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Biochemical and Phytochemical Constituents of Stem of *Toddalia asiatica*. L, A Wild Woody Liana

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ABSTRACT

In India, many indigenous plants are used in herbal medicine to cure diseases and heal injuries. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. *Toddalia asiatica* (L.) Lam. (Rutaceae) is widely used as a folk medicine in India. The roots are used in the treatment of indigestion and influenza and the leaves to treat lung diseases and rheumatism. The root and its bark have been used to treat fever, malaria, cholera, diarrhoea and rheumatism [1]. Despite the use of this plant for such purposes, there is little information on the biochemical composition of *Toddalia asiatica*. L.Var . floribunda.. This work is therefore aimed at documenting the biochemical compositions of *Toddalia asiatica*. L. Var . floribunda. From the earlier reports qualitative phytochemical results reveals that the methanolic stem extract of *Toddalia asiatica.L.* contain secondary metabolites like Alkaloids, Flavonoids, Saponins, Steroids, Glycosides, Tannins. From the quantification result of various biochemical and phytochemical constituents like carbohydrates, proteins, total ascorbic acid, phenols, tannins and flavanoids content, it was noted that alkaloid found to be the highest with phenol as the next active constituents among the secondary metabolites in the stem. From the results it reveals *Toddalia asiatica* has the potential to be represented as an effective biochemical and therapeutic constituent for the future use in the medical field.

Keywords: *Toddalia asiatica*, ascorbic acid, alkaloids, phenols, tannins, flavanoids.

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INTRODUCTION

India is called the botanical garden of the world for its wide spread natural resources. Over 6000 plants in India are used in traditional, folklore and herbal medicine has been identified. Among 1500 medicinal plants, 500 are commonly used [2]. These plants are gifts of nature to cure limitless number of diseases among human beings and other living organisms [3]. Some important chemical components found in plants are alkaloids, carbon compounds, hydrogen, nitrogen, glycosides, essential oils, fatty oils, resins, mucilage, tannins, gums and others [4]. Most of these are potent bioactive compounds found in different parts of medicinal plant and can be used for therapeutic purpose or as precursors for the synthesis of useful drugs [5]. *Toddalia asiatica* is native of tropical Asia & Africa. All the parts of the plant are very pungent, especially the roots when freshly cut. The fresh barks of the root are administered for the cure of hill fever. The fresh leaves are eaten raw for pain in the bowels. The medicinal plants were nutritionally important. Even though the young leaves and shoots of this plant is popularly consumed as tonic vegetables in traditional cuisines, there are no reports that provide information on the quantitative analysis of their active components, especially the phenolic content, which has been reported to be related to antioxidant activity [6]. In order to explore the nutritive and therapeutic potential of *Toddalia asiatica,* the biochemical estimations are done. This work is aimed at documenting the biochemical and phytochemical composition of *Toddalia asiatica*. Var . floribunda stem.

MATERIALS AND METHOD

Plant material

The plant material (stem) of *Toddalia asiatica*, a woody liane was collected at Kolli hills, Namakkal District which is rich in wide variety of medicinal flora. The collected sample was identified and confirmed by BSI, Coimbatore. The stem part was air dried and powdered and was used for estimations.

Preparation of the methanolic extract

10 grams pulverized material were dissolved in 100 ml of solvent methanol and kept in a shaker for overnight. The obtained extract was filtered with Whatmann No.4 filter paper and the filtrate was collected and used for quantitative analysis of phytochemicals.

Biochemical Estimations

Estimation of protein [7]

Weigh 2g of the sample and grind well with a pestle and mortar in 10 ml of the phosphate buffer. Centrifuge the homogenate at 1000 rpm for 20 minutes and use the supernatant. The total protein content of the stem was determined by Lowry's method.

Estimation of total carbohydrate [8]

Weigh 100mg of the sample into a boiling tube. Hydrolysis was done by keeping it in a boiling water bath for three hours with 5ml of 2.5N hydrochloric acid and cool at room temperature. Neutralise it with solid sodium carbonate until effervescence stops. Make up to the volume to 100ml centrifuge. Collect the supernatant and take 0.5 and 1.0 ml aliquot for analysis. Then add 4ml of anthrone reagent. Heated for 8 minutes in a boiling water bath. Cool rapidly and read the green to dark green colour at 630nm.

Estimation of ascorbic acid

Ascorbic acid content in plant material was estimated as per the method described by Sadasivam and Manikam [9]. 50mg plant material was homogenized in 10 ml 4% oxalic acid and centrifuged at 5000 rpm for 15 min. The supernatant were collected and bromine water was added drop wise with constant stirring to give a yellow color. The excess bromine was expelled by blowing in air with a pipette and made the final volume 25 ml with 4% oxalic acid. 2 ml of brominated extract was adjusted to 3 ml with distilled water. This was allowed to react with 1 ml of 2% DNPH filtered and used, followed by 1-2 drops of Thiourea (10%). Blank was prepared as above with distilled water in the place of Ascorbic acid or extract and incubated at 37^{o}C for three hours. The orange red Osazone crystals were dissolved by adding 7 ml of 80% H_2SO_4. The absorbance was measured at 540 nm using UV-Vis spectrophotometer. The results were expressed as milligrams of ascorbic acid equivalent per gram of dry weight.

Estimation of phenols [10]

Phenolic compounds in the methanolic extract of stem was estimated by colorimetric assay, based on the procedure described by the Singleton and Rossi with some modifications.1 ml of sample was mixed with 1 ml of folin's ciocalteau phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 ml with distilled water. The reaction was kept in dark for 90 min, after which the absorbance was read at 725 nm using spectrophotometer. Tannic acid was used as the standard and the results were expressed as mg of tannic acid equivalents per g of extract.

Estimation of flavanoids [11]

Flavanoids content in the methanolic stem extract was estimated by aluminium chloride colorimetric method. To 4 ml of the sample, 0.3 ml of 5% sodium nitrite and after 5 min 0.3 ml of

10% aluminium chloride was added and kept for incubation for 6 mins. Then 2 ml of 1M sodium hydroxide was added. The volume was made upto 10 ml with distilled water. Read the absorbance at 510 nm.

Estimation of tannins [12]

The tannin content in the samples was estimated by the method of Price and Butler [13] with modifications. 60mg of plant sample was shaken constantly for 60 s with 3ml of methanol in a test tube and filtered. The tube was quickly rinsed with an additional 3 ml of methanol and the contents were poured at once into the filtering funnel. The filtrate volume was made up to 50 ml with distilled water and analyzed within an hour. 3ml of 0.1 M $FeC1_3$ in 0.1 N HCl was added to the extract, followed immediately by timed addition of 3ml of 0.008 M $K_3Fe(CN)_6$. The absorbance was measured after 10 min spectrophotometrically at 720 nm. The tannin content was expressed for the samples as tannic acid equivalence.

Estimation of Tocopherol [14]

The plant sample (2.5g) were homogenized in a small volume of 0.1N sulphuric acid and the volume was finally made up to 50 ml by adding 0.1N sulphuric acid slowly, without shaking and the contents were allowed to stand overnight. The contents of the flask were shaken vigorously on the next day and filtered through Whatman No.1 filter paper. Aliquots of the filtrate were used for the estimation Into 3 stoppered centrifuge tubes, 1.5ml of plant tissue extract, 1.5ml of the standard and 1.5ml of water were pipetted out respectively. To all the tubes,1.5ml of ethanol and 1.5ml of xylene were added, mixed well and centrifuged. 0.1ml of the xylene layer was transferred into another stoppered tube and 0.1ml of 2, 2'dipyridyl reagent was added to each tube and mixed. Pipetted out 1.5ml of the mixture into a spectrophotometer cuvette and the extinction was read at 460nm. 0.33ml of ferric chloride solution was added and mixed well, and after exactly 15 minutes, the absorbance of the red colour produced was read against a blank at 520nm.

Extraction and determination of Alkaloids [15]

Alkaloids were extracted by well established methods after preliminary detection of alkaloids. The result obtained was presented in the previous article as 3.3 ± 0.22 g/100g [16].

RESULTS AND DISCUSSION

The plants produce natural source of primary metabolites namely the biochemical constituents for their growth and function. Secondary metabolites are sought because they are known to exhibit numerous protective effects that promote positive health effects.

The quantitative determination of biochemical constituents of *Toddalia asiatica*. Var floribunda. was done and the results are summarized in Table 1 and that of phytochemical constituents in Table 2.

Table 1: Biochemical constituents of *Toddalia asiatica* stem

Biochemical constituents	Result
Carbohydrate	26±0.816mg/gm
Total Proteins	29±0.801 mg/gm

Results are mean triplicate value on a dry weight basis ± standard deviation

Table 2: Phytochemical constituents of *Toddalia asiatica* stem

Phytochemical constituents	Results
Ascorbic Acid	3.2 ± 0.58 ascorbic acid equivalence (mg/g)
Phenols	18 ± 0.816 Tannic acid equivalence (mg/g)
Flavanoids	0.020 ± 0.004 catechol equivalence (mg/g)
Tannins	1.05 ± 0.04 Tannic acid equivalence (mg/g)
Tocopherol	9 ± 0.816 (µg/g)

Results are mean triplicate value on a dry weight basis ± standard deviation

The estimation of carbohydrates plays an important part in plant physiology. Carbohydrates are synthesized in plants from a series of reactions involving photosynthesis and are then utilized by animals and humans in metabolism to produce energy and other compounds. It plays important role in the cellular metabolic pathways [17]. Proteins play many critical roles and they have many potential therapeutic uses in preventing and curing diseases and disorders [18].

The quantification results of biochemical constituents namely, the total carbohydrate and proteins signifies that the *Toddalia asiatica* holds effective prospects for its nutritive value. It also indicates that *Toddalia asiatica* has the potentiality to be represented as an effective biochemical constituent for the future.

Plant produces many phytochemicals to protect themselves from bacteria and other predatorial invaders, but research has discovered that plants with phytochemical abilities may also protect humans from illness. Alkaloids are one of the diverse groups of secondary metabolites which found to have antimicrobial activity [19] and many other therapeutic actions. Flavanoids are one of the phenolic compounds and acts as natural biological modifiers. The biological functions of flavonoids apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors [20]. There exists a direct relationship between the levels of phenolic compounds and antioxidant prospective of plants [21]. Total ascorbic acid, phenols, tannins, vitamin E and flavanoids may constitute for the antioxidant and therapeutic value of the plant. From the results obtained from the stem of *Toddalia*

asiatica, alkaloids were found to be the highest with phenol as the next active constituents in the stem of the plant. These are the important secondary metabolites that act as bioactive compounds which can be used for therapeutic purpose. The biologically active compounds like alkaloids, flavonoids, tannins and phenolic compounds are the main reason for the medicinal value of plants that produce a definite physiological action on the body if it is administered.

CONCLUSION

A good and commandable store of carbohydrate content and the protein level in the T.A stem gives a positive indication that it holds a good store of energy rich use, and can be recommended for dietary supplementation in near future. From the result, it was proved that *Toddalia asiatica* Stem can have more advantageous effects with the presence of many active secondary metabolites like alkaloids, flavonoids, tannins which may likely to boost the immune system and to defend diseases like cancer, cardio-vascular diseases etc. So, the results of the above study conclusively validate the biochemical and phytochemical treasures indulged in *Toddalia asiatica.* Stem.

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