Current Evaluation of the *Tubiflora acaulis* Kuntze (Acanthaceae): Pharmacognostical, Preliminary Phytochemical Investigation and Antioxidant Activity

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**Authors’ contributions**

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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**ABSTRACT**

**Aim:** The present work was aimed at evolving with Pharmacognostical, Preliminary Phytochemical investigation and Antioxidant activity of plant *Tubiflora acaulis* Kuntze (Synonym *Elytraria acaulis*) belong to family Acanthaceae.

**Study Design:** The permission was taken from Maharashtra State Biodiversity Board, Nagpur for plant collection, collected and authenticated and evaluated Pharmacognostical character including morphological and microscopical as well as Physical Evaluation, phytochemical process and antioxidant activity.

**Place and Duration of Study:** The study was carried out at PSGVPMs College of Pharmacy, Shahada, Dist- Nandurbar (MS) Affiliated to KBCNMU, Jalgaon University, Jalgaon (MS), during 2019-21.

**Methodology:** The plant material firstly taken permission from Maharashtra State Biodiversity...
Keywords: Tubiflora acaulis; (Synonym Elytra acaulis) pharmacognostical; phytochemicals.

ABBREVIATIONS

R & D : Research and Development
WHO : World Health Organisation
DMSO : DIMETHYLSULFOXIDE
DPPH : 2,2-diphenyl-1-picrylhydrazyl

1. INTRODUCTION

Natural products are known to play an important role in pharmaceutical biology. Plants have been an exemplary source of medicine since ancient age. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional medicines [1]. Plants have always been a major component of traditional system of healing in developing countries, which have also been an integral part of their history and culture. Medicinal plants offer alternative remedies with tremendous opportunities. India has rich medical heritage with a large number of traditional practices, systems and medicines as a part of its total health care scenario, some of them are more than 3,000 years old. In spite of remarkable achievements of modern medicines and research, these ancient systems continue to play a major role in the control or alleviation of diseases [2]. Plant drugs have been the major source for treatment of diseases for a long time. They have been used in traditional medicine on the basis of experiences and practice [3]. Modernization is posing serious threats to medicinal plants and associated systems. Public are attracted to the modern system of medicine, which provides quick relief, at lower cost. But, in recent times, there has been an increasing awareness about the significance of medicinal plants and their use. There has been revival of interest in knowing about many medicinal plants and their by products which are inherently safer and more efficacious than the modern, potent synthetic drugs which very often produce undesirable side effects in man. This prompted the people to return to the ancient and traditional system of phytomedicines or herbal medicines. With the result, the use of natural medicines or herbal drugs has gained momentum and the demand for herbal raw drugs and other products is increasing many folds.

The R & D thrust in the pharmaceutical sector is focused on development of new innovative indigenous plant based drugs through investigation of leads from the traditional system of medicine [4]. The World Health Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Proven agro industrial technologies need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines [5]. Herbal medicines are in great demand in the developed as well as developing countries for primary health care because of their wide biological and medicinal activities, higher safety margins and lesser costs. Approximately 1500 medicinally active plant species are reported from this region, which having miracles healing properties against several diseases. Yet, there may be several plants that are yet to be revealed and identify scientifically. The authentic identification, as well as preservation of the traditional knowledge of medicinal plants are equally important. WHO has given a clear guideline about the importance, processes of characterization and identification of ethnomedicinal plants. The assessment of quality, standard, and purity of the plant-derived material may vary depends on several parameters, hence, for better and safe use of medicinal plants, proper identification and characterization like morphological,
microscopical, macroscopical and pharmacognostical and phytochemical are required to be performed [6]. In this present research work, the plant *Tubiflora acaulis* Kuntze, belonging to family Acanthaceae was selected. *Tubiflora acaulis* Kuntze was Stemless perennial herb with 1-several unbranched flowering stems with a short creeping sometimes branched root stock, up to 30 cm tall. Leaves in a basal rosette, subsessile, elliptic to obovate, up to 18 cm long, hairy, particularly on the veins below; margin subentire to scallop in the upper part. Flowers in 1-several spikes held in tight apiculate, overlapping bracts. Bracts and flowering stem bluish green. Corolla white, lower lip and lateral lobes spreading, 2-lobed. Flowers often not opening. Capsule 5.5-6.5 mm long, hairless [7]. Traditionally Leaf powder of *Tubiflora acaulis* Kuntze with water is given for urinary complains [8]. The whole plant is internally and as well as externally used in the case of deworming by traditional users in Villupuram district of Tamil Nadu, India [9]. Leaf powder with water for treatment of Kidney stone and Urinary tract troubles in Aravali regions of Rajasthan [10]. Decoction of root is mixed in equal amount of local liquor and one cup of this mixture is taken daily for 3-4 days in the morning for easy expulsion of guinea worm. Half teaspoon root extract is given to children once a day for two days for asthma by tribals of Southern Rajasthan [11]. With this various use of plant it is not yet well explored as there are only few literatures on that plant. The methanol extract contain two pyrazole alkaloids withasomnine (120 mg) and 4-hydroxy withasomnine (30 mg) [12] and 4H-1-Benzo pyran-4-one,3-((6-deoxy-beta-Lmannofuranosyl)-beta-Ogalactofuranosyl) oxy)-7-(3-deoxy-beta-betamannopyranosyl) oxy)-5-hydroxy-2-(4-hydroxyphenyl), having molecular formula C27 H30O15 and molecular weight is 595.518 g/mol [13]. But studies for the identification of biologically active compounds for this species are very less. So it is important to evaluate the phytochemicals present in this plant species. The present study is focused on the Pharmacognostic characters of the plant and its bioactive compounds. Pharmacognostical evaluation includes morphological and microscopical characters, physicochemical properties.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The Leaves of *Tubiflora acaulis* was collected from Satpuda hills, Akkalkuwa, Dist: Nandurbar, Maharashtra, India, cleaned and dried at room temperature in shade and away from direct sunlight. The dried aerial part was coarsely powdered in grinder. Large difference in particle size of crude drug results in long extraction time as the coarse particles increases the extraction time and fine may form bed, so the powdered material was sieved through 60-120 mesh to remove fine and the powder was subjected for further study.

2.2 Authentication of the Plant Material

The plant authenticated by Dr. Priyanka A. Ingale, Scientist B, Botanical Survey of India, Pune (Voucher Specimen number-01) by comparing morphological features and a sample voucher specimen of plant was deposited for future reference also authenticated by Dr. S.K Tayade Head Department of Botany PSGVP Mandal arts, science & commerce college Shhada (MS).

2.3 Plant Material Collection Permission

According to the Biological Diversity Act, 2002, permission granted from Maharashtra State Biodiversity Board, Nagpur by sending the Proposal for permission of collection of biological resources as an herbal plant for research work to Maharashtra State Biodiversity Board, Nagpur. (Ref. No. MSBB/Research/342/2021-22) Date-12/08/2021)

2.4 Pharmacognostic Study

2.4.1 Macroscopic evaluation

Plant morphology of *Tubiflora acaulis* Kuntze study was carried out by standard methods to determine the different external parameters of stems, roots, and leaves that were observed. The macroscopy or morphology characteristic of this plant include size, colour, odour, taste, etc. were noted [14,15].

2.4.2 Microscopic examination

Thin transverse section of fresh Leaf, Root were taken, stained with various solutions and observed under 10X and 45X. The transverse sections were taken. The Stomatal Number, Stomatal Index, Vein islet Number, Vein Termination Number etc were determined. The microscopic powder characteristics also performed [14,15,16].
2.4.3 Physical Evaluation

In Physical Evaluation parameter foreign organic matter, loss on drying, ash value, Total ash, sulphated ash, acid -insoluble ash, extractive value were performed [14,15,16].

2.4.3.1 Determination of foreign organic matter

Foreign organic matter means the material which is not collected from the original plant source, part of organ other than mentioned, insects, moulds or the animal contamination. For determination of foreign organic matter 5 gm of air dried coarsely powdered drug was spread in a thin layer. The sample was inspected with the unaided eye. The foreign organic matter was separated manually as completely as possible. Sample was weighed and percentage of foreign organic matter was determined from the weight of the drug taken.

2.4.3.2 Determination of loss on drying

Loss on drying is the amount of both water and volatile matter which evaporates during drying. For determination loss on drying accurately weighed flat and thin porcelain dish was dried and 2g of sample was transferred, the weight was taken and sample was distributed evenly. Then loaded porcelain dish was kept in oven at 100°C. The sample was dried to constant weight. After drying it was collected to room temperature in desiccators. Weighed and calculated loss on drying in terms of percent w/w.

2.4.3.3 Determination of ash value

Ash is the residue remains after incineration. Ash value is used to determine quality and purity of crude drug. Ash value contains inorganic radicals like phosphates carbonates and silicates of sodium, potassium, magnesium, calcium etc. sometimes inorganic variables like calcium oxalate, silica and carbonate content of the crude drug affects total ash value. Such variables are then removed by treating with acid and then acid insoluble ash value is determined.

2.4.3.4 Determination of total ash

Accurately weighed 2gm of the air-dried crude drug was taken in a tarred silica dish and incinerated at a temperature not exceeding 450°C until free from carbon, cooled in a desiccator and weight was taken. The process was repeated till constant weight was obtained.

2.4.3.5 Determination of water soluble ash

The percentage of ash was calculated with reference to air-dried drug.

2.4.3.6 Determination of acid insoluble ash

The ash obtained as per the method described above boiled for 5 minutes with 25 ml of water, filtered, and collected the insoluble matter on an ash less filter paper, washed with hot water, ignited for 15 minutes at a temperature not exceeding 450°C and weight was taken. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug.

2.4.3.7 Determination of sulphated ash

Silica crucible was heated to redness for 10 minutes and allowed to cool in desiccator and weighed. 2 gm of air-dried drug was weighed and ignited gently until the substance was charred cool. The residue was moistened with 1 ml sulphuric acid. It was heated gently until the white fumes no longer evolved and ignited at 800°C ±25°C until all black particles had disappeared. Ignition was conducted in place protected from air currents. Crucible was cooled and few drops of sulphuric acid were added and ignited. Then it was allowed to cool and weighed.

2.4.3.8 Determination of extractive value

The total soluble constituents of the drug in any particular solvent or mixture of solvents may be called as extractive value. Different extractive values like water soluble extractive, alcohol soluble extractive, chloroform soluble extractive and petroleum ether-soluble extractive value were determined by standard method.

2.4.3.9 Determination of water soluble extractive value

5 gm of air dried coarsely powdered drug was macerated with 100 ml of chloroform water in a
closed flask for 24 hours, and it was shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of water-soluble extractive value was calculated with reference to air-dried drugs.

2.4.3.10 Determination of alcohol soluble extractive value

5 gm of air-dried coarsely powdered drug was macerated with 100 ml of ethanol of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allows standing for 18 hours. Then it was filtered, during filtration precaution was taken against loss of ethanol, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to air-dried drugs.

2.4.3.11 Determination of chloroform soluble extractive value

5 gm of air-dried coarsely powdered drug was macerated with 100 ml of chloroform of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allows standing for 18 hours. Then it was filtered, during filtration precaution was taken against loss of chloroform, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of chloroform soluble extractive value was calculated with reference to air-dried drugs.

2.4.3.12 Determination of petroleum ether soluble extractive value

5 gm of air-dried coarsely powdered drug was macerated with 100 ml of petroleum ether of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allows standing for 18 hours. Then it was filtered, during filtration precaution was taken against loss of petroleum ether, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105°C and weighed. Percentage of petroleum ether soluble extractive value was calculated with reference to air-dried drugs.

2.5 Extraction of Powdered Plant Material

The leaves of Tubiflora acaulis Kuntze (Acanthaceae) was collected and dried in the shade and then pulverized in a grinder. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. The powdered was utilized for successive extraction by prescribed in standard reference using Petroleum ether, chloroform and methanol as solvent for extraction of powdered plant [15].

2.6 Preliminary Phytochemical Screening

2.6.1 Qualitative phytochemical analysis

Phytochemical tests were performed using Petroleum ether, chloroform and methanol extracts to determine the presence of different phytochemicals following established standard protocol. The plant extracts were subjected for the test of alkaloid-like substances, carbohydrates, fixed oils and fats, glycosides (Cardiac, Anthraquinone, Saponin), phenolic compounds and tannins, proteins and amino acids, flavonoids, lignin, terpenoids, and diterpenes. Qualitative phytochemical examinations were carried out for all extracts of leaves as per the standard methods [15,17].

2.6.2 Quantitative phytochemical analysis

Quantitative phytochemical analysis were carried out for different parts of plant (dissolved in water) and all extracts of leaves (dissolved in DMSO) as per following methods.

2.6.2.1 Determination of total carbohydrate content

For determination of total carbohydrate content 2 ml of a test solution was mixed with 1 ml of 5% aqueous solution of phenol and 5 ml of concentrated sulfuric acid in a test tube. The resulting mixture allowed to stand for 10 min and vortexed for 30 second and then placed for 20 min in a water bath at room temperature for color development. Then the intensity of absorption of developed color was recorded at 490 nm by using spectrophotometer. Glucose was used as standard solution [17,18].

2.6.2.2 Determination of total protein content

The Bradford assay is a based on simple colorimetric measurement. Bradford reagent (light brown) binds with protein results in change in color. For determination of total protein content the equal volume of test solution was treated with Bradford reagent and incubated for 5 min. Then
the intensity of absorption of developed color was recorded at 595 nm by using spectrophotometer. Albumin was used as standard solution. Solution [17].

2.6.2.3 Determination of total saponin content

For determination 2 gm of sample was heated with 200 ml of 20% ethanol at 55°C for four hour in conical flask and filtered. The procedure was repeated and both extracts were combined and reduced up to 40 ml. resulting solution was mixed with 20 ml of diethyl ether into 250 ml separating funnel and aqueous layer was recovered. The process was repeated for several time and the aqueous layer combined. The 60 ml of butanol was added in the above solution and washed with 10 ml of 5% aqueous sodium chloride solution and the remaining solution was heated up to dryness on water bath and placed in hot air oven until constant mass observed [19].

Percentage of total saponin =
Weight of residue / Percentage of total saponin X 100

2.6.2.4 Determination of total steroid content

Liberman-Burchard reaction involves oxidation of steroids which results pentaenylc cation with one or more double bond as compare to original compound. The oxidation product SO₃ reduces to SO₂ which form characteristic blue-green color. For determination equal volume of test solution was treated Liberman-Burchard reagent in test tube and covered by black paper then allowed to stand for 15 minutes. Then the intensity of absorption of green color complex was recorded at 640 nm by using spectrophotometer. Diosgenin was used as standard solution [17,20].

2.6.2.5 Determination of total alkaloid content

For determination 1 ml of test solution was treated with 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution in separating funnel then extracted with 5 ml of chloroform. Then chloroform extract was transferred in to 10 ml volumetric flask and volume adjusted with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. Atropin was used as standard solution [21].

2.6.2.6 Determination of total flavonoid content

The principle of determination of total flavonoid content by aluminum chloride method is that aluminum chloride forms acid stable complex with C-4 keto group and either C-3 or C-5 hydroxyl groups of flavonoids. In addition it forms acid liable complexes with orthodihydroxyl groups of flavonoid which can be detected by UV spectroscopy [17]. For determination 5 ml of test solution was treated with 0.3 ml of 5% sodium nitrite and 3 ml 10% aluminum chloride in 10 ml volumetric flask and after 6 minute 2 ml of sodium hydroxide solution was added then volume adjusted up to 10 ml with distilled water. The solution was mixed well again and the absorbance was measured against a blank at 510 nm with UV spectrophotometer. Quercetin was used as standard solution [22].

2.6.2.7 Determination of total tannin content

Tannins reduce phosphotungustomolybdic acid in alkaline media and produce blue colored solution which is proportional to concentration of tannin. For determination 1 ml of test solution was treated with 0.5ml of Folin-Denis reagent and 1 ml of sodium carbonate solution in 10 ml volumetric flask. Final volume was made up to 10 ml with distilled water. The solution was mixed well and kept for about 30 minutes then absorbance was measured against a blank at 760 nm by UV spectrophotometer. Tannic acid was used as standard solution and calibration curve was prepared [23].

2.6.2.8 Determination of total phenolic content

Folin-Ciocalteau method is useful for determination of phenolic and polyphenolic compounds it work by measuring the amount of the substance being tested needed to inhibit the oxidation of reagent. However, this reagent will reacts with any other reducing substance therefore reagent measure total reducing capacity of substance not just the level of phenolic compounds [17]. For determination 1 ml of test solution was treated with 0.5ml of Folin-Ciocalteau reagent and 1 ml of 2% sodium carbonate solution in 10 ml volumetric flask. Final volume was made up to 10 ml with distilled water. The solution was mixed well again and kept for about 3 hour with intermittent shaking then the absorbance was measured against a blank at 760 nm by UV spectrophotometer. Gallic acid was used as standard solution and calibration curve was prepared [24].

2.7 Evaluation of Anti-oxidant Activity

2.7.1 DPPH scavenging activity

Equal volume 100 µM DPPH in ethanol was mixed with test solutions (10 µg/ml to 100 µg/ml) and allowed to stand for 30min at room
temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard. IC50 value was calculated from % inhibition which was calculated by following formula [24].

\[
\text{% Inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100
\]

2.7.2 Nitric oxide scavenging activity

Equal volume of Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration of test solutions (10 µg/ml to 100 µg/ml) and the tubes were incubated at 25º C for 150 min. then 2 ml incubated solution was removed and diluted with equal volume of Griess reagent. The absorbance of chromophore formed was read at 546nm. The experiment was repeated for three times. Ascorbic acid was used as standard. IC50 value was calculated from % inhibition which was calculated by following formula [24].

\[
\text{% Inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100
\]

2.7.3 Reducing power assay

1 ml of different concentration of test solutions (10 µg/ml to 100 µg/ml) was added in a mixture of 2.5 ml of phosphate buffer solution (pH 6.6) and 2.5 ml of potassium ferricyanide solution (1% w/v) and incubated at 50 ºC for 20 min. Then 2.5 ml of tri-chloro acetic acid (10% w/v) was added to the mixture, which was then centrifuged for 10 min at 1000 rpm. The 2.5 ml of upper layer of solution was mixed with equal volume of distilled water and 0.5 ml of FeCl3 solution (0.1% w/v), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reductive potential [25].

3. RESULTS AND DISCUSSION

In the present study the leaves of Tubiflora acaulis Kuntze (Acanthaceae) was evaluated for pharmacognostic, phytochemical analysis, antioxidant activity which revealed the following results.

Common Names [7]

| Language  | Name         |
|-----------|--------------|
| Marathi   | Vismuli, Burandya |
| Hindi     | Patharchatta |
| Tamil     | Pumikatampam  |
| Telugu    | Yeddadugu    |
| Synonym   | Elytraria acaulis |

3.1 Pharmacognostic Study

3.1.1 Macroscopic evaluation

Leaves in a basal rosette, subsessile, elliptic to obovate, up to 24 cm long, 7 cm width, hairy, particularly on the veins below; margin subentire to scallop in the upper part and mostly Found in all over in Satpuda region in moist and shady habitats. (Fig. 2, Table 1).

3.1.2 Microscopic evaluation

3.1.2.1 Qualitative microscopic evaluation

The fresh leave was transversely sectioned through the midrib region and was mounted and stain with different reagent like phloroglucinol, dil. HCl. Shows Upper and lower epidermis, palisade cell along with collenchyma, xylem and phloem. Collenchymas available at below upper and lower epidermis, midrib contain vascular bundle in which xylem is surrounded with phloem. Starch grains and calcium oxalate crystals are also found.

Fig. 1. Tubiflora acaulis Kuntze Plant

3.1.2.2 Quantitative microscopic evaluation

The results of stomatal number and index, palisade ratio, vein-islet number and vein termination number are given in Table 2.

3.1.3 Physical parameter

The physical constant evaluation of the drugs play significant role in identification of adulteration or improper handling of drugs. Thus all parts of plant were subjected for physical evaluation. The results are mentioned in Table 3.

3.2 Extraction of Powdered Plant Material

Leaves were selected for further study on the basis of ethno-medicinal survey, phytochemical
evaluation and subjected for extraction by various solvent (pet. ether, chloroform, methanol respectively) (Table 4).

3.3 Preliminary Phytochemical Screening

3.3.1 Qualitative phytochemical analysis

Qualitative phytochemical analyses were carried out on Tubiflora acaulis Kuntze leaf separately for different extract like pet. Ether, chloroform and methanol. Petroleum ether extract contain steroids, alkaloids the chloroform extract contain steroid, alkaloids, flavonoids, tannins the methanolic extract contain saponins, alkaloids, glycosides, flavonoids, tannins, carbohydrates, proteins and amino acids. (Table 5) It gives a preliminary insight into various compounds present in a plant, based on which further study towards the biological activities of the compounds can be tracked.

3.3.2 Quantitative phytochemical analysis

3.3.2.1 Total carbohydrate content

Total carbohydrate content was determined by phenol sulphuric acid method using glucose as standard for preparation of calibration curve. The absorbances of standard glucose for determination of total carbohydrate content were shown in Table 6. The total carbohydrate content of leaf methanol extract 07.39 ± 0.06 µg/ml (Graph 1).

3.3.2.2 Total protein content

Total protein content was determined by Barford method using albumin as standard for preparation of calibration curve. The absorbances of standard albumin for determination of total protein content were shown in Table 7. The total protein content of leaf methanol extract 04.98 ± 0.19 µg/ml (Graph 2).

Table 1. Macroscopic characters of leaves of Tubiflora acaulis Kuntze (Acanthaceae)

| Sr. No. | Parameter | Morphological characters of Leaf |
|---------|-----------|----------------------------------|
| 1       | Color     | Green                            |
| 2       | Odour     | Characteristic                   |
| 3       | Taste     | Characteristic                   |
| 4       | Size      | Mature leaf Size 24.00 X 7.00 Cm |
| 5       | Shape     | Elliptic to obovate              |

Fig. 2. Leaves of Tubiflora acaulis Kuntze
Table 2. Quantitative microscopy of *Tubiflora acaulis* Kuntze (Acanthaceae)

| Sr. No. | Parameter                     | Values          |
|--------|-------------------------------|-----------------|
| 1      | Stomatal number               | 110 per mm²     |
| 2      | Stomatal index                | 27 per mm²      |
| 3      | Palisade ratio                | 3 per cell      |
| 4      | Vein-islet number             | 17 per mm²      |
| 5      | Vein-termination Number       | 12 per mm²      |

Table 3. Physical Parameters of *Tubiflora acaulis* Kuntze (Acanthaceae)

| Sr. No | Parameter                          | Leaf            |
|--------|------------------------------------|-----------------|
| 1      | Foreign organic matter             | 0.19 ± 0.03     |
| 2      | Loss on drying                     | 0.86 ± 0.02     |
| 3      | Ash value                          | 1.04 ± 0.02     |
| 4      | Water soluble ash                  | 0.63 ± 0.04     |
| 5      | Acid insoluble ash                 | 0.57 ± 0.03     |
| 6      | Sulphated ash                      | 0.21 ± 0.02     |
| 7      | Pet ether soluble extractive value | 0.40 ± 0.02     |
| 8      | Chloroform soluble extractive value| 0.62 ± 0.03     |
| 9      | Alcohol soluble extractive value   | 0.65 ± 0.04     |
| 10     | Water soluble extractive value     | 1.12 ± 0.03     |
Table 4. Yield of various extracts obtained from the leaf of *Tubiflora acaulis* Kuntze (Acanthaceae)

| Sr. No. | Extract     | Color            | Yield (%)w/w |
|---------|-------------|------------------|--------------|
| 01      | Petroleum ether | Yellows brown    | 5.10         |
| 02      | Chloroform  | Dark green       | 8.21         |
| 03      | Methanol    | Dark brown       | 11.21        |

Table 5. Preliminary phytochemical analysis of various extracts of *Tubiflora acaulis* Kuntze (Acanthaceae)

| Sr. No. | Parameter          | Pet. ether Extract | Chloroform Extract | Methanol Extract |
|---------|--------------------|--------------------|--------------------|------------------|
| 1       | Carbohydrate       | -                  | -                  | +                |
| 2       | Protein            | -                  | -                  | +                |
| 3       | Amino acid         | -                  | -                  | +                |
| 4       | Steroids           | +                  | +                  | -                |
| 5       | Cardiac glycosides | -                  | -                  | -                |
| 6       | Anthraquinone glycosides | -                  | -                  | -                |
| 7       | Saponin glycosides | -                  | -                  | +                |
| 8       | Cyanogenetic glycosides | -                  | -                  | -                |
| 9       | Coumarin glycosides | -                  | -                  | -                |
| 10      | Alkaloids          | +                  | +                  | +                |
| 11      | Flavonoids         | -                  | +                  | +                |
| 12      | Tannins            | -                  | +                  | +                |

+ Positive; - Negative

Table 6. Absorbance of standard glucose for total carbohydrate content

| Concentration (µg/ml) | Absorbance |
|-----------------------|------------|
| 3 µg/ml               | 0.142      |
| 6 µg/ml               | 0.291      |
| 9 µg/ml               | 0.441      |
| 12 µg/ml              | 0.521      |
| 15 µg/ml              | 0.616      |
| 18 µg/ml              | 0.735      |
| 21 µg/ml              | 0.861      |
| 24 µg/ml              | 0.971      |
| 27 µg/ml              | 1.191      |
| 30 µg/ml              | 1.301      |

Graph 1. Concentration response curve for Glucose at different concentration

\[ y = 0.0415x + 0.023 \]

\[ R^2 = 0.9913 \]
Table 7. Absorbance of standard albumin for total protein content

| Concentration (µg/ml) | Absorbance |
|-----------------------|------------|
| 2 µg/ml               | 0.051      |
| 4 µg/ml               | 0.081      |
| 6 µg/ml               | 0.12       |
| 8 µg/ml               | 0.131      |
| 10 µg/ml              | 0.16       |
| 12 µg/ml              | 0.19       |
| 14 µg/ml              | 0.213      |
| 16 µg/ml              | 0.248      |
| 18 µg/ml              | 0.273      |
| 20 µg/ml              | 0.271      |

Graph 2. Concentration response curve for Albumin at different concentration

3.3.2.3 Total saponin content

Total saponin content was determined by simple solubility method. The total saponin content of leaf methanol extract 02.19 ± 0.31 µg/ml.

3.3.2.4 Total steroid content

Total steroid content was determined by Liberman-Burchard reaction method using diosgenin as standard for preparation of calibration curve. The absorbances of standard diosgenin for determination of total steroid content were shown in Table 8. The total steroid content of petroleum ether and chloroform extract of leaf have 18.20 ± 0.07 and 08.05 ± 0.18 µg/ml respectively (Graph 3).

3.3.2.5 Total alkaloid content

Total alkaloid content was determined by bromocresol green reagent using atropin as standard for preparation of calibration curve. The absorbances of standard atropin for determination of total alkaloid content were shown in Table 9. The total alkaloid content of petroleum ether, chloroform and methanolic extract of leaf have 02.86 ± 0.12, 03.01 ± 0.04 and 04.89 ± 0.15 µg/ml respectively (Graph 4).

Table 8. Absorbance of standard diosgenin for total steroid content

| Concentration (µg/ml) | Absorbance |
|-----------------------|------------|
| 2 µg/ml               | 0.1512     |
| 4 µg/ml               | 0.1912     |
| 6 µg/ml               | 0.2156     |
| 8 µg/ml               | 0.2712     |
| 10 µg/ml              | 0.3324     |
| 12 µg/ml              | 0.3521     |
| 14 µg/ml              | 0.3864     |
| 16 µg/ml              | 0.4012     |
| 18 µg/ml              | 0.4121     |
| 20 µg/ml              | 0.5321     |
3.3.2.6 Total flavonoid content

Total flavonoid content was determined by aluminum chloride colorimetric method by using quercetin as standard for preparation of calibration curve. The absorbances of standard quercetin for determination of total flavonoid content were shown in Table 10. The total flavonoid content of chloroform and methanolic extract of leaf have 14.23 ± 0.09 and 17.60 ± 0.04 µg/ml respectively. (Graph 5).

Table 9. Absorbance of standard atropine for total alkaloid content

| Concentration (µg/ml) | Absorbance |
|-----------------------|------------|
| 1 µg/ml               | 0.015      |
| 2 µg/ml               | 0.025      |
| 3 µg/ml               | 0.029      |
| 4 µg/ml               | 0.041      |
| 5 µg/ml               | 0.051      |
| 6 µg/ml               | 0.062      |
| 7 µg/ml               | 0.075      |
| 8 µg/ml               | 0.091      |
| 9 µg/ml               | 0.101      |
| 10 µg/ml              | 0.115      |

3.3.2.7 Total tannin content

Total tannin content was determined by Folin Denis reagent by using tannic acid as standard for preparation of calibration curve. The absorbances of standard tannic acid for determination of total tannin content were shown in Table 11. The total tannin content of chloroform and methanolic extract of leaf have 09.07 ± 0.15, 12.03 ± 0.05 µg/ml respectively (Graph 6).
Table 10. Absorbance of standard quercetin for total flavonoid content

| Concentration (µg/ml) | Absorbance  |
|-----------------------|-------------|
| 2 µg/ml               | 0.0321      |
| 4 µg/ml               | 0.0412      |
| 6 µg/ml               | 0.0698      |
| 8 µg/ml               | 0.1141      |
| 10 µg/ml              | 0.1301      |
| 12 µg/ml              | 0.1641      |
| 14 µg/ml              | 0.1901      |
| 16 µg/ml              | 0.2131      |
| 18 µg/ml              | 0.2511      |
| 20 µg/ml              | 0.2901      |

Graph 5. Concentration response curve for quercetin at different concentration

Table 11. Absorbance of standard tannic acid for total tannin content

| Concentration (µg/ml) | Absorbance  |
|-----------------------|-------------|
| 2 µg/ml               | 0.1701      |
| 4 µg/ml               | 0.2501      |
| 6 µg/ml               | 0.3198      |
| 8 µg/ml               | 0.4256      |
| 10 µg/ml              | 0.481       |
| 12 µg/ml              | 0.5281      |
| 14 µg/ml              | 0.601       |
| 16 µg/ml              | 0.6335      |
| 18 µg/ml              | 0.6864      |
| 20 µg/ml              | 0.7351      |

Graph 5. Concentration response curve for tannic acid at different concentration
3.3.2.8 Total phenolic content

Total phenolic content was determined by Folin-Ciocalteu method by using gallic acid as standard for preparation of calibration curve. The absorbances of standard gallic acid for determination of total phenolic content were shown in Table 12. The total phenolic content of chloroform and methanolic extract of leaf have 15.36 ± 0.09, 16.05 ± 0.02 µg/ml respectively (Graph 6).

3.4 Anti-oxidant Activity

3.4.1 DPPH scavenging activity

DPPH scavenging activity was performed on different extracts (10 µg/ml to 100 µg/ml) of Tubiflora acaulis Kuntze by using Ascorbic acid as standard antioxidant (10 µg/ml to 100 µg/ml). The result shows that DPPH scavenging activity is directly proportional to all extracts and ascorbic acid. (Table 13) The IC₅₀ of petroleum ether, chloroform, methanolic and ascorbic acid was found to be 85.00, 64.55, 37.09 and 33.39 µg/ml respectively (Graph 7).

3.4.2 Nitric oxide scavenging activity

Nitric oxide scavenging activity was performed on different extracts (10 µg/ml to 100 µg/ml) of Tubiflora acaulis Kuntze by using Ascorbic acid as standard antioxidant (10 µg/ml to 100 µg/ml). The result shows that Nitric oxide scavenging activity is directly proportional to all extracts and ascorbic acid. (Table 14.) The IC₅₀ of petroleum ether, chloroform, methanolic and ascorbic acid was found to be 89.83, 76.30, 57.71 and 48.38 µg/ml respectively (Graph 8).

3.4.3 Reducing power assay

Reducing power assay was performed on different extracts (10 µg/ml to 100 µg/ml) of Tubiflora acaulis Kuntze by using Ascorbic acid as standard antioxidant (10 µg/ml to 100 µg/ml). The result shows that absorbance of Reducing power assay is directly proportional to concentration in all extracts and ascorbic acid. (Table 15) The increase in absorbance indicates highest reducing power which also describe graphically in Graph 9.

The above results indicate that methanol extract have significant anti-oxidant activity as compare to other (Table 16). The quantitative phytochemical analysis for different extract of Tubiflora acaulis Kuntze reveals that methanolic extract contain high concentration of flavonoids, tannins and phenolic compounds as compare to other.

Table 12. Absorbance of standard gallic acid for total phenolic content

| Concentration (µg/ml) | Absorbance |
|-----------------------|------------|
| 2 µg/ml               | 0.031      |
| 4 µg/ml               | 0.059      |
| 6 µg/ml               | 0.101      |
| 8 µg/ml               | 0.119      |
| 10 µg/ml              | 0.141      |
| 12 µg/ml              | 0.181      |
| 14 µg/ml              | 0.219      |
| 16 µg/ml              | 0.243      |
| 18 µg/ml              | 0.256      |
| 20 µg/ml              | 0.271      |

Table 12. Quantitative phytochemical analysis of various extract of Tubiflora acaulis Kuntze (Acanthaceae) leaf

| Sr. No. | Parameter | Pet. ether Extract | Chloroform Extract | Methanol Extract |
|---------|-----------|--------------------|--------------------|------------------|
| 1       | Carbohydrate | -                  | -                  | 07.39 ± 0.06     |
| 2       | Protein    | -                  | -                  | 04.98 ± 0.19     |
| 3       | Saponin    | -                  | -                  | 02.19 ± 0.31     |
| 4       | Steroids   | 18.20 ± 0.07       | 08.05 ± 0.18       | -                |
| 5       | Alkaloids  | 02.86 ± 0.12       | 03.01 ± 0.04       | 04.89 ± 0.15     |
| 6       | Flavonoids | -                  | 14.23 ± 0.09       | 17.60 ± 0.04     |
| 7       | Tannins    | -                  | 09.07 ± 0.15       | 12.03 ± 0.05     |
| 8       | Total Phenolic | -                | 15.36 ± 0.09       | 16.05 ± 0.02     |
Table 13. DPPH scavenging activity of different extracts of *Tubiflora acaulis* Kuntze leaf

| Concentration (µg/ml) | % Scavenging activity of Leaf extract |  |  |  |
|----------------------|-------------------------------------|----------------|----------------|----------------|
| 10 µg/ml             | 36.33 + 0.54                        | 37.08 + 0.25   | 43.33 + 0.77   | 44.15 + 0.41   |
| 20 µg/ml             | 36.13 + 0.54                        | 39.45 + 0.46   | 47.08 + 0.63   | 46.34 + 0.61   |
| 30 µg/ml             | 39.65 + 0.48                        | 42.66 + 0.28   | 49.75 + 0.75   | 48.74 + 0.73   |
| 40 µg/ml             | 42.29 + 0.41                        | 46.28 + 1.18   | 51.06 + 1.27   | 52.34 + 0.74   |
| 50 µg/ml             | 43.15 + 0.52                        | 48.62 + 0.36   | 53.47 + 0.56   | 54.19 + 0.50   |
| 60 µg/ml             | 45.33 + 0.61                        | 50.05 + 0.26   | 54.02 + 1.38   | 57.52 + 0.43   |
| 70 µg/ml             | 46.86 + 0.68                        | 51.94 + 0.69   | 54.14 + 0.79   | 58.21 + 0.58   |
| 80 µg/ml             | 47.33 + 0.35                        | 52.92 + 0.80   | 58.82 + 0.94   | 60.3 + 0.26    |
| 90 µg/ml             | 50.27 + 0.26                        | 53.05 + 0.57   | 60.10 + 0.59   | 62.72 + 0.85   |
| 100 µg/ml            | 55.02 + 0.49                        | 57.57 + 0.38   | 63.91 + 0.68   | 62.64 + 1.03   |

Graph 6. Concentration response curve for gallic acid at different concentration

Graph 7. DPPH scavenging activity of different extracts of *Tubiflora acaulis* Kuntze leaf
Table 14. Nitric oxide scavenging activity of different extracts of *Tubiflora acaulis* Kuntze leaf

| Concentration (µg/ml) | % Scavenging activity of Leaf extract | Pet. Ether extract | Chloroform extract | Methanolic extract | Ascorbic acid |
|-----------------------|--------------------------------------|--------------------|--------------------|--------------------|---------------|
| 10 µg/ml              | 36.06 + 0.73                        | 37.16 + 1.02       | 41.51 + 1.22       | 39.82 + 0.35       |
| 20 µg/ml              | 37.82 + 1.06                        | 40.76 + 0.75       | 43.55 + 0.70       | 44.86 + 1.02       |
| 30 µg/ml              | 40.91 + 1.07                        | 42.18 + 0.43       | 46.68 + 0.33       | 46.66 + 0.29       |
| 40 µg/ml              | 42.13 + 0.39                        | 42.32 + 0.54       | 46.29 + 0.64       | 48.67 + 0.35       |
| 50 µg/ml              | 42.95 + 0.11                        | 45.24 + 0.58       | 47.65 + 0.21       | 49.65 + 0.29       |
| 60 µg/ml              | 43.25 + 0.81                        | 46.24 + 0.57       | 49.68 + 1.27       | 50.46 + 0.59       |
| 70 µg/ml              | 44.62 + 0.73                        | 50.56 + 0.82       | 53.31 + 0.23       | 54.37 + 0.47       |
| 80 µg/ml              | 47.86 + 0.64                        | 51.25 + 0.55       | 53.53 + 0.84       | 58.23 + 0.63       |
| 90 µg/ml              | 50.36 + 0.71                        | 52.37 + 0.56       | 55.26 + 0.58       | 59.44 + 0.48       |
| 100 µg/ml             | 53.48 + 0.42                        | 53.43 + 0.91       | 58.26 + 1.16       | 64.21 + 1.21      |

Graph 8. Nitric oxide scavenging activity of different extracts of *Tubiflora acaulis* Kuntze leaf

Table 15. Absorbance of reducing power assay of different extracts of *Tubiflora acaulis* Kuntze leaf

| Concentration (µg/ml) | % Scavenging activity of Leaf extract | Pet. Ether extract | Chloroform extract | Methanolic extract | Ascorbic acid |
|-----------------------|--------------------------------------|--------------------|--------------------|--------------------|---------------|
| 10 µg/ml              | 0.051 + 0.001                        | 0.052 + 0.002      | 0.044 + 0.004      | 0.055 + 0.002     |
| 20 µg/ml              | 0.054 + 0.001                        | 0.057 + 0.003      | 0.054 + 0.002      | 0.06 + 0.004     |
| 30 µg/ml              | 0.055 + 0.001                        | 0.061 + 0.001      | 0.059 + 0.001      | 0.068 + 0.002     |
| 40 µg/ml              | 0.057 + 0.001                        | 0.065 + 0.002      | 0.063 + 0.003      | 0.072 + 0.000     |
| 50 µg/ml              | 0.057 + 0.002                        | 0.067 + 0.009      | 0.068 + 0.009      | 0.079 + 0.001     |
| 60 µg/ml              | 0.064 + 0.002                        | 0.071 + 0.008      | 0.068 + 0.001      | 0.082 + 0.002     |
| 70 µg/ml              | 0.062 + 0.001                        | 0.074 + 0.001      | 0.079 + 0.000      | 0.086 + 0.001     |
| 80 µg/ml              | 0.066 + 0.001                        | 0.084 + 0.002      | 0.081 + 0.001      | 0.098 + 0.002     |
| 90 µg/ml              | 0.079 + 0.002                        | 0.086 + 0.004      | 0.099 + 0.001      | 0.104 + 0.003     |
| 100 µg/ml             | 0.081 + 0.003                        | 0.09 + 0.000       | 0.103 + 0.005      | 0.109 + 0.006    |

Table 16. IC$_{50}$ Value of different extracts of *Tubiflora acaulis* Kuntze leaf

| Method of evolution                          | Leaf extract | Pet. Ether extract | Chloroform extract | Methanolic extract | Ascorbic acid |
|---------------------------------------------|--------------|--------------------|--------------------|--------------------|---------------|
| DPPH Scavenging activity                    | 85.00        | 64.55              | 37.09              | 33.39              |
| Nitric oxide scavenging activity            | 89.83        | 76.30              | 57.71              | 48.38              |
| Reducing power assay*                       | 0.081        | 0.09               | 0.103              | 0.109              |

* indicate absorbance value
4. CONCLUSION

Pharmacognostic studies and phytochemical screening can serve as a basis for proper identification of a plant. Medicinal plants are the local heritage with the global importance. World is endowed with a rich wealth of medicinal plants. The transverse section of the fruit of Tubiflora acaulis Kuntze shows the presence of Upper and lower epidermis, palisade cell along with collenchyma, xylem and phloem, vascular strand and calcium crystals. Phytochemical analysis of Tubiflora acaulis Kuntze of Petroleum ether extract shows steroids, the chloroform extract shows Saponin glycosides, alkaloids, the methanolic extract shows saponins, alkaloids, glycosides, flavonoids, tannins, carbohydrates, proteins and amino acids, steroids. Compared to other extracts methanolic extract showed more number of phytochemical constituents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The permission was taken from Maharashtra State Biodiversity Board, Nagpur for plant collection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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