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Phytochemical analysis with reference to the root and leaf of *Toddalia asiatica* (L.) Lam. (Rutaceae)

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Abstract
Plants been used in traditional medicine for several thousand years. India is a home to a variety of traditional medicine systems that relay to a very large extent on native plant species for their raw drug materials. There are many reports on the use of plants in the traditional healing by either tribal people or indigenous communities of India. Now there is a need to look back owards the traditional medicine which can serve as novel therapeutics. *Toddalia asiatica*, has been in folklore use in India and China from 18th century. Since, this plant possess many medicinal properties, the present study was designed to evaluate the phytochemicals of stem extract of *Toddalia asiatica* L. The phytochemicals of stem extract of *Toddalia asiatica* L. was analysed qualitatively and the presence of some phytochemicals are confirmed by HPLC analysis. The results of the above study conclusively validate the phytochemical treasures indulged in *Toddalia asiatica.*

Keywords: *Toddalia asiatica*, methanolic extract, phytochemicals, leaf, root and HPLC

1. Introduction
Medicinal plants acts as a raw material base for the elaboration of more complex semi-synthetic chemical compounds. Many of these isolations from the medicinal plants were based on the uses of the agents in traditional medicine. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use. There has been a resurgence in the consumption and demand of medicinal plants. Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for the conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the future. Now there is a growing interest in correlating each phytochemical constituent of a plant with its pharmacological activity (Gupta, 1994) [1]. 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 (Hemashenpagam *et al*., 2009) [2].

Toddalia asiatica is known as 'Milagarani' in Siddha system of medicine it is knowm as Kanchana in Ayurveda. *Toddalia asiatica* a very variable rambling, prickely, sarmentose shrub, distributed almost throughout India, ascending to an altitude of 2,500m. In south India, the plants are very common in the Nilgiri and palani hills and also in the scrubby jungles of Orissa. In the plains, particularly in dry situations, the plant assumes the form of a low shrub with smaller and narrower leaflets. Plant contains coumarins toddaalone, toddanol norbraylin, and 5, 7, 8-trimethoxy coumarin.

Root contains benzophenanthridine alkaloid, hexacosanoic acid, Bsitosterol, arnottianamide. Root bark contains coumarins toddalenol, toddalosin, 5-methoxysuberenon, toddalenone, 8-formylli-alkaloidsbenzo(c)phenanthridine, alkaloids (des-N-Methylchelerythrine, oxychelerythrine, arnottianamide, oxyauicine, avicine, chelerythrine and chelerythrine psicyanide), quinoline alkaloids-N-methyl flindersine, 4-methoxy-1, methyl-2, quinolone, skimmianine, integriquinone, triterpenoid B-amyrin, toddalinine, pimpinellin, isopimpinellen, chelerythrine and dihydro chelerythrine. Leaves yield essential oil (Yuganarasimhan, 2000) [3]. Root-bark is used to cure diaphoretic stomachic and antipyretic. It considered being a potent antimalarial drug showing both antiperiodic, antipyretic effects similar to those of cinchona alkaloids. Leaves chewed for stomach disorders. Local tribes used this plant for multiple applications like stomach problems, fever, cough and cold. It is also used in the treatment of various ailments a cough, Influenza, indigestion, rheumatic arthritis, sprains, bronchitis, nausea, diarrhea, and chest pain (Orwa *et al*., 2008) [4]. *Toddalia asiatica* is used traditionally in Kenya by many communities for the treatment of malaria, toothaches.

2. Materials and Methods

2.1 Plant Material

Plant material of *Toddalia asiatica was* collected from Curzon Estate, Kotagiri - 643217, The Niligiris District, Tamil Nadu, during the month of December 2017. The plant specimen was identified with Gambles Flora of the Presidency of Madras and the identity is confirmed with the herbarium specimen deposited in Department of Botany, Periyar EVR College (Autonomous) Tiruchirappalli, Tamil Nadu.

2.2 Preparation of the Extract

Plant materials leaf and root was washed with distilled water and shade dried. The dried samples were manually ground to a fine powder. The plant materials was identified and authenticated by Botanical Survey of India (Southern Circle, Coimbatore Tamil Nadu, India).

A voucher specimen of both has been deposited for future reference in the Department of Botany Periyar EVR College (Autonomous), Tiruchirappalli - 620 023, Tamil Nadu. *Toddalia asiatica* leaves and root were chopped into small pieces, shade dried. Dried samples were powdered in a Wiley mill. Powdered samples were stored in polythene containers at room temperature. The leaves and root samples were taken for analysis to detect the presence of certain biologically active compound(s). The extract contains polar components of the material and 2µl sample of the solution was employed in HPLC for analysis of different compounds.

2.3 Extraction

50 g of *Toddalia asiatica* (Leaves) and (Root) coarse sample using Soxhlet method, extraction 24 hrs and using Methanol (MT) solvent.

2.4 Preliminary phytochemical screening

The condensed extracts of different solvent used for preliminary phytochemical screening were carried out using standard procedures to test the presence of bioactive compounds (Amarasingham *et al*., 1964) [3], (Chabra *et al*., 1984) [4], Harborne (1984) [5].

Qualitative Analysis of phtyochemicals: The analysis of phytochemicls from the solvent free extract of *Toddalia asiatica* leaves was individually carried out using various qualitative test for alkaloids, flavonoids, protein, amino acid, tannins, phenolics, glycosides, saponins and carbohydrates compounds. Extraction of phytochemicals. The individual phytochemical was extracted in the appropriate solvent and stored in air tight containers at 4 ºC till further use.

2.5 Test for alkaloids

The small portion of extract were stored separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal agents, such as Mayer's reagent (White precipitate or turbidity). Test for flavonoids Shinoda test One ml of the extract was treated with magnesium turnings and 1-2 drops of concentrated Hcl. Formation of pink or red colour shows the presence of flavonoids.

2.6 Test for protein and amino acids

Ninhydrin test: One ml of the extract, 2 drops of freshly prepared 0.2 per cent ninhydrin reagent was added and heated. The appearance of blue colour indicates the presence of proteins, peptides or amino acids.

2.7 Test for tannins and phenolic compounds

One ml of the extract was treated with few ml of the gelatin solution; a white precipitate reveals the presence of tannins and phenolic compounds.

2.8 Test for glycosides Legal test

The extract was dissolved in pyridine and freshly prepared sodium nitoprusside solution was added. The formation of pink to red colour indicates the presence of glycosides.

2.9 Test for saponins

To 1 ml of the extract, alcoholic vanillin solution and a few drops of concentrated sulphuric acid were added. A deep violet colour confirms the presence of saponins. Test for carbohydrates Benedict's test Five ml of Benedict's solution was added to the extract and boiled in water bath. The appearance of red yellow or green precipitate indicates the presence of reducing sugars.

2.10 Maceration

Powdered dried leaves (1g) and root (1 g) were macerated with methanol: water (1:1; v/v, 10 mL) and left at rest (7 days, room temperature). The material was filtered and the crude extract obtained was analyzed directly by HPLC-UV. This procedure was repeated in triplicate.

3. Results and discussion

It was observed when similar kind of weight of sample was taken the analysed herbs shows the peak area maximum in case of mint. The rest of herbs showed lower content. So mint can be used as a rich source of Plant contains coumarins toddaalone, toddanol norbraylin, and 5, 7, 8-trimethoxy coumarin. Phytochemical screening of the test was performed on methanol extract of *Toddalia asiatica* leaves **(**Table 1, 2, 3, 3.1 and Figure 2.1 and 3.2).

Table 1: Preliminary phytochemical analysis of Leaf powder extracts of *Toddalia asiatica*

Extract Name	Constituents
Methanol	Alkaloids
	Anthraquinone
	Phenol
	Quinine
	Steroid
	Tannins
	Terpenoid
	Reducing sugar
	Xanthoprotein
	Fixed oil

Table 2: HPLC analysis of leaf extract of *Toddalia asiatica*

Peak #	Ret. Time	Area	Area%
1	1.380	6626222	91.450
2	1.995	11401	0.157
3	2.620	2796	0.039
4	2.941	15355	0.212
5	3.595	60080	0.829
6	4.495	171510	2.367
7	5.137	54559	0.753
8	5.515	35838	0.495
9	5.914	72924	1.006
10	6.363	72069	0.995
11	7.192	9975	0.138
12	7.360	15937	0.220
13	7.469	5448	0.075
14	7.552	6329	0.087
15	7.707	10627	0.147
16	7.814	7261	0.100
17	7.864	5714	0.079
18	7.947	4014	0.055
19	8.032	6847	0.095
20	8.117	8354	0.115
21	8.213	2600	0.036
22	8.258	7527	0.104
23	8.459	11256	0.155
24	8.630	5282	0.073
25	8.949	7829	0.108
26	9.237	5827	0.080
27	9.397	1047	0.014
28	13.376	1083	0.015
	Total	7245709	100.000

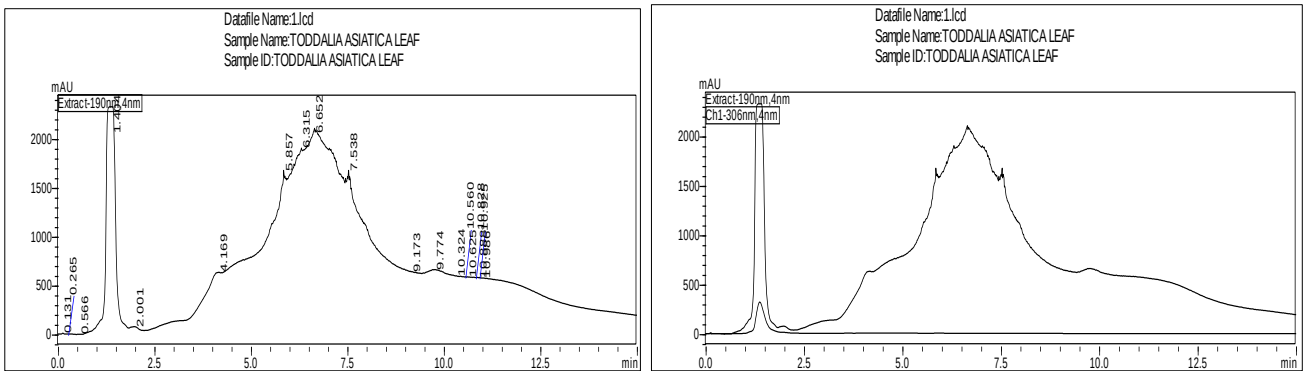

Fig 1: HPLC analysis of leaf extract of *Toddalia asiatica*

Table 3: Preliminary phytochemical analysis of Root powder extracts of *Toddalia asiatica*

Extract Name	Constituents
Methanol	Alkaloids
	Catechin
	Flavonoid
	Phenol
	Steroids
	Reducing sugar
	Glycoside

Table 4: HPLC analysis of root extract of *Toddalia asiatica*

Peak #	Ret. Time	Area	Area%
1	0.404	1183	0.003
2	1.365	34295288	99.961
3	2.267	12308	0.036
	Total	34308779	100.000

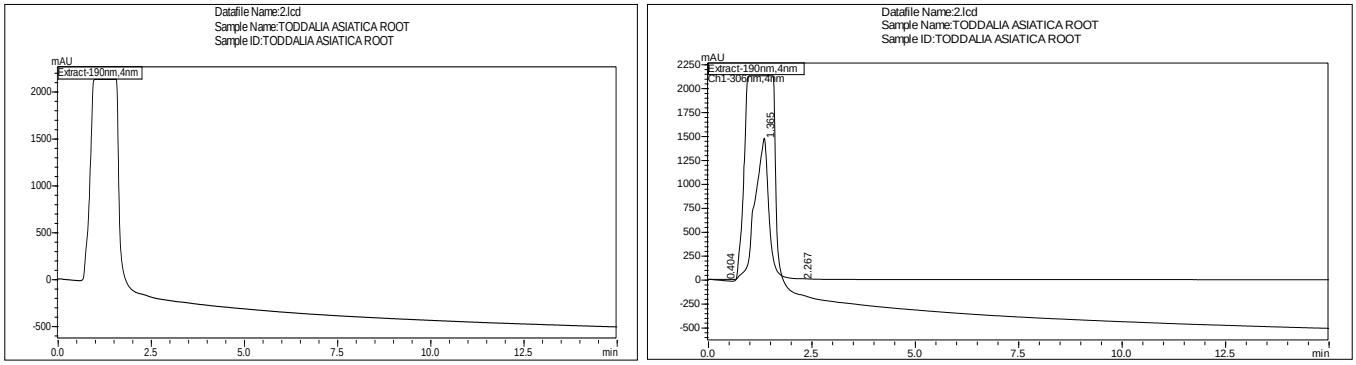

Fig 2: HPLC analysis of root extract of *Toddalia asiatica*

3. Conclusion

In conclusion, from the results of the present investigation, it could be inferred that *Toddalia asiatica* leaf and root is found to have significant medicinal activities. Phytochemical screening and HPLC study substantiate that *Toddalia asiatica* Leaf and root methanol extract contain pharmacologically active principles. The phytochemical analysis of the extracts revealed the existence of various constituents including steroids, terpenoids, esters, acids, tannins etc. The active constituent needs to be isolate and should be considered for further *in vivo* or *in vitro* studies to confirm the tradition. Biological study inferred that, seed extract active as anti-oxidant.

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