

Original Research Article

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Phytochemical Analysis of Wild *Meethi* *Neem* [*Murraya koenigii* (L.) Spreng] Collections of Himachal Pradesh

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ABSTRACT

Murraya koenigii (L.) Spreng commonly known as 'Curry patta' or 'Meethi Neem' is a native plant of India. The leaves are widely used for flavoring food items for the Indian preparations like curries, chutneys, sambhar, buttermilk, and preparations of egg, fish and meat. Fresh leaves of *Meethi Neem* contain carotene, nicotinic acid, vitamin C, alkaloids, flavonoids, tannins, volatile oil and also have reported to possess anti-oxidant, antibacterial, antifungal, anti-carcinogenic, hypoglycemic and antihypertensive activity. Fifteen wild *Meethi Neem* leaf samples collected at three stages viz. pre-flowering, flowering and fruiting stages from 15 diverse locations of Kangra and Mandi district of Himachal Pradesh were analyzed to evaluate phytochemical constituents i.e. total chlorophyll, carotenoid content ($\mu\text{g/g}$) and ascorbic content. The range of variation for phytochemical constituents at three stages varied significantly viz. total chlorophyll (0.79 to 1.43, 1.31 to 1.94 and 0.88 to 1.10 mg/g), total carotenoids (30.55 to 49.25, 49.09 to 60.81 and 31.24 to 41.42 $\mu\text{g/g}$), ascorbic acid (7.01 to 9.82, 3.58 to 6.06 and 2.99 to 4.93 mg/100g), respectively. Among all picking stages highest value of total chlorophyll and carotenoid content was observed at flowering stage whereas ascorbic acid content showed highest value at pre-flowering stage.

Keywords

Ascorbic acid,
Flowering stage,
Chlorophyll,
Carotenoid content,
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Introduction

Mountains support an estimated one-third of terrestrial species diversity (Körner, 2004) and host half of all 34 global biodiversity hotspots (Chape *et al.*, 2008). The Himalayan mountains is well known as a source of medicinal and aromatic plants, which contain variety of bioactive agents. Since the beginning of human civilization, medicinal

plants have been used by mankind for its therapeutic value. Medicinal plants are generally tree, shrub, herb, annuals, biennial, tubers, rhizomes and climbers containing bioactive compounds. Some of the medicinal plant includes *tulsi*, *neem*, *babul*, *lavender*, *brahmi*, *curry patta* (*Meethi Neem*), *datura*, *satavar*, *arjun* and *sarpagandhha*. *Meethi Neem* (*Murrayakoenigii*) is one among them which has been extensively used for culinary

preparations in Indian kitchen. *Murrayakoenigii* (L.) Sprengis a native plant of India commonly known as 'Curry patta' or 'Meethi Neem' in Indian dialects, and belongs to the family *Rutaceae*. It is a shrub or a small tree found throughout India up to an altitude of 1,500m (Ajay *et al.*, 2007).

The plant occurs both in wild and cultivated forms. Wild forms in India are found growing in the hills of Assam, Central India, Himachal Pradesh, Kerala, Punjab, Sikkim, Tamil Nadu, Uttarakhand, West Bengal and Western Ghats (Dastur, 1970). In Southern parts of India, it is cultivated for its aromatic leaves for dish preparations. Flowering starts from the middle of April and ends in the middle of May.

The fruiting continues from the middle of July to the end of August. *Meethi neem* is extensively known for its medicinal uses, especially aromatic characteristics of leaves and contains carotene, nicotinic acid, vitamin C, vitamin A, calcium, oxalic acid, glycosides alkaloids, flavonoids, tannins, volatile oil and also have reported to possess anti-oxidant, antibacterial, antifungal, anti-carcinogenic, hypoglycemic and antihypertensive activity (Narasimhan *et al.*, 1975).

Curry leaves are also used in the treatment of various diseases like diabetes mellitus, body pain, inflammation, kidney pain, blood disorders and piles. The leaves are widely used for flavouring food items like buttermilk, egg and meat and for the Indian preparations like curries, chutneys and sambhar. There are several evidences about the use of these medicinal plants and their derived products in traditional Chinese, Ayurveda, Siddha, Unani and Tibetan medicines. Ancient literature such as Rigveda, Yajurveda, Atharvaveda, Charak Samhita and Sushrut Samhita also describes their use for the treatment of various health problems (Sekar *et al.*, 2011). Human being uses numerous plants and plant derived

products to cure and get relief from various physical and mental illnesses (Balunas and Kinghorn, 2005). The natural compounds in medicinal plants act in a synergistic manner within the human body and provide unique therapeutic properties with minimal or no side-effects. Phytochemical properties of *Meethi Neem* leaves belonging to higher altitudinal ranges of Kangra and Mandi districts of Himachal Pradesh have not been studied earlier. Hence, the present attempt was made to identify the promising genotype for active photochemical constituents of *Meethi Neem* available in two high altitude districts of Himachal Pradesh.

Materials and Methods

Sampling

The research material for the present study comprised of 15 wild *Meethi Neem* leaves samples which were collected during 2013-14 at three different stages *i.e.* before flowering, at the time of flowering and at fruiting stage (Fig. 1). Approx. 500 gm fresh leaves were collected from 15 randomly selected plants from 15 different locations of Kangra and Mandi Districts of Himachal Pradesh (Table 1).

Extraction and estimation of total chlorophyll and carotenoids

Total chlorophyll and total carotenoids in fresh *Meethi Neem* leaves were estimated by the methods of Jayraman (1981) followed by Davies (1976). 0.2 g fresh leaves were ground in pestle and mortar with the addition of 5 ml of 80 per cent acetone. This paste was centrifuged for 15 min at 4,000 rpm. The supernatant was transferred to a 50 ml beaker.

The chlorophylls and carotenoids were repeatedly extracted out with 20 ml (5-5 ml repeatedly, 4 times) 80 per cent acetone until

residues became colourless. Finally, the volume was made up to 20 ml with 80 per cent acetone and absorbance of the extracted solution was measured at 663 nm, 645 nm and 480 nm on spectrophotometer Model Merck SpectroquantPharo 100.

The extent of chlorophylls (mg/ g) was calculated by the following equation:

$$\text{Total chlorophylls} = [20.2 (A_{645}) + 8.02 (A_{663})] \times V / (1000 \times W)$$

The amount of total carotenoids ($\mu\text{g/g}$) was calculated with following equation:

$$\text{Total carotenoids} = [A_{480} + 0.114 (A_{663}) - 0.638 (A_{645})] \times V / W$$

Where, A= absorbance at specific wavelengths, V= final volume extract, W= fresh weight of tissue

Extraction and estimation of Ascorbic acid

Ascorbic acid content in *Meethi Neem* leaves was analyzed by the method of AOAC, 1990. Twenty-five gram of fresh *Meethi Neem* leaves ground with 25 ml of 2.0 per cent oxalic acid in pestle and mortar to get slurry.

The total weight of slurry was recorded. 10 g of this slurry was taken in a beaker and volume was made up to 25 ml with 1 per cent oxalic acid. The content of the beaker was filtered through Whatman No.1 filter paper. 5.0 ml of this filtrate was pipette out and titrated against a dye solution (2, 6 dichlorophenol indophenol). Three concordant readings were recorded. L-ascorbic acid was used as a standard titrated against dye. The amount of ascorbic acid (mg /100 g) was calculated using following formula:

$$\begin{aligned} & \text{Ascorbic acid (mg/100g)} \\ & \text{Standard conc. of ascorbic acid} \\ & \times A \times \text{Volume made up} \times B \\ & = \text{-----} \times 100 \\ & \text{Titer value of Standard} \times \\ & \text{Wt. of slurry used} \times S \\ & \times \text{Vol. used for titration} \end{aligned}$$

Where A = Titer value of sample, B = Total weight of slurry, S = Weight of sample (g)

Statistical analysis

All phytochemical analysis was carried out in triplicate to reduce the experimental error to a minimum. Statistical analyses were performed using Statistical Analysis Software, Version 9.2 (SAS, 2009). Duncan's Multiple Range Test (DMRT) is used for variation analysis.

Results and Discussion

The *Meethi Neem* collections were collected from 31°47' - 32° 50' N and 76 °25' - 76° 56' E and 689 -1339 meter altitudinal range of Kangra and Mandi Districts of Himachal Pradesh. The results revealed that there were significant differences among the 15 *Meethi Neem* collections with respect total chlorophyll, carotenoids and ascorbic acid content. Variation in chlorophyll, carotenoids and ascorbic acid content of *Meethi Neem* collections at different picking stages viz. pre-flowering, flowering and fruiting stage are mentioned (Table 2).

Total chlorophyll content

Data in respect of total chlorophyll content of *Meethi Neem* collections at different stages are depicted in Table 2. A significant variation was observed in total chlorophyll content. The value ranged from 0.79 to 1.43, 1.31 to 1.94 and 0.88 to 1.10 mg/g at pre-flowering, flowering and fruiting stage, respectively. At pre-flowering stage, the highest value for total

chlorophyll content was observed in Dhramn (1.43 mg/g) followed by Tang (1.38) and Sagned (1.37 mg/g) whereas its lowest value was noticed in Madi (0.79mg/g).Subsequently at flowering stage, Mlan (1.94 mg/g) showed maximum value of total chlorophyll content followed by Kunnu (1.93 mg/g) and Dharampur (1.87 mg/g) whereas lowest value was found in Pudva (1.31 mg/g). At fruiting stage, Dhira (1.10 mg/g) showed highest value of total chlorophyll content followed by Sagned (1.06 mg/g) and Kunnu (1.05 mg/g).

Kotropi (0.88 mg/g) had lowest value of total chlorophyll content at fruiting stage. In general, leaf chlorophyll content increased during flowering stage and thereafter decreased more rapidly during the fruiting stage. The decrease in chlorophyll content might be due to translocation of metabolites from leaves to growing grain. Garg *et al.*, (2012) reported the total chlorophyll content of *Meethi Neem* was 0.026 g/ L i.e. 2.6 mg/g which was nearly close to the present value of total chlorophyll content.

Table.1 Sampling details of 15 different wild *Meethi Neem* collections

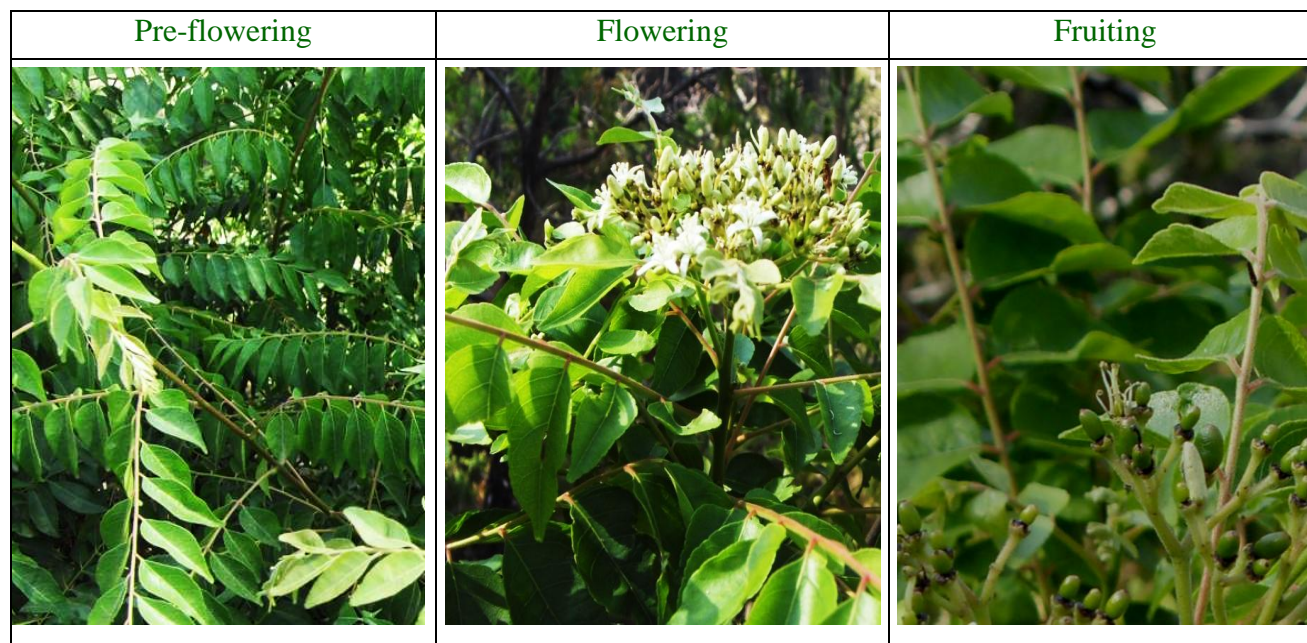
Sr. No.	Collections Name	Tehsil, District	Altitude range (msl)	Latitude/longitude
Kangra				
1	Pudva	Palampur, Kangra	709 m	31 ⁰ 57'N/76 ⁰ 26'E
2	Dhramn	Jaisinghpur, Kangra	807 m	31 ⁰ 59'N/76 ⁰ 32'E
3	Dhira	Dhira, Kangra	946 m	32 ⁰ 01'N/76 ⁰ 27'E
4	Mlan	NB, Kangra	960 m	32 ⁰ 07'N/76 ⁰ 25'E
5	Malghota	Baijnath, Kangra	1047 m	32 ⁰ 03'N/76 ⁰ 38'E
6	Tang	Dharamshala, Kangra	1069 m	32 ⁰ 09'N/76 ⁰ 25'E
Mandi				
7	Nagri	Palampur, Kangra	1278 m	32 ⁰ 07'N/76 ⁰ 28'E
8	Sagned	Sarkaghat, Mandi	689 m	31 ⁰ 48'N/76 ⁰ 45'E
9	Dharampur	Dharampur, Mandi	751 m	31 ⁰ 47'N/76 ⁰ 44'E
10	Lad Badhol	Lad Badhol, Mandi	777 m	31 ⁰ 55'N/76 ⁰ 42'E
11	Neri	Jogindernagar, Mandi	827 m	31 ⁰ 49'N/76 ⁰ 46'E
12	Madi	Sandhol, Mandi	1016 m	31 ⁰ 48'N/76 ⁰ 44'E
13	Drang	Mandi, Mandi	1121 m	31 ⁰ 49'N/76 ⁰ 56'E
14	Kunnu	Padar, Mandi	1201 m	32 ⁰ 50'N/76 ⁰ 55'E
15	Kotropi	Padar, Mandi	1339 m	31 ⁰ 54'N/76 ⁰ 53'E

Table.2 Variation in total chlorophyll, carotenoids and ascorbic acid content of *Meethi Neem* collections at different picking stages

Sr No.	Collection Name	Total chlorophyll Content(mg/g)			Total carotenoids content (µg/g)			Ascorbic content (mg/100g)		
		Pre-Flowering	Flowering	Fruiting	Pre-Flowering	Flowering	Fruiting	Pre-Flowering	Flowering	Fruiting
1	Pudva	1.11 ^f	1.31 ^a	0.99 ^e	44.84 ⁱ	53.17 ^d	32.31 ^b	8.49 ^h	5.89 ⁿ	4.20 ^h
2	Dhramn	1.43 ^m	1.76 ^g	1.03 ^h	48.73 ⁿ	58.78 ^j	38.95 ^g	8.60 ⁱ	4.62 ^e	4.93 ^m
3	Dhira	1.24 ⁱ	1.83 ^j	1.10 ^k	45.27 ^k	57.69 ^h	33.06 ^d	8.21 ^f	4.39 ^c	3.53 ^e
4	Mlan	1.06 ^e	1.94 ^o	0.99 ^d	43.12 ^h	59.79 ^l	35.48 ^t	8.87 ^j	4.59 ^d	3.58 ^t
5	Malghota	0.83 ^b	1.50 ^d	1.00 ^{ef}	40.21 ^d	52.13 ^c	32.81 ^c	9.72 ⁿ	5.02 ^h	3.23 ^c
6	Tang	1.38 ^l	1.84 ^k	1.03 ^h	47.46 ^m	57.18 ^g	35.66 ^f	9.82 ^o	4.89 ^g	3.11 ^b
7	Nagri	1.26 ^j	1.86 ^l	0.98 ^d	42.21 ^g	59.11 ^k	40.21 ⁱ	9.66 ^m	5.05 ⁱ	4.89 ^l
8	Saged	1.37 ^l	1.43 ^b	1.06 ^j	49.25 ^o	49.09 ^a	34.49 ^e	9.11 ^k	5.35 ^k	2.99 ^a
9	Dharampur	1.27 ^k	1.87 ^m	0.96 ^b	41.23 ^f	60.44 ^m	41.27 ^j	7.64 ^d	3.58 ^a	3.56 ^{ef}
10	Lad Badhol	1.23 ^h	1.83 ⁱ	1.02 ^g	46.87 ^l	58.03 ⁱ	39.64 ^h	7.01 ^a	4.21 ^b	3.26 ^d
11	Neri	1.17 ^g	1.53 ^e	1.03 ^h	40.48 ^e	52.15 ^c	33.18 ^d	7.18 ^c	4.81 ^f	3.83 ^g
12	Madi	0.79 ^a	1.82 ^h	1.01 ^f	30.55 ^a	55.51 ^f	31.24 ^a	9.47 ^l	5.67 ^m	4.82 ^j
13	Drang	0.93 ^c	1.49 ^c	0.97 ^c	38.19 ^c	49.28 ^b	31.34 ^a	7.95 ^e	6.06 ^o	4.60 ⁱ
14	Kunnu	0.83 ^b	1.93 ⁿ	1.05 ⁱ	35.19 ^b	60.81 ⁿ	41.42 ^j	7.18 ^b	5.49 ^l	3.56 ^f
15	Kotropi	1.03 ^d	1.60 ^t	0.88 ^a	45.08 ^j	54.64 ^e	35.55 ^t	8.36 ^g	5.14 ^j	4.85 ^k
Mean		1.13	1.70	1.01	42.58	55.85	35.78	8.49	4.98	3.93
Range		0.79- 1.43	1.31 -1.94	0.88 -1.10	30.55 -49.25	49.09 -60.81	31.24 - 41.42	7.01 -9.82	3.58 - 6.06	2.99 - 4.93

*Figures having same superscripts are non-significant at 95% confidence level

Fig.1 Plants of *Murraya koenigii* (L.) Spreng showing three different stages



Total carotenoid content

Variability in total carotenoids content was evaluated (Table 2). It is evident from the data that at pre-flowering, flowering and fruiting stages the total carotenoids significantly varied from 30.55 to 49.25, 49.09 to 60.81 and 31.24 to 41.42 ($\mu\text{g/g}$), respectively. At pre-flowering stage, highest value for this parameter was noticed in Sagned ($49.25\mu\text{g/g}$) followed by Dhramn ($48.73 \mu\text{g/g}$) and Tang ($47.46 \mu\text{g/g}$). The other collections showed significantly lower value of carotenoids content with minimum value exhibited by Madi ($30.55 \mu\text{g/g}$). At flowering stage, Kunnu ($60.81 \mu\text{g/g}$), Dharampur ($60.44 \mu\text{g/g}$) followed by Mlan ($59.787 \mu\text{g/g}$) showed the highest value of total carotenoids content. The lowest value was recorded in Madi ($49.09\mu\text{g/g}$). At fruiting stage, Kunnu ($41.42 \mu\text{g/g}$), Dharampur ($41.27 \mu\text{g/g}$) followed by Nagri ($40.21 \mu\text{g/g}$) were recorded to have the highest total carotenoids content. Madi ($31.24 \mu\text{g/g}$) showed lowest value for this parameter. Among all collections, Sagned and Kunnu showed highest value of total carotenoid

content. Singh *et al.*, (2014) reported carotene content in curry leaves as $7560 \mu\text{g/g}$ ($75.60 \mu\text{g/g}$) in fresh leaves which is nearly close to the present findings.

Ascorbic acid content

The variability in ascorbic acid content at different stages in *Meethi Neem* samples was analyzed and depicted (Table 2). Ascorbic acid content showed a variation ranging from 7.01 to 9.82, 3.58 to 6.06 and 2.99 to 4.93 (mg/100g) at pre-flowering, flowering and fruiting stage, respectively. At pre-flowering stage Tang (9.82 mg/100g) followed by Malghota (9.72 mg/100g) and Nagri (9.66 mg/100g) exhibited higher ascorbic acid content as compared to other collections.

Singh *et al.*, (2014) reported the vitamin C content of *Meethi Neem* as 4mg/100g . The minimum value of ascorbic acid was showed by Lad Badhol (7.013 mg/100g). At flowering stage Drang (6.06 mg/100g), Pudva (5.89 mg/100g) accompanied by Madi (5.67 mg/100g) showed maximum ascorbic acid

content. The minimum value at flowering stage was exhibited by Dharampur (3.58 mg/100g). At fruiting stage, Dharamn (4.93 mg/100g), followed by Nagri (4.89 mg/100g) and Kotropi (4.85 mg/100g) were observed to possess highest ascorbic acid content. The lowest value was recorded in Saged (2.99 mg/100g). Based on the three picking stages Tang, Drang and Dhramn exhibited highest value of ascorbic acid content. Singh *et al.*, (2014) reported the vitamin C content of *Meethi Neem* as 4mg/100g. A higher level of ascorbic acid in the developing leaves could be due to the fact that ascorbic acid is essential at the early growth stages.

Plants at this stage are metabolically more active as they require higher concentrations of essential compounds for growth. The lower values in mature plants might be due to oxidative stress in plant at mature stage (Uddin *et al.*, 2012).

Besides its use as flavoring food items, curry leaves are considered as medicinal and reported to possess active principle for several diseases like diabetes mellitus, body pain, inflammation, kidney pain, blood disorders and piles in human beings. Based on the three picking stages viz. pre flowering, flowering and fruiting stages Mlan, Kunnu and Tang collections exhibited highest value of total chlorophyll, carotenoids and ascorbic acid content. Phytochemical composition and diversity in wild populations of curry leaves is enormous. Information on chemodemes of *Murraya koenigii* (L.) Spreng from higher altitudes of Terai and northern Himalayan ranges will be rewarding. Hence, a comprehensive programme on acquisition of the material from mountainous hilly ranges of Himalaya will not only help to save the species from extinction but also in identification of phytochemically superior lines for conservation and future research programmes.

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