ISSN 2320-3862
JMPS 2016; 4(4): 282-286

Received: 27-05-2016
Accepted: 28-06-2016

Kavitha Chandran CI
Department of Pharmacognosy, Govt. Medical College, Thiruvananthapuram, Kerala State, India.

Indira G
Department of Pharmacognosy, Govt. Medical College, Thiruvananthapuram, Kerala State, India.

Correspondence
Kavitha Chandran CI
Department of Pharmacognosy, Govt. Medical College, Thiruvananthapuram, Kerala State, India.

Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of *Strobilanthes Kunthiana* (Neelakurinji)

Kavitha Chandran CI and Indira G

Abstract
Strobilanthes kunthiana (Nees) (Neelakurinji) is a shrub that is found in the shola forests of the Western Ghats in South India. Present study was to estimate total phenolic, flavanoids, tannin and chlorophyll content of leaves of *Strobilanthes kunthiana*. Total phenolic content was assessed using Folin-Ciocalteu's method. Estimation of Total Flavanoids was carried out by aluminium chloride colorimetric method. Tannic acid was used as a standard and the total tannin content were expressed as tannic acid equivalents (TAE). Absorbance was measured using a spectrophotometer at 700nm. The chlorophyll content in the sample was estimated by Arnon's method. These studies showed that the aqueous extract of leaves of *Strobilanthes kunthiana* have statistically significant amount of phenolic, flavonoid and tannin content as compared to ethanolic extract.

Keywords: *Strobilanthes kunthiana,* flavonoid, tannin, chlorophyll, phenolic

Introduction
India has an ancient heritage of traditional medicine. Materia Medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products [1, 2]. Even though such plants are used by traditional physicians, a scientific study of many plants is lacking. *Strobilanthes kunthiana* (Nees) is a shrub that is found in the shola forests of the Western Ghats in South India [3, 4]. Nilgiri Hills, which literally means the blue mountains, got their name from the purplish blue flowers of Neelakurinji that blossoms gregariously only once in 12 years. Paliyan tribal people apparently used it to calculate their age[5]. This plant belongs to the genus *Strobilanthes* which was first scientifically described by Christian Gottfried Daniel Nees von Esenbeck in the 19th century [6, 7]. The plant *Strobilanthes kunthiana* (Nees) (Neelakurinji) belonging to family Acanthaceae, so far not much has been done on leaves of this plant. The leaves of *Strobilanthes kunthiana* (Neelakurinji) has been selected for the present research work. Present study was to estimate total phenolic, flavanoids, tannin and chlorophyll content of leaves of *Strobilanthes kunthiana.*

Materials and methods
Plant material
The plant specimens (leaves) for the proposed study were collected from localities of Devikulam, Moonar, Iduki Dist. The Collected plants were carefully examined and authenticated by Dr. G.Valsala Devi, Curatior, Professor and Head, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. A voucher specimen (Voucher No.KUBH-5868) has been deposited for future reference.

Preparation of *Strobilanthes kunthiana leaves* extract
The collected leaves were washed with running tap water to remove adhering materials and cut into small pieces. Then, the leaves were dried at a temperature not exceeding 50 °C. These dried materials were cut into small pieces and then pulverized mechanically into coarse powder. The fine powder was separated by passing through sieve No: 60. The coarse powder obtained by passed through the sieve No. 18. Then this coarse powder was extracted by macerated with different solvents like ethyl alcohol and distilled water. The different extracts were obtained in rotary evaporator. The semisolid mass of ethanolic and aqueous extract was stored in a dessicator.

Quantitative Estimation

Estimation of Total Chlorophyll content [8-10]

Procedure

The chlorophyll content in the sample was estimated by Arnon's method. A known quantity of fresh tissue was homogenized in 10 ml 80% acetone. The homogenate was filtered, centrifuged at 5,000 rpm at 5 min. The supernatant was collected and made up to 5 ml, read the absorbance at 645 nm and 663 nm against the blank. The amount of chlorophyll present in the sample was calculated by Arnon's formula.

Chl a (mg g-1) = [(12.7 × A663) - (2.6 × A645)] × ml acetone / mg leaf tissue

Chl b (mg g-1) = [(22.9 × A645) - (4.68 × A663)] × ml acetone / mg leaf tissue

Total Chl = Chl a + Chl b.

Estimation of Total Phenolic Content [11-14]

The total phenolic content of dry extracts was performed with Folin-Ciocaltaeu assay. 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin Ciocalteu's phenol reagent. After 5 minutes, 10 ml of 7% sodium carbonate solution was added to the mixture followed by the addition of 13ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 minutes at 23 ^{0}C, after which the absorbance was read at 760 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE)/g of dried sample.

Estimation of Total Tannin Content [15, 16]

The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 mi of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. a set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Estimation of Total Flavonoid Content [17-19]

Procedure

Preparation of standard solution

10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100mcg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 25, 50, 75, 100, 125 and 150 mcg/ml were prepared. To each of these 4ml water was added followed by 0.3ml of 5% sodium nitrite. After 5min 0.3ml of 10% Aluminium chloride solution and at the 6th minute 2ml of 1M Sodium hydroxide was added. The total volume was made up to 10ml with distilled water. A blank was prepared without addition of aluminium chloride solution. The solutions were mixed well and the absorbance was measured against the blank at 510nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

Preparation of sample solution

The total flavanoids content of each plant extract was estimated by method described by Zhishen *et al*. Based on this method, each sample (1.0 ml) was mixed with 4 ml of distilled water and subsequently with 0.30ml of NaNO2 solution (10%). After 5 min, 0.30ml AlCl3 solution (10%) was added followed by 2.0ml of NaOH solution (1%) to the mixture. Immediately the mixture was thoroughly mixed and absorbance was then determined at 510 nm versus blank. Standard curve of quercetin was prepared (0-12 mg/ml) and the results were expressed as quercetin equivalents (mg quercetin/ g dried extract).the estimation of flavonoid content was carried out in triplicate.

Results

Preliminary Phytochemical screening of successive extracts indicated the presence of Flavanoids, Saponins, Tannins, Carbohydrates, Terpenoids and Steroids in *Strobilanthes kunthiana* leaves.

Estimation of Chlorophyll content.

Quantitative estimation of chlorophyll content was carried out by Arnon's method. Absorbance was taken at 645 and 663 nm.

Table 1: Absorbance of the sample

Wave length in nm	Absorbance of the sample
645	1.2815±0.0003
663	2.0360±0.0005

Table 2: Amount of Chlorophyll present *S. kunthiana*

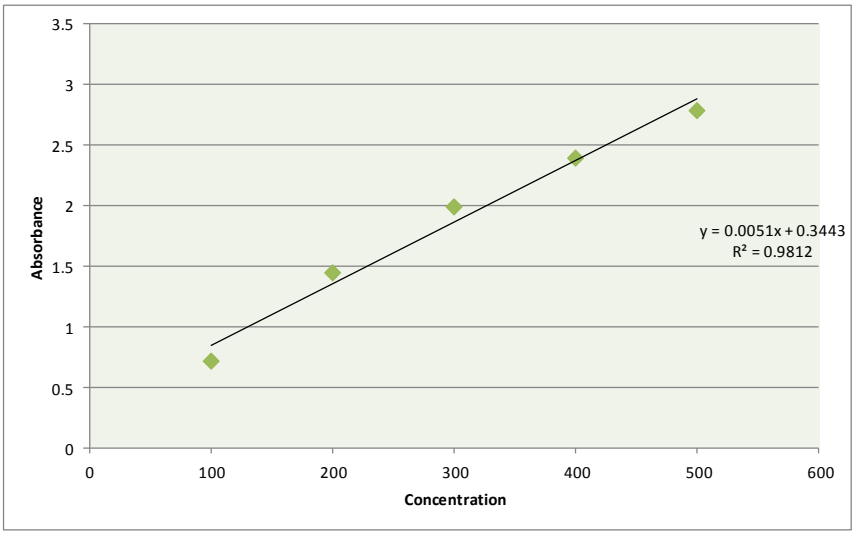
Sl.No.	Type of Chlorophyll	Amount(mg/L)
1	Chlorophyll a	0.5602±0.0004
2	Chlorophyll b	0.4954±0.0003
3	Total Chlorophyll	1.0553±0.0003

Estimation of Total Phenolic Content

Table 3: Absorbance of standard and sample at 760nm

Drug	Concentration (µg/ml)	Absorbance
Gallic acid [standard]	100	0.7214 ± 0.0001
	200	1.4453 ± 0.0002
	300	1.9916 ± 0.0005
	400	2.394 ± 0.0009
	500	2.7861 ± 0.0004
S.kunthiana ethanolic extract	100	0.0347±0.0005
S.kunthiana Aqueous extract	100	0.2313±0.0001

n= 3, values are given in SEM



Graph 1: Standard curve of Gallic Acid

Table 4: Total Phenolic Content of different extracts of leaves of *Stroblanthes kunthiana.*

Sample	Total phenolic content GAE mcg/ml
Ethanolic extract 100µg/ml	22.54± 0.101
Aqueous extract 100µg/ml	61.86 ± 0.125

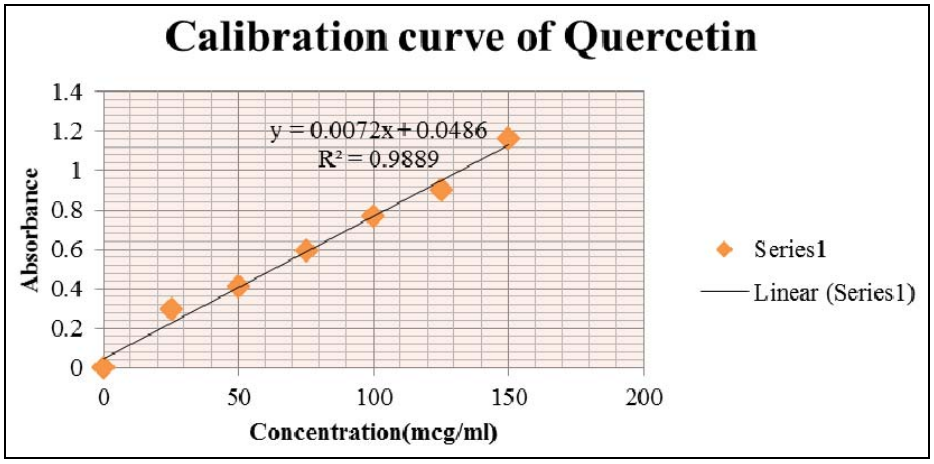
n=3, values are given in SEM

Estimation of Total Flavonoid Content

Table 5: Absorbance of standard and different extracts

Sl. No	Concentration of Quercetin (µg/ml)	Mean absorbance
1	25	0.296 ± 0.0002
2	50	0.412 ± 0.0002
3	75	0.592 ± 0.0003
4	100	0.769 ± 0.0001
5	125	0.902 ± 0.0005
6	150	1.163 ± 0.0002
7	*S.kunthiana* ethanolic extract (100µg/ml)	0.1667±0.0002
8	*S.kunthiana* Aqueous extract (100µg/ml)	0.1819±0.0001

n=3, values are given in SEM



Graph 2: Standard graph of quercetin

Table 6: Total Flavonoid content of different extract of leaves of *Stroblanthes kunthiana*

Sl. No.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mcg/ml
1	Ethanolic extract	16. 9571 ± 0.220
2	Aqueous extract	19.1285± 0.002

n=3, values are given in SEM

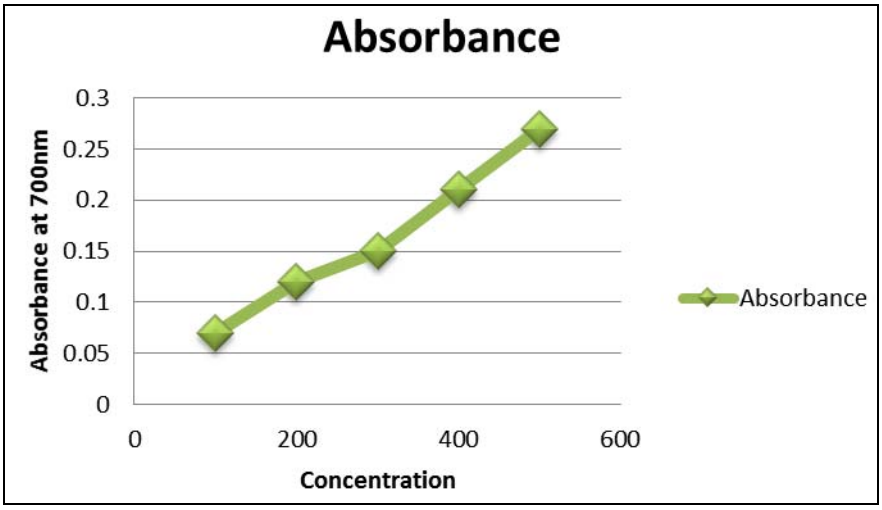
Estimation of Total Tannin Content

Tannic acid was used as a standard and the total tannin content Were expressed as tannic acid equivalents (TAE). Absorbance was measured using a spectrophotometer at 700nm.

Table 7: Absorbance of standard and different extracts

Sl. No	Concentration of Tannic acid (µg/ml)	Mean absorbance
1	100	0.07 ± 0.0002
2	200	0.12 ± 0.0002
3	300	0.15 ± 0.0003
4	400	0.21 ± 0.0001
5	500	0.27 ± 0.0005
6	*S.kunthiana* ethanolic extract (100µg/ml)	0.053±0.0002
7	*S.kunthiana* Aqueous extract (100µg/ml)	0.082±0.0001

n=3, values are given in SEM



Graph 3: standard graph of Tannic acid

Table 8: Total Tannin Content of different extracts of leaves of *Stroblanthes kunthiana*

Sl. No	Extracts100 µg/ml	Tannin content -Tannic acid equivalent (mcg/ml)
1	Ethanolic extract	40.01 ± 0.020
2	Aqueous extract	60.22± 0.010

n=3, values are given in SEM

These studies showed that the aqueous extract of leaves of *Strobilanthes kunthiana* have statistically significant amount of phenolic, flavanoid and tannin content as compared to ethanolic extract. The chlorophyll content was determined by Arnon's method. Total chlorophyll content measured in mg/L.

Discussion

Medicinal plants since ancient time are lauded for their diverse pharmacological actions which could be attributed to the presence of secondary plant metabolites such as alkaloids, flavanoids, glycosides, tannins, steroids etc. some of these plants are important source of natural antioxidants that have been shown to reduce the risk and progression of certain acute and chronic diseases such as cancer, heart diseases and srtoke by scavenging free radicals which are implicated in the pathogenesis of many diseases. The present study indicated that the aqueous extract have more amount of total phenolic, flavonoid and tannin content as compared to ethanolic extract. Phytochemical studies to isolate components are also required to be done to locate the molecules responsible for pharmacological activity. Also further spectral characterization of the isolated compounds can yield promising drugs of future use.

References

1. Mark A Carine, Jake M. Alexander, Robert W. Scotland. A Revision of *Strobilanthes Kunthiana*- group (phlebophyllum sensu Bremekamp) (Acanthaceae). KEW bllettin. 2004, 59:1-25.
2. Brijyog Brijesh Singh, Supriya Das, Anup Maithi. Antioxidant Property for lipophilic extract of *Strobilanthes kunthiana* flowers. Indian Journal of Research in Pharmacy and Biotechnology, 2014; 2(1):1005-1009.
3. Paulsamy S. Ecological Status of medicinal and other economically important plants in the Shola understories of Nilgiris, the Western Ghats. Narural Products Radiance, 2007; 6(1):55-61.
4. Elizabeth C Moylan. Phylogenetic relationships among *Strobilanthes* S.L (Acanthaceae): Evidence from its NR DNA, TRNL-F CpDNA, and Morphology. American Journal of Botany. 2004; 91(5):724-735.
5. Preethi F, Suseem SR. A Comprehensive Study on an Endemic Indian Genus- Strobilanthes. International Journal of Pharmacognosy and Phytochemical Researh, 2014; 6(3):459-466.
6. Anonymous II. Indian pharmacopoeia Government of India,

Ministry of Health and Welfare, Controller of publications. Delhi. 1985; 2:A-75.
7. Anonymous III. Quality control methods of Medicinal plant materials. WHO, Geneva. A.I.T.B.S Publishers & Distributors. Delhi. 2002, 28-30.
8. Kokate CK. *Practical Pharmacognosy*. Fourth edition. Vallabh Prakasan. Delhi; 2001; 107-111, 123-125:130.
9. Patra Arjun, Jha Shivesh, Narasimha Murthy P. Pharmacognostical standardisation of Leaves of *Hygrophila spinosa* T. Anders. Phcog J. 1(2):82-87.
10. Khandelwal KR. *Practical Pharmacognosy- Techniques and Experiments*. Nirali Prakasan. Pune; 2003; 160:149-156.
11. Dr. Khaabadi SS, Dr. Deore SL, Baviskar BA. Experimental Phytopharmacognosy A Comprehensive Guide. First edition, Nirali prakashan, Pune. 2011; 4(13).
12. Vijay D Tambe, Rajendra S Bhambar. Estimation of Total Phenol, Tannin, Alkaloid and Flavanoid in *Hibiscus Tiliaceus* Linn. Wood Extracts. Journal of Pharmacognosy and Phytochemistry. 2014; 2(4):41-47.
13. Mudasir Sultana, Pawan Kumar Verma, Rajinder raina, Shahid Prawez, Dar M A. Quantitative Analysis of Total Phenolic, Flavanoids and Tannin Contents in Acetone and n- Hexane Extracts of *Ageratum conyzoides*. International Journal of Chem Tech Research, 2012; 4(3):996-999.
14. Fatma Alhakmani, Sokindra Kumar, Shah Alam Khan. Estimation of total Phenolic content, in- vitro Antioxidant and anti inflammatory activity of *Moringa Oleifera*. Asian Pacific Journal of Tropical biomedicine. 2013; 3(8):623-627.
15. Naima Saeed, Muhammad R Khan, Maria Shabbir. Antioxdant activity, total Phenolic and Total Flavanoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complementary and Alternative medicine, 2012; 12:221.
16. Govindappa M, Naga Sravya S, Poojashri MN, Sadananda TS, Chandrappa CP. Antimicrobial, Antioxidant and *in virto* Anti-inflammatory activity of Ethanol extract and Active Phytochemical screening of *Wedelia trilobata* (L.) Hitchc. Journal of Pharmacognosy and Phytochemistry. 2011; 3(3):43-51.
17. Naima Saeed, Muhammad R Khan, Maria Shabbir. Antioxdant activity, total Phenolic and Total Flavanoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complementary and Alternative medicine, 2012; 12:221.
18. Abubakkar B Aliyu, Free Radical scavenging ANF Total antioxidant capacity of Root extracts of Anchomanes difformis Engl (Araceae). Acta Poloniae Pharmaceutica-Drug Research, 2013; 70(1):115-121.
19. Tauheeda Riaz. Phytochemical Screening, free radical scavenging, Antioxidant activity and phenolic content of *Dodonaea viscose* Jacq. Journal of the Serbian Chemical Society, 202; 77(4):423-435.