



Studies on anthelmintic activity of *Naringi crenulata*

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Abstract Plants extracts have been used since time immemorial for curing various ailments in traditional medicine in India. Infection of helminth parasite is a serious problem in rural areas which results in physical impairments among children from age five to thirteen years. *Naringi crenulata* (or) *Limonia crenulata* is a Rutaceae family, spinous glabrous small tree, grows tropical Africa and Asia. It is commonly known as “Mahavilvam” in Tamil and Kukka velaga in Telugu. It is a wide spread of the genus (*Naringi*) grown as under large trees in evergreen forests up to 200mts. The leaves are aromatic just like any other citrus leaves. The root extract is used for vomiting, dysentery and colic disorders. Fruit decoction is used as an antidote to insect poison. In the present study an attempt is made to know the anthelmintic activity of leaf of *Naringi crenulata*. The *in vitro* studies were done with extracts of leaves, stem bark and roots of the plants of concentration 10, 20, 40, 60, and 100 mg/ml were used to test the *Ascaris* worms for plant potency while albendazole 20mg was used as control. Paralysis time and death were determined within 4 hours in the petri dish while unrestrained movements by the worms before and after extracts administration were recorded. Time of paralysis and time of death were significantly reduced at all concentrations compared to the vehicle treated group ($P \leq 0.05$). The study showed that the extracts *Naringi crenulata* exhibited *Anthelmintic* activities on the intestinal worms. Further studies are needed to arrive at comprehensive conclusions. Then only it can be used as an oral medication for these worms infestation in children. The present research was therefore designed to scientifically validate some widely used ethno botanicals for their anthelmintic activity in children in Siddeswaram sacred groove Vempalli toka village tribal community in SPSR Nellore district. A.P.

Keywords Anthelmintic activity; *Naringi crenulata*, *in vitro* studies, *Ascaris*

Introduction

Naringi crenulata (Roxb.) Nicolson is a tropical medicinal plant belonging to Rutaceae family. It is popularly called as Ekadasa Bilvam or Kukka velaga in Telugu language. From immemorial period it is used for treating various disorders such as vomiting, dysentery and digestive disorders. The bark is used for muscle sprains. Earlier studies shows that the bark of this plant is having anticancer [1], hepatoprotectivity [2], anti microbial activities [3-4]. The tribes of Vempalli toka village of Siddeswaram Sacred groove are using *Naringi crenulata* leaf bark; root and stem are used deworming humans, goats and cattle. The present research was therefore designed to scientifically validate some widely used ethno botanicals for their anthelmintic activity by doing *in vitro* studies.

Materials and Methods

Location:

Siddeswaram (Latitude: 17.608751, Longitude: 83.187155) is a sacred groove near Vempalli toka village of Sitharamapuram mandal, SPSR Nellore district, Andhra Pradesh India. A small but effective village having lord Shiva temple also known as Siddeswaradu, Kasi Nayana, Istakameswari Devi Ayyappa, Agasthya Rishi



Thapovan, Veera Bhoga Vasantaraya temples. It had a rich flora and fauna. It had a lush Eastern Ghats, dry forests and short bushes.

Naringi crenulata

Trees, grows up to 10 m, armed with sharp solitary or paired maxillary spines; bark yellowish-grey, smooth, corky; blaze yellow; branch lets angled, glabrous. Leaves imparipinnate, alternate, estipulate; rachis 2.5-10 cm long, winged, wings obovate-oblong, glabrous, punctuate; leaflets 3-7, opposite, sessile, estipulate; lamina 1-6 x 0.7-2.4 cm, elliptic or elliptic-obviate, base acute or obliquely acute, apex obtuse to emarginated, margin crenate, glabrous, profusely pellucid-gland dotted; glands dimorphic; curvaceous; lateral nerves 4-10 pairs, pinnate, slender, faint, intercostae reticulate, faint. Flowers bisexual, white, in few flowered axillary racemes; pedicel 8-10 mm long; sepals 4, free or united at base, ovate-orbicular, glandular; petals 4, free, elliptic or oblong, white, glandular, glabrous; stamens 8, sub equal, free, inserted round the disc; filaments subulate; anthers yellow, apiculate, disc thin, glandular; ovary superior, globose, 4-celled, glabrous, glandular, ovule 1 in each cell.; style stout; stigma capitates; Fruit a berry, 6-8 mm across, subglobose, bluish-black, glandular; seeds 1-4, dull yellow, smooth.

Material

Fresh leaves were collected from Siddeswaram Sacred grove of Sitharamapuram mandal of SPSR Nellore District. During the field visit leaves are collected air-dried and pulverized and sieved through 40 meshes. Adult *Ascaris lumbricoidis* were obtained from the gastro intestinal tracts of goats slaughtered in the local markets. Small and large intestines were collected. They were opened in a plastic bucket separately and the contents were washed in tap water. The process was repeated for several times until the sediment becoming transparent. Then the adult female worms were collected with the help of a needle and placed in a petridish containing PBS (Phosphate Buffer Saline). Petri-dish containing the worms was kept in incubator at 38°C until required for experiment on the same day [6]. A minimum of ten worms were exposed in three replicates to each of the following treatments in separate petri dishes at room temperature (25-30°C): The inhibition of motility and/ or mortality of the worms kept in the above treatments were used as the criterion for anthelmintic activity. The motility was observed after 0, 2, 4, 6, 8 and 12 hour intervals. Finally, the treated worms were kept for 30 minutes in the lukewarm fresh PBS to observe the revival of motility. The number of dead and survived worms was recorded for each treatment

Treatments

1. The first group was control and animals were kept in phosphate buffer saline
2. The second group is also a negative control and the animals were treated with 20 mg Albendazole an anthelmintic drug.
3. Rest of the groups is treated with aqueous extracts of stem, bark, leaf and root at different concentrations such as 10, 20, 40, 60, and 100 mg/ml in methanol.

Results

The data regarding the effect of different parts of the *Naringi crenulata* on the anthelmintic activity on *Ascaris lumbricoidis* is presented in Table 1.

The data shows that there is a significant difference in the time taken for paralysis and time taken for death for different treatments of Methnolic extracts of *Naringi crenulata*. Concentrations above 60 mg are giving better results than the lower concentrations.

Discussion

Helminths are major threat for rearing cattle and sheep. They cause anemia, weight loss, poor growth, diarrhea and stomachache. Various anthelmintic drugs such as, albendazole. Bactericide. Diethylcarbamazine. Emverm. Hetrazan. Ivermectin and Mebendazole etc are used to control them. Sometimes development of disease resistance [5] and drug toxicity will be caused due to these drugs [6]. Tribals are using these herbs directly for



controlling parasites. There are no studies on the pharmacological preparations, dosages and mode of action of *Naringi crenulata*. Hence in this study preliminary in vitro studies were made. The results shows that Methnolic extracts of leaf, stem, bark, and root extracts of can control *Ascaris lumbricoidis* by paralyzing them and causing death within a stipulated time at higher concentration that is above 60 mg concentrations per ml.

Table 1: Effect of different parts of *Naringi crenulata* on anthelmintic activity on *Ascaris lumbricoidis*. (Values are mean of ten observations and three replicates)

S. No.	Official part used	Treatments	Concentration (mg/ml)	Time taken for paralysis (in minutes)	Time taken for death (in minutes)
1	Control	Phosphate Buffer saline		29	45
		Albendazole	20mg	6.8	7.4
2	Root	Methnolic Extract	10mg	15.6	34.3
			20mg	12.33	29.4
			40mg	9.6	9.4
			60mg	8.7	9.23
			100mg	8.2	9.2
3	Stem	Methnolic Extract	10mg	22.464	49.392
			20mg	17.7552	42.336
			40mg	13.824	13.536
			60mg	12.528	13.2912
			100mg	11.808	13.248
4	Seed	Methnolic Extract	10mg	8.976	9.768
			20mg	16.2756	38.808
			40mg	12.672	12.408
			60mg	11.484	12.1836
			100mg	10.824	12.144
5	Leaf	Methnolic Extract	10mg	8.16	8.88
			20mg	14.796	35.28
			40mg	11.52	11.28
			60mg	10.44	11.076
			100mg	9.4	10.5

ANOVA: Two-Factor

SUMMARY	Count	Sum	Average	Variance
Row 1	2	74	37	128
Row 2	2	14.2	7.1	0.18
Row 3	2	49.9	24.95	174.845
Row 4	2	41.73	20.865	145.6925
Row 5	2	19	9.5	0.02
Row 6	2	17.93	8.965	0.14045
Row 7	2	17.4	8.7	0.5
Row 8	2	71.856	35.928	362.5586
Row 9	2	60.0912	30.0456	302.1079
Row 10	2	27.36	13.68	0.041472
Row 11	2	25.8192	12.9096	0.291237
Row 12	2	25.056	12.528	1.0368
Row 13	2	18.744	9.372	0.313632
Row 14	2	55.0836	27.5418	253.8545
Row 15	2	25.08	12.54	0.034848
Row 16	2	23.6676	11.8338	0.24472



Row 17	2	22.968	11.484	0.8712
Row 18	2	17.04	8.52	0.2592
Row 19	2	50.076	25.038	209.7971
Row 20	2	22.8	11.4	0.0288
Row 21	2	21.516	10.758	0.202248
Row 22	2	20.88	10.44	0.72
Column 1	22	283.5968	12.89076	26.11533
Column 2	22	438.6008	19.9364	197.5551

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	3586.625	20	179.3312	3.523934	0.003487	2.124155
Columns	563.2302	1	563.2302	11.06771	0.003363	4.351243
Error	1017.79	20	50.8895			
Total	5167.645	41				

Conclusion

The *Naringi crenulata* appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The anti-inflammatory, antispasmodic, analgesic and diuretic can be attributed to their high phenols, tannins, triterpenoids, saponins and flavonoids. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation of techniques like extraction, purification, separation, crystallization and identification.

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