon gas supply was used.

Statistical analysis

Microsoft Excel was used for all the statistical calculations. SPSS software was used for IC_{50} value calculation. The experiments were all done in triplicates. The final result was shown as mean ± standard deviation (SD).

RESULTS

Extract preparation of the plant

The moisture content percentage of the plant was found as 84.09 ± 8.99 g/100g wet weight, and the crude extract percentage was found to be 18.43 ± 2.33 g/100g dry powder.

Qualitative phytochemical analysis

Qualitative phytochemical analysis was done for different plant solvent extracts and is represented in Table 1. A total of 15 phytochemicals were studied, out of which anthracyanine and glycosides were absent in all the extracts. It was found that most phytochemicals were absent in hex extracts except for alkaloids, flavonoids, coumarins, triterpenoids, carbohydrates, and saponins were present in very few amounts. In DE extract tannins, flavonoids, phenols and coumarins were found in high amounts, whereas anthracyanine, glycosides, triterpenoids and phlorotannins were found in very low amounts. Whereas in EA extract, alkaloids, tannins, phenol and coumarins were found in very high amounts. Furthermore, methanolic extract, quinones, steroids, saponins and proteins were found in very high amounts.

Quantitative phytochemical analysis

The solvent fractions of the plant have been studied for their phytochemical content. Figure 1 shows the protein, carbohydrate, TPC, and TFC of different plant solvent extracts. EA has shown the highest protein content with 147.47±1.96 µg/mg and TPC with 242.96±6.25 µg/mg of the four solvents. Whereas hex has shown the highest carbohydrate content with 205.93±5.07 µg/mg, and DE has the highest TFC with 140.02±3.75 µg/mg. Meanwhile, the lowest protein content and TPC has shown by hex with 52.33 ± 2.67 µg/mg and 68.82±4.91 µg/mg, respectively, and the lowest carbohydrate content with 37.35 ± 5.97 µg/mg and TFC 46.11±2.27 µg/mg were shown by methanol compared to all fractions solvent extracts.

Antioxidant study

The antioxidant activity of the solvent fractions has been studied. Figure 2 shows the TAC and FRAP activities of the plant. Of the four solvents, DE fraction showed the strongest TAC with 154.65±1.18 µg/mg, and the strongest FRAP activity was shown by the EA fraction with 173.79±6.40 µg/mg activity. In contrast, hex has shown the weakest activity for both TAC (115.68±3.88 µg/mg) and FRAP (7.32±0.04 µg/mg), respectively.

Regarding DPPH activity, the EA fraction of *H. japonicum* showed the strongest activity, followed by methanol, DE and hex extract of the plant. Figure 3 showed the dose-dependent scavenging of DPPH free radicals with IC_{50} values 33.29±0.38 µg/ml, 59.77±0.30 µg/ml, 78.95±2.82 µg/ml and 203.19±1.50 µg/ml for EA, methanol, DE and hex fractions of the plant, respectively. The ABTS free radical scavenging activity study also showed the highest activity in the ethyl acetate fraction of *H. japonicum* (Figure 4). The IC_{50} concentration of ABTS assay were 14.36±0.75, 17.83±0.47, 25.94±1.56, and 45.16±0.86 µg/ml for EA, DE, methanol and hex extract, respectively. A similar result is observed in TBARS antioxidant assay. Inhibition of lipid peroxidation was observed highest in EA with 7.68±0.12 µg/ml and lowest in hex fraction with 267.13±5.27 µg/ml of *H. japonicum*. The lipid peroxidation inhibition property of *H. japonicum* with the increase of plant extract is shown in Figure 5.

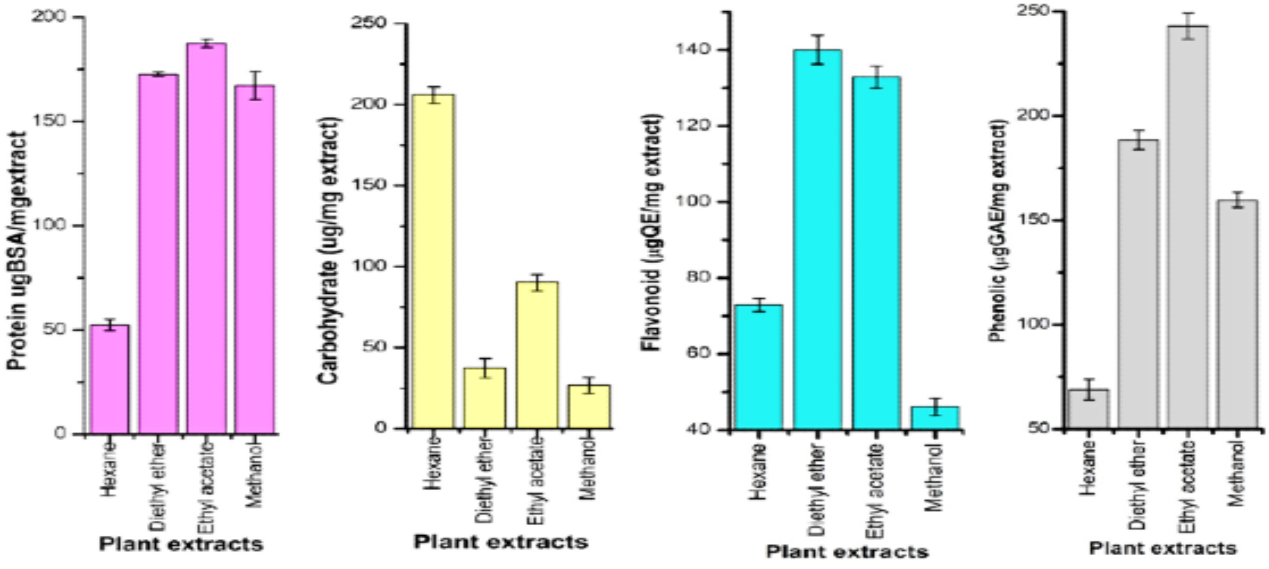

Figure 1: Protein, Carbohydrate, TPC, TFC of different solvent fractions of *Hypericum japonicum*. Values are expressed as mean ± SD, *n*=3.

Trace elements and heavy metal analysis

Five heavy metals- Zinc (Zn) and Copper (Cu) are two essential elements, and Cadmium (Cd), Lead (Pb) and Chromium (Cr) are three toxic elements, were analyzed for *Hypericum japonicum*. These metals were analyzed because heavy metals in excess can cause toxicity to the plant, harmful to humans if consumed. The concentrations of the different metals are shown in Table 2. According to our study, it was found that all the concentrations of the metals are in negligible amounts. The highest concentration was shown in Zn, with 1.430 ppm in concentration. According to WHO,[37] limitations for plant herbal medicines for different heavy metals are Zn-100 ppm, Cu-150 ppm, Cd-0.3 ppm, Pb-10 ppm and Cr-2 ppm. Therefore, *H. japonicum* can be consumed for medicinal purposes since it was found that toxic metals are very much negligible.

DISCUSSION

The plant-derived phytochemicals or secondary metabolites are a group of compounds with very high biological activity which provides several benefits related to health in various ways, like co-factors for enzyme activity, enzyme stimulator or inhibitors, scavengers of toxic or reactive chemicals, substrates for biochemical reactions, etc.[38] Free radicals can cause several diseases like cancer, neurodegenerative diseases, AIDS etc. Antioxidants are useful for managing such kinds of diseases.[39] The present study of phytochemical analysis conducted on the plants of four different solvent extracts, namely hexane, diethyl ether, ethyl acetate and methanol, revealed 13 phytochemicals out of 15 studied in one solvent extract or other. The phytochemicals such as alkaloids, terpenoids, flavonoids, coumarins, steroids, carbohydrates and proteins were present in all the solvents, whereas anthracyanine and glycosides were completely absent. The result showed different types of results in different solvents. Similarly, a study done on *Taraxacum officinale* confirmed the presence of several phytocompounds like tannins, alkaloids, flavonoids, terpenoids, saponins, glycosides and phenols and with a different result in different solvents studied.[40] In another study, it was found that out of twelve phytochemicals studied, eleven were found in one of the solvents.[41]

The phenols are strong antioxidants which act as structure-dependent. They can act against reactive oxygen species (ROS), that helps initiating harmful free radical reactions. Flavonoids are one of the most crucial natural phenols found,

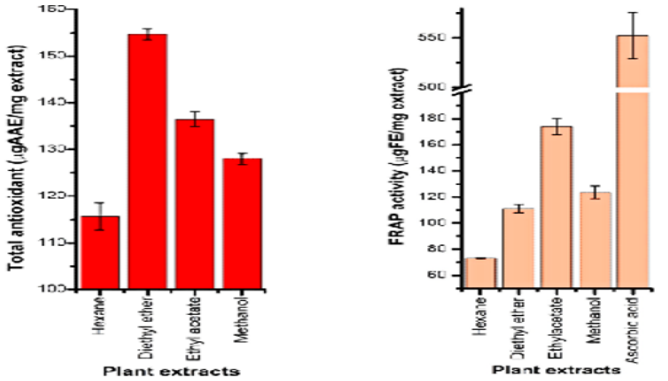

Figure 2: Total antioxidant capacity (TAC) and ferric reducing (FRAP) activity of different solvent fractions. Values are expressed as mean ± SD, *n*=3.

Table 2: **Trace Element Composition of *Hypericum japonicum*.**

Heavy metals	Concentration (in ppm)
Chromium (Cr)	0.436
Copper (Cu)	0.182
Zinc (Zn)	1.430
Cadmium (Cd)	0.010
Lead (Pb)	0.081

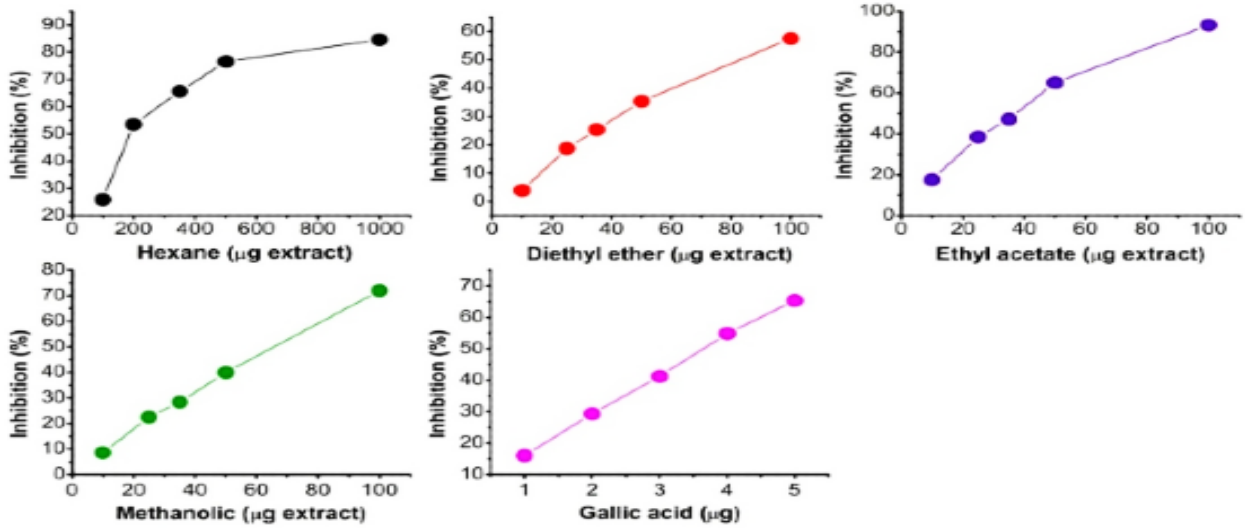

Figure 3: DPPH scavenging activity of different solvent fractions of *Hypericum japonicum*. Values are expressed as mean ± SD, *n*=3.

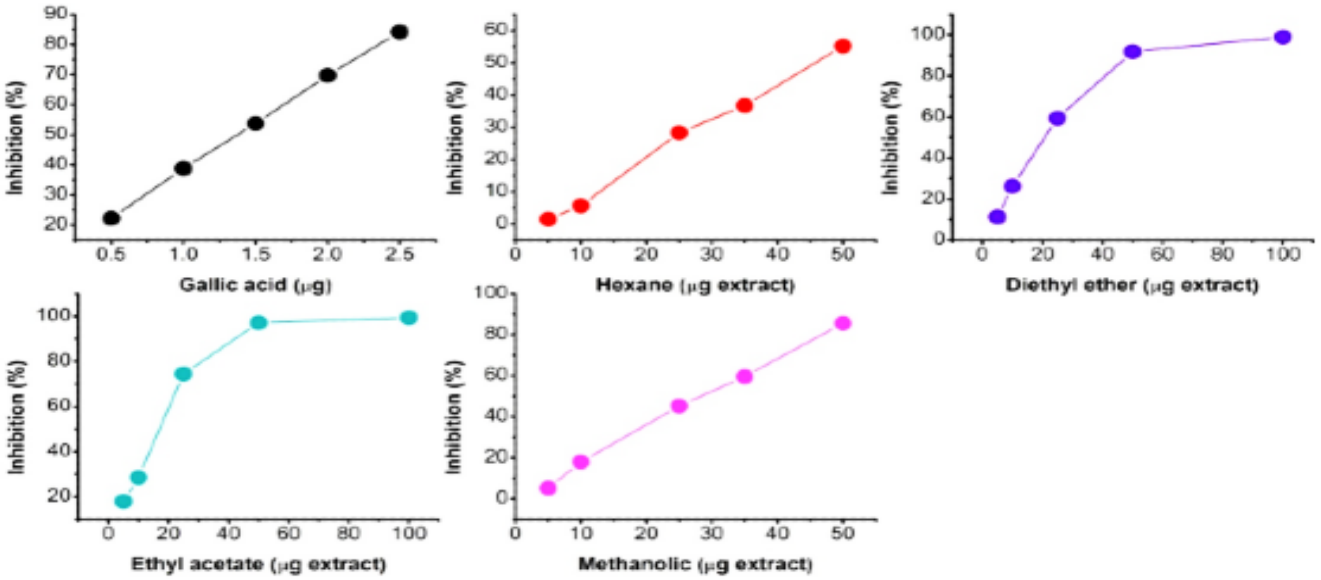

Figure 4: ABTS activity of different solvent fractions of *Hypericum japonicum*. Values are expressed as mean ± SD, *n*=3.

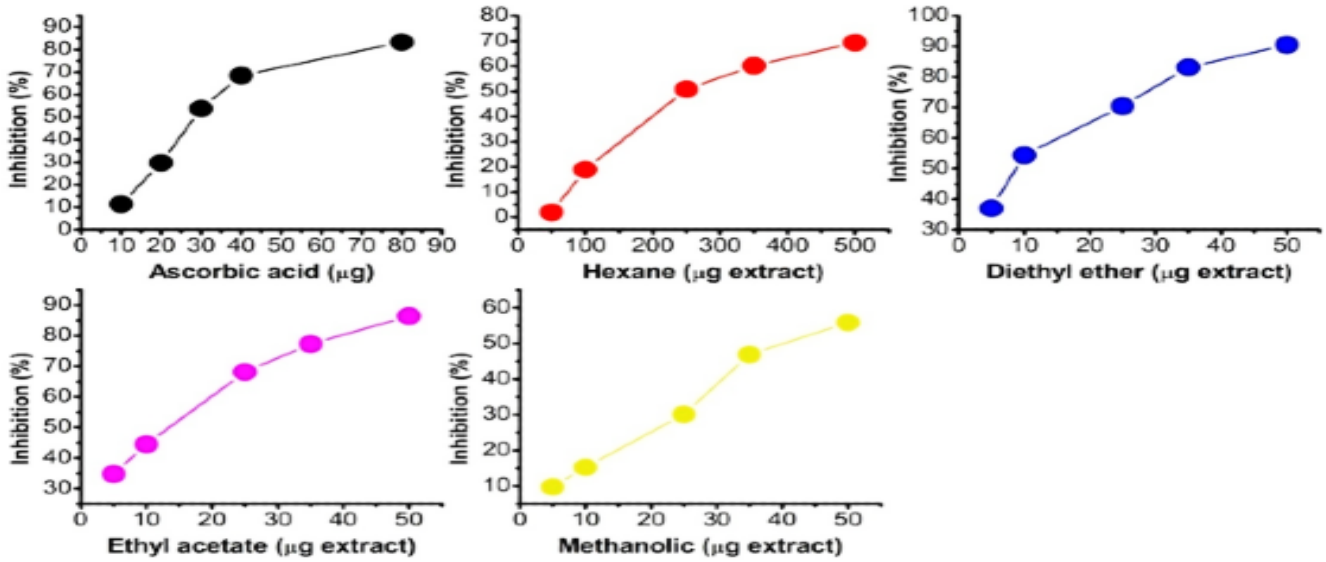

Figure 5: TBARS activity of different solvent fractions of *Hypericum japonicum*. Values are expressed as mean ± SD, n=3.

which have highly diverse and widespread natural compounds. The flavonoid has very high antioxidant activities containing multiple hydroxyl groups.[42] The total phenolic content was highest in hexane, ranging from 68.82±4.91 µg/mg to 242.96±6.25 µg/mg, and total flavonoid content ranged from 46.11±2.27 µg/mg to 140.02±3.75 µg/mg.

In comparison with our study in another study, phenols and flavonoids, which were present in *Kirkia wilmsii* tubers, were found to be slightly higher than our result. They found that the TPC found were at a range of 45.32 mg GAE/g to 122.84 mg GAE/g, and TFC found from 206.26 to 917.02 mg QE/g.[43] The ethyl acetate fraction has shown the strongest FRAP activity in the present study, whereas diethyl ether has shown the strongest for TAC, and hexane has shown the weakest for the FRAP and TAC. In our earlier study done in two plants *Clerodendrum infortunatum* and *Citrus grandis*, a similar result was shown for the FRAP, with ethyl acetate showing the strongest and hexane showing the weakest for both the plants. In contrast, in the case of TAC, *Citrus grandis* has shown the strongest activity in diethyl ether, similar to the present study and hexane has shown the weakest activity in *Clerodendrum infortunatum*.[44] In another finding the FRAP and TAC content has found higher than the present result with FRAP content ranging from 1180±20 to 3400±28 µmol/g and TAC ranging from 650±20 to 2500±33 µM/g.[45] Ethyl acetate has shown potent activity for DPPH with 23.04±4.49 µg/ml and hexane with least activity 203.19±1.50 µg/ml. A similar result was found in *Dillenia suffruticosa* leaves with ethyl acetate showing the most potent activity 29.42±0.49 µg/ml and hexane showing the weakest with 2923.47±114.10 µg/

ml.[46] Many such type study has been done where good DPPH antioxidant activity has been shown in different solvents of the plants.[47,48] In our present study, ethyl acetate has also shown the most potent activity in the case of ABTS and TBARS activity and hexane the least potent antioxidant activity, same as the DPPH, showing that the plant has good antioxidant properties. Many other authors have revealed that the medicinal plants have good antioxidant activity, similar to our result.[10,49,50]

The efficiency of medicinal plants is highly determined by the presence of essential oils and secondary metabolites. However, if heavy metals like Pb, Cd, Zn, Ni and other impurities are found in the medicinal plant above the threshold concentrations can cause health problems.[51] Additionally, food quality as well as nutrition of minerals can get directly affected because of the presence of heavy metals.[37] The present work was done on five heavy metals; Zn, Cu, Cr, Cd and Pb. The study revealed that heavy metals found in *H. japonicum* are in a very negligible amount. Contrasting our result, we found that the heavy metal concentrations in medicinal plants of the Eastern Mediterranean Region of Turkey were at slightly higher levels collected from nearby industrial regions, mining and farming sites.[16] However, there are many studies done by different authors showing very similar results concluding the concentrations of heavy metals with significantly less concentrations in medicinal plants.[11,52-54]

CONCLUSION

The present study conveys that the plant *Hypericum japonicum* is rich in phytochemicals and antioxidant properties. From the phytochemical qualitative analysis, most phytochemicals were present except anthracyanine and glycosides in all the extracts. Ethyl acetate has shown the highest protein content and TPC of the four solvents. Whereas hexane has shown the highest carbohydrate content, diethyl ether has the highest flavonoid content. Ethyl acetate has the most potent antioxidant activity compared to other three solvent extracts. Thus, the plant extracts have shown good antioxidant properties. Heavy metals were found in very negligible amounts. The study, therefore, observed that the plant extract of *H. japonicum* could be a potential source of phytochemicals and antioxidants. However, further studies need to be carried out to explore the pharmacological properties of the plant.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

***H. japonicum*:** *Hypericum japonicum*; **hex- n:** hexane; **DE:** Diethyl ether; **EA:** Ethyl acetate, **ml:** milli litre; **µg:** micro gram; **GAE:** gallic acid equivalent; **TPC:** Total phenolic content; **TFC:** Total flavonoid content; **TAC:** Total antioxidant capacity assay; **FRAP:** Ferric reducing antioxidant power assay; **DPPH:** 1,1Diphenyl2picrylhydrazyl radical scavenging activity; **ABTS:** 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonate; **TBARS:** Thiobarbituric acid reactive species assay: **NEHU:** North Eastern Hill University; **SAIF:** Sophisticated Analytical Instrument Facility.

AUTHORS'S CONTRIBUTION

MKR has carried out the all the phytochemical and antioxidant analysis along with all the other experiments and manuscript drafting. AS has carried out the work design as well as writing of the manuscript.

SUMMARY

Plants are a good source of medicine which possess various bioactive compounds having many biological activities. *Hypericum japonicum* Thunb. is an important medicinal plant of western Assam. The heavy metals were also found in very negligible amount. The plant showed to be a good source of phytochemical and also showed high antioxidant activity.

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