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Preliminary phytochemical assessment and antioxidant activity of *Neptunia triquetra*

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Abstract
In the present study, preliminary phytochemical screening was carried out to the plant *Neptunia triquetra* which are commonly called as a yellow sensitive plant. This plant belongs to the *Fabaceae* family and *Mimosaceae* clade. The plant is native to India with wide distribution and well known medicinal properties. The various extracts of the leaves of the plant such as Hexane extract, Chloroform extract, Methanol extract and Aqueous extract (water) were subjected to preliminary phytochemical screening for qualitative and quantitative identification of diverse phytochemicals present. The extracts were also subjected to antioxidant activity using DPPH assay and FRAP assay. The results revealed that the *Neptunia triquetra* is the potential source for diverse phytochemicals. The Methanol crude extract exhibited potential high antioxidant activity when compared to other extracts. As phenols and flavonoids contribute to the major medicinal properties of the plant, these two phytochemicals were analyzed quantitatively.

Keywords: Preliminary phytochemical, assessment, *Neptunia triquetra*

1. Introduction
India is the land of biodiversity with abundant availability of medicinal plants which are best owed with traditional touch to heal the ill health. They have vast genetic diversity among the wide range of toplogy and climate [1]. Since the antique time medicinal plants made significant contribution to the primary health care of people as plants and plant products were used to treat humans and other animals for diverse diseases [2]. As per the World Health organization (WHO) report, 75-95% of the world population is mainly dependent on traditional therapies by using conventional medicine extracted from the plants as phytochemicals. Due to historic and traditional reasons the traditional medicine became primary approach to improve the health. Herbal medicines have been widely used in developing countries for the treatment and prevention of various diseases in the place where infectious disease are endemic and modern health facilities and services are insufficient [3]. Most interestingly, Natural products are extensively used in pharmaceutical research and drug developing sector which are isolated from the medicinal plants. Flavonoids, alkaloids, tannins, phenolic compounds *etc*., are some of the active constituents present in the plant extracts.
With increase in world population followed by intensification and indiscriminate usage of antibiotics for treatment to human and animals leads to the development of antibiotic resistance and multidrug resistance in microbes [4]. Apart from this, side effects of several synthetic drugs, high cost of medicines, *etc*., contributed to the deterioration of usage of synthetic drugs. Always there is a strong need for developing diverse constituents originated from natural products that are capable of curing ill health without any side effects. On the other hand, Instead of synthetic medicine herbal medicine is safer as plant extract phytochemicals targets the biochemical pathway. The rational design of novel drugs from traditional medicine indeed offers new prospects in modern healthcare.
Oxidative stress generated as part of metabolism is the major reason behind generation of free radicals. Free radicals as well as other reactive oxygen species (ROS) were identified as agents associated with human aging, illness such as asthma, atherosclerosis, Alzheimer's and Parkinson's diseases, diabetes, hypertension, heart disease, and causes cancer [5,6]. Free radicals were also produced by many cells as a part of protective mechanism. Neutrophils produce them to destroy pathogens [7]. As free radicals play an important role in the diseases scenario of an individual, a thorough understanding of the various physiologically significant free radicals is of paramount importance. The free radicals and ROS were scavenged and ameliorated by certain compounds which are commonly called as antioxidants. The literature

revealed that some of the selected herbal drugs are known to possess antioxidant activity either by superoxide, or hydroxyl, or DPPH radical scavenging and lipid peroxidation inhibition activities. There is no detailed study on free radical scavenging activity on each plant. Hence, a detailed study was carried out on Hexane, Chloroform, Methanol, Aqueous (water) extracts of *Neptunia triquetra* for scavenging activity of DPPH and FRAP radical.

Neptunia triquetra are small herb with yellow flowers. The plant is commonly called as "Yellow Sensitive Plant" whereas in telugu language called as "chinna nidra kanti". The plant has characteristic features of small tiny shrub or herb while the leaves are tiny, small and are sensitive to touch (Thigmonasty). Flowers are yellow and small. This sensitive plant belongs to the *Fabaceae* (Touch-me-not) family. The other synonyms include *Desmanthus triquetris*, *Desmanthus triquetrus*, *Mimosa triquetra*. Yellow Sensitive Plant grows on land, even though it is a cousin of the sensitive water plant. The plant has potential medicinal properties and a few of them include treatment to the Jaundice. The entire plant is edible and used for medicinal purposes. For instance, flowers were used to treat eye diseases [8]. As per the available research reports on the *Neptunia triquetra*, it is revealed that the plant is less explored scientifically. Hence, in the present study, it is contemplated to screen the small herb *Neptunia triquetra*, for qualitative and quantitative preliminary phytochemical screening followed by assessing its potential antimicrobial and anti-oxidant activities.

The image of plant along with its flowers and classification was represented in figure-1.

Fig 1: Figure corresponds to the images of plant *Neptunia triquetra*. Image (a) corresponds to the plant with flower Image (b) corresponds to the whole plant Image (c, d) corresponds to the stem of the plant.

2. Materials and Methods

Plant collection: The plant *Neptunia triquetra* was widely distributed in the state of Andhra Pradesh, India. The plant material was collected from in and around the surroundings of Guntur district, Andhra Pradesh, India. After authentication by taxonomist, a voucher specimen was deposited in herbarium. The collected plant material was washed thoroughly and shade dried. After several weeks of drying, the dried leaves were crushed and sieved through No. 22 sized mesh followed by labeling as *Neptunia*, stored in cool and dry place until further use.

Extraction of phytochemicals from *Neptunia triquetra*

The *Neptunia triquetra* leaves powder was taken in a soxhlet apparatus and subjected to successive soxhlet extraction. The various solvents such as Hexane, Chloroform, Methanol and Water were used for the extraction of phytochemicals. After loading approximately 100gms dry leaves powder in soxhlet apparatus, the successive extraction was carried out with continuous heating and cooling for 30-40 cycles in apparatus and each extract was collected, rotary evaporated and labeled for further use.

Preliminary screening of phytochemicals of *Neptunia triquetra* (Qualitative analysis)

All the reagents required for screening the presence of various phytochemicals were prepared as per the standard procedures and protocols. The reagents include Benedict's reagent, Dragendorff's reagent, Fehling's solution A& B, Liebermann-Burchard reagent, Mayer's reagent and Molisch's reagent. Standard screening tests of plant extracts of *Neptunia triquetra* were carried out to know the presence / absence of various secondary metabolites such as alkaloids, anthraquinones, flavonoids, phenolic compounds, saponins, steroidal compounds, and tannins *etc*.

Detection of alkaloids: The availability of alkaloids in the extracts of medicinal plant *Neptunia triquetra* was evaluated by using the tests a) Dragendorff's reagent test and b) Mayer's reagent test.

a) Dragendorff's reagent test: 0.5gms of each of the *Neptunia triquetra* extract was warmed with 2% H_2SO_4 for few minutes. The contents were filtered and added with 2-3 drops of Dragendroff's reagent, mixed well. Observation of Orange red precipitate indicates the positive test for the presence of alkaloids [9].

b) Mayer's reagent test: 5g of crude extract from *Neptunia triquetra* was taken in a test tube, added with 1% aqueous HCl and heated on water bath at 60°C with stirring and then filtered. To the filtrate, few drops of Mayer's reagent (Potassium Mercuric Iodide) were added, mixed well. Formation of buff- colored precipitate is considered as positive test for the alkaloids.

Detection of flavonoids: The crude extract was dissolved in aqueous solvents, filtered and each filtrate was mixed with 5mL of diluted ammonia solution. Mixed well and further added with concentrated H_2SO_4. Appearance of a yellow colour confirms the presence of flavonoids [10]. The presence of flavonoids was further confirmed by a) alkaline reagent test and b) Shinoda's test [11].

a) Alkaline reagent test: The crude extract was dissolved in distilled water, mixed well and filtered. To the filtrate 2 mL of 2.0% NaOH was added and was mixed well. Formation of yellow color was observed. It became colorless when added with few drops of diluted HCl. This result indicates the positive test for the presence of flavonoids.

b) Shinoda's test: To the aqueous crude extracts of *Neptunia triquetra*, few pieces of magnesium crystals and Conc. HCl were added, mixed well. After few minutes of incubation formation of pink colour is observed, which indicates the positive test for flavonoids.

Detection of anthraquinones (Borntrager's test): Few grams of *Neptunia triquetra* extract was taken in a dry test tube and added with 15 mL of Chloroform. The test tube was heated in boiling water bath for 5minutes. The obtained extract was filtered and equal volume of 10% ammonia solution was added. The contents were mixed well and observed for colour change. Appearance of bright pink colour on the surface indicates the positive test for the presence of Anthraquinones [12].

Detection of sterols: 0.5gms of *Neptunia triquetra* extract was taken in a dry test tube and added with 10mL of Chloroform, mixed well and filtered. The obtained filtrate was divided in to two parts and used for testing. The presence of steroids was assessed using different tests such as a) Liebermann-Burchard's test and b) Salkowski's test.

a) Liebermann-Burchard's test: To the few mL of filtrate, 1mL of acetic anhydride ($C_4H_6O_3$) was added and mixed well. To this 1mL of conc. sulfuric acid (H_2SO_4) was added carefully along the sides of test tube. Appearance of green colour indicates the positive test for the steroids.

b) Salkowski's test: To the filtrate conc. Sulfuric acid (H_2SO_4) was added carefully along the walls of the test tube to form a lower layer. A reddish brown color at the interface indicates the presence of a steroid ring.

Detection of tannins: The presence of tannins was evaluated using different tests such as a) Ferric chloride test, b) Lead Acetate test, and c) Potassium Dichromate test.

a) Ferric chloride test: To the 2mL of *Neptunia triquetra* extract dissolved in water, added with 2mL of 10% Ferric chloride solution and mixed well. Appearance of blackish blue colour infers the presence of gallic tannins and appearance of green-blackish colour indicates the positive test for the catechol tannins.

b) Lead acetate test: To the 2mL of *Neptunia triquetra* extract dissolved in water, added with few mL of 10% Lead acetate [Pb $(C_2H_3O_2)_2$] solution and mixed well. Formation of yellow precipitate indicates the positive test for the presence of tannins.

c) Potassium dichromate test: The *Neptunia triquetra* extract was dissolved in distilled water and to it potassium dichromate ($K_2Cr_2O_7$) solution was added. Yellow colour precipitate indicates presence of tannins and phenolic compounds.

Detection of Phlobatannins: Approximately 200mg of *Neptunia triquetra* extract was dissolved in 10mL of double distilled water and mixed well. To this few drops of 1% HCl was added and boiled. Appearance of red precipitate indicates the positive test for the presence of Phlobatannins [13].

Detection of saponins (Froth test): The *Neptunia triquetra* extract was added with 20mL of distilled water and was shaken in a graduated cylinder for 10 minutes. Allowed for incubation for 5 minutes and observed. Formation of a layer of "honey comb" froth indicates the positive test for the presence of saponins.

Detection of anthocyanins: To the 2 mL of *Neptunia triquetra* extract, equal volume of 2 N HCl was added and mixed well. The appearance of pink-red colour was observed. It turns into purplish blue colour after addition of ammonia. It indicates the positive test for the presence of anthocyanins [14].

Detection of leucoanthocyanins: The *Neptunia triquetra* extract was added to 5 mL of Isoamyl alcohol and mixed well. Appearance of red colour on the upper layer indicates the positive test for the presence of leucoanthocyanins.

Detection of coumarins: 3mL of 10% NaOH was added to the *Neptunia triquetra* extract and mixed well. Appearance of a yellow colour indicates the positive test for the presence of coumarins.

Tests for carbohydrates: The presence of carbohydrates was tested using different tests such as a) Molisch's test, b) Barfoed's test (for detecting mono saccharides), c) Fehling's test "(To assess the presence of free reducing sugars), d) Test for ketones and e) Test for pentoses.

a) Molisch's test: The extract was dissolved in distilled water and 2 mL of this was taken and added with few drops of Molisch's reagent. Few mL of conc. sulfuric acid (H_2SO_4) was also added along the walls of test tube. The interface of two layers was observed for the appearance of purple colour which infers the positive test for the presence of carbohydrates.

b) Barfoed's test:
The extract was dissolved in distilled water and 2mL of this was taken in a test tube. To this 2 mL of Barfoed's reagent was added and mixed well. The contents after mixing were placed in a boiling water bath for 10minutes and observed for the colour change. Formation of red colour precipitate at the bottom of test tube indicates the positive test for the presence of mono saccharides.

c) Fehling's test: The extract was dissolved in distilled water and 2mL of solution was taken in a test tube. To this 5mL of Fehling's solution A and 5mL of Fehling's solution B was added and mixed well. The contents were boiled in a boiling water bath for 5minutes. Appearance of brick red colour indicates the positive test for the presence of free reducing sugars.

d) Test for ketones: The extract was dissolved in 2mL of distilled water and added with few crystals of resorcinol ($C_6H_6O_2$). To this equal volume of HCl was added and mixed well. The contents were heated using a spirit lamp and observed for the colour change. Appearance of pink red colour indicates the positive test for the presence of ketones.

e) Test for pentoses: The extract was dissolved in 2 mL of distilled water and added with 2 mL of HCl and few drops of phloroglucinol ($C_6H_6O_3$). The contents were mixed well and heated using a spirit lamp for few minutes. The contents were observed for colour change and appearance of red colour is indicative for the positive test for the presence of pentoses.

Quantitative estimation of phyto constituents from *Neptunia triquetra*
The presence of various phyto constituents were further tested for the quantitative estimation. For this purpose total phenol content and total flavonoid content were estimated as per the

standard modified protocols [15].

Determination of total phenol content

The amount of total phenol content, in different solvent extracts of *Neptunia* was determined by Folin- Ciocalteu's reagent method [16]. For this purpose, 0.5 mL of the extract was mixed with 0.1mL of (0.5N) Folin- Ciocalteu's reagent. The contents were mixed well and were incubated at room temperature for 15 minutes. Then 2.5mL of saturated sodium carbonate solution was added and further incubated for 30 minutes at room temperature and the absorbance was measured at 760nm using UV-Visible spectroscopy (Thermo Scientific, USA). Gallic acid was used as a positive control. Total phenol content was expressed in terms of Gallic acid equivalent (mg/g of extracted compounds).

Determination of total flavonoid content:

The amount of flavonoid content in different solvent extracts of *Neptunia triquetra* was estimated by aluminum chloride colorimetric method [17]. The reaction mixture was made by adding 1 mL of extract with a concentration of 1 mg/mL, 0.5 mL of 1.5% aluminum chloride and 0.5 mL of 120 mM potassium acetate. The contents were mixed well and were incubated at room temperature for 30 minutes. The absorbance of all samples was measured at 415nm using UV-Visible spectroscopy. Rutin was used as positive control. The flavonoid content is expressed in terms of rutine quivalent (mg/g of extracted compound).

Anti-oxidant activity

The anti-oxidant activity was assessed by studying the diphenyl picrylhydrazine (DPPH) radical scavenging ability and ferric reducing antioxidant power (FRAP) assay.

DPPH (2, 2-diphenyl-l-picryl hydrazyl) radical scavenging assay

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging assay. DPPH solution (0.004% w/v) was prepared in 95% ethanol and allowed for overnight incubation in the dark for generation of DPPH radical. A stock solution of Hexane, Chloroform, Methanol, Aqueous (water) extracts of *Neptunia triquetra* and standard ascorbic acid were prepared in the concentration of 100mg/100mL (1mg/mL). From each stock solution 1mL, 2 mL, 3 mL, 4 mL & 5 mL of this solution were taken in five test tubes respectively. With same solvent made the final volume of each test tube up to 10mL whose concentration was then 100µg/ mL, 200µg/ mL, 300µg/ mL, 400µg/ mL & 500µg/ mL respectively. 2mL of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 15 minutes and thereafter the optical density was recorded at 517nm against the blank. For the control, 1mL of DPPH solution in ethanol was mixed with 10ml of ethanol and the optical density of the solution was recorded after 30 minutes. The assay was carried out in triplicate. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation [18, 19, 20].

$$\text{DPPH Scavenged (\%)} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} X100$$

Where "A control" is the absorbance of the control reaction and "A test" is the absorbance of the sample of the extracts. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. All the experiments were repeated for three times and mean values were taken and IC_{50} values were calculated and represented in results

FRAP ferric reducing antioxidant power assay

The 20µL of different extracts of *Neptunia triquetra* with a concentration of 0.5mg/mL was taken in a test tube and mixed with 20µL of 0.2 M potassium phosphate buffer (pH 7.2). The contents were mixed well and added with potassium ferricyanide (1 % w/v, 20µL). The contents were boiled in water bath at 50°C for 25 minutes. After cooling TCA (10 % w/v, 20µL), distilled water (75µL) and ferric chloride (0.1 % w/v, 20µL) were added and the reaction mixture was further incubated for 30 minutes at room temperature. Absorbance was measured at 630nm. Ascorbic acid was used as standard. The results were represented as ascorbic acid equivalents per mg sample (µg AAE/mg) [21].

$$\text{FRAP scavenging activity (\%)} = \left[\frac{(A0 - As)}{A0}\right] X100$$

Where, A_0 is the absorbance of the control and As is the absorbance of the plant sample,

Concentration of working extract is 1mg/mL.

All the experiments were repeated for three times and mean values were taken and IC_{50} values were calculated and represented in results.

3. Results

Phytochemical analysis

Qualitative analysis

The *Neptunia triquetra* is the richest source for diverse phytochemicals. The alkaloids were present in all the extracts except Hexane extract. Similar characteristic feature of presence was exhibited by flavonoids. There are no traces of appearance of anthocyanins, coumarins and leuco anthocyanins class of phytochemicals in any of the crude extracts of *Neptunia triquetra*. The carbohydrates were present in aqueous extract. The phytochemical evaluation of Hexane, Chloroform, Methanol and Aqueous extracts of *Neptunia triquetra*., leaves were given in table-1.

Table 1: Comparative Analysis of Phytochemical Analysis of *Neptunia* triquetra leaves.

S. No	Name of the phytochemical screened	Hexane Extract	Chloroform Extract	Methanol extract	Aqueous (Water) extract
01.	Detection of Alkaloids				
	a) Dragendorff's reagent test	Negative	Positive	Positive	Positive
	b) Mayer's reagent test	Negative	Positive	Positive	Positive
02.	Detection of Flavonoids				
	a) Alkaline reagent test	Negative	Positive	Positive	Positive
	b) Shinoda's test	Negative	Positive	Positive	Positive

03.	Detection of Anthraquinones (Borntrager's test)	Negative	Negative	Negative	Negative
04.	Detection of sterols				
	a) Liebermann-Burchard's test	Positive	Positive	Positive	Positive
	b) Salkowski's test	Positive	Positive	Positive	Positive
05.	Detection of Tannins				
	a) Ferric chloride test	Negative	Negative	Positive	Positive
	b) Lead Acetate test	Negative	Negative	Positive	Positive
	c) Potassium Dichromate test	Negative	Negative	Positive	Positive
06.	Detection of Phlobatannins	Positive	Positive	Positive	Negative
07.	Detection of Saponins (Froth Test)				
08.	Detection of Anthocyanins	Negative	Negative	Negative	Negative
09.	Detection of Leucoanthocyanins	Negative	Negative	Negative	Negative
10.	Detection of Coumarins	Negative	Negative	Negative	Negative
11.	Tests for carbohydrates				
	a) Molisch's test	Negative	Negative	Negative	Positive
	b) Barfoed's Test	Negative	Negative	Negative	Positive
	c) Fehling's test	Negative	Negative	Negative	Positive
	d) Test for ketones	Negative	Negative	Negative	Positive
	e) Test for pentoses	Negative	Negative	Negative	Positive

Quantitative analysis

Based upon the preliminary phytochemical analysis qualitative determination of phyto constituents were carried out for various extracts of *Neptuinia triquetra* by various standard methods and found that high amount of total phenols and total flavonoids. These two phytochemicals appeared highest concentration in Methanol extract and concentration increased with increase in extracts. For 500µg/mL of crude extract % of phenol content µg GAE/µg was 74.33±0.26. For similar concentration of Methanol extract total flavonoid content was 42.37±0.22 which is equal to µg rutin/µg. The results of total phenol content are represented in table-2 and whereas for the total flavonoid content were represented in table-3 respectively.

Table 2: Total phenol content of *Neptunia triquetra* leaves

Concentration of extracts (µg/mL)	% of Phenol content µg GAE/µg			
	Hexane Extract	Chloroform Extract	Methanol Extract	Water Extract
100	16.27±0.23	19.40±0.42	24.31±0.40	22.44±0.39
200	24.28±0.26	28.43±0.28	36.40±0.30	32.39±0.25
300	35.41±0.27	40.55±0.41	49.41±0.30	45.31±0.35
400	40.44±0.43	49.45±0.41	61.52±0.35	56.44±0.38
500	46.27±0.33	56.35±0.30	74.33±0.26	65.35±0.32

Table 3: Total Flavonoid content *Neptunia triquetra* leaves

	% of flavonoid content µg rutin/µg			
Concentration of extracts (µg/mL)	Hexane extract	Chloroform extract	Methanol extract	Aqueous (water) extract
100	0.04±0.03	10.16±0.14	12.25±0.19	10.12±0.12
200	1.15±0.07	14.29±0.20	18.31±0.18	15.29±0.14
300	2.17±0.14	22.39±0.25	26.32±0.21	22.39±0.18
400	5.48±0.20	28.26±0.16	34.53±0.25	29.48±0.21
500	6.23±0.14	36.31±0.18	42.37±0.22	10.12±0.12

Anti-oxidant activity

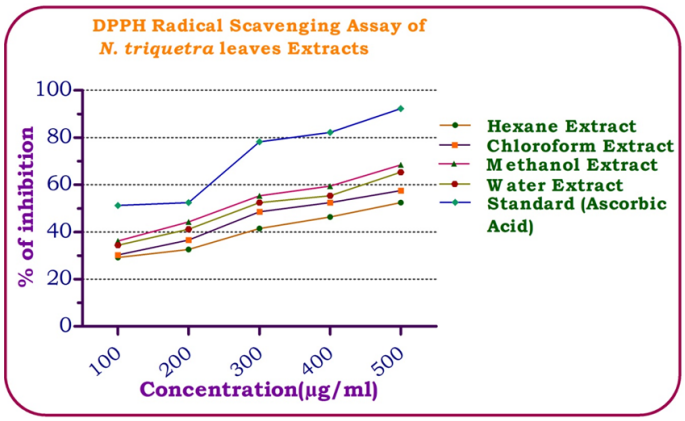
On the basis of phytochemical investigation, all extracts (Hexane, Chloroform, Methanol and Aqueous) were chosen for the antioxidant studies. DPPH Radial Scavenging Activity of *Neptunia* was represented in table-4 and table-5 respectively.

Table 4: DPPH radical scavenging antioxidant activity of *Neptunia triquetra* leaves

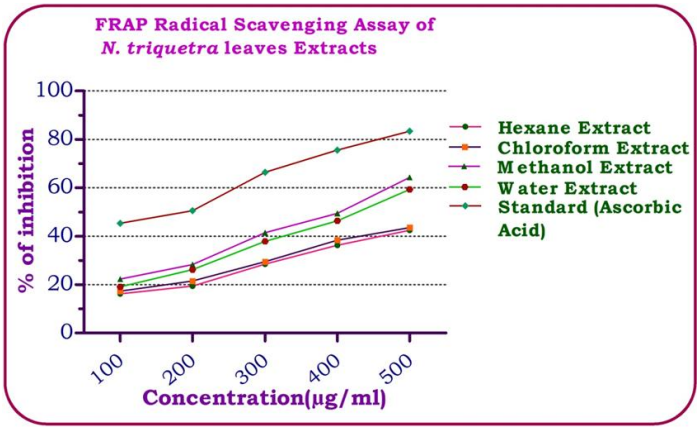
Conc. (µg/mL)	Hexane extract	IC_{50}	Chloroform extract	IC_{50}	Methanol Extract	IC_{50}	Water extract	IC_{50}	Standard % of inhibition (Ascorbic acid)	IC_{50}
100	29.20±0.24	459.24±4.06	30.27±0.18	370.28±4.21	36.18±0.22	265.69±2.84	34.38±0.23	305.25 ± 6.06	51.23±0.15	109.79 ± 1.64
200	32.61±0.64		36.55±0.28		44.27±0.20		41.21±0.21		52.45±0.25	
300	41.46±0.20		48.51±0.38		55.35±0.23		52.44±0.25		78.21±0.21	
400	46.37±0.23		52.49±0.18		59.43±0.28		55.34±0.32		82.23±0.19	
500	52.43±0.27		57.49±0.37		68.41±0.26		34.38±0.23		92.25±0.24	

Table 5: FRAP radical scavenging antioxidant activity of *Neptunia triquetra* leaves

Conc. µg/ml	Hexane	IC_{50}	Chloroform	IC_{50}	Methanol	IC_{50}	Aqueous	IC_{50}	Ascorbic Acid	IC_{50}
100	16.23±0.22	609.15±3.19	17.30±0.20	587.96±3.11	22.35±0.23	385.29±3.12	19.16±0.18	421.77±1.03	45.38±0.25	159.02±2.94
200	19.42±0.20		21.51±0.32		28.32±0.22		26.23±0.20		50.51±0.36	
300	28.53±0.38		29.53±0.11		41.47±0.23		37.85±0.95		66.39±0.26	
400	36.23±0.21		38.37±0.23		49.42±0.28		46.33±0.23		75.56±0.39	
500	42.47±0.20		43.53±0.25		64.29±0.19		59.32±0.25		83.35±0.22	



Graph 1: DPPH radical scavenging assay with the crude extracts of *Neptunia triquetra*



Graph-2: FRAP assay with the crude extracts of *Neptunia triquetra*.

4. Conclusion

From the ethno pharmacognosy point of view *Neptunia triquetra* is the potential medicinal plant with wide range of medicinal properties. A large number of diseases and illness were combated with the extracts of this plant. However, there is lack of scientific evidence for the key ingredients and diverse class of phytochemicals present in this plant. Hence, in the present study preliminary phytochemical screening for the crude extracts of *Neptunia triquetra* was carried out and as per our results, the plant is the richest source for the diverse class of phytochemicals. Both qualitative and quantitative analysis was performed in the present study. However, the chemical structure of all these phytochemicals needs to be elucidated. The *Neptunia triquetra* also have the anti-oxidant activity as demonstrated from the results of DPPH radical scavenging assay and FRAP assay.

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