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Derris trifoliata Lour*:* a legume fodder shrub for the coastal regions of India

Edna Antony[1*], K. Sridhar[1], Sultan Singh[2], Nilamani Dikshit[2] and G. Sahay[2]
[1]Southern Regional Station, ICAR-IGFRI, Dharwad-580005, India
[2] ICAR-Indian Grassland and fodder Research Institute, Jhansi-284003, India
*Corresponding author e-mail: edna.antony@icar.gov.in
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Abstract
Derris trifoliata is a climbing leguminous shrub distributed in coastal India as a mangrove associate. It can grow in mudflats and survive in soils with different grades of salinity. Its morpho-physiological characteristics of leaf and stem confirmed features of salinity tolerance. Lenticels in the stem are likely an adaptation to survive in saline marshes, akin to pneumatophores. Animals readily consumed *D. trifoliata* leaves, indicating its acceptability. Its foliage contained 16.47% crude protein (CP), 52.75% neutral detergent fibre (NDF), 35.49% acid detergent fibre (ADF) and 23.06% cellulose. It had total digestible nutrients (TDN), digestible energy (DE) and metabolisable energy (ME) contents of 58.76%, 2.59 Kcal/ g and 2.13 Kcal/g, respectively with 61.25% digestible dry matter (DDM) and 108.1 of relative feed value (RFV). Rotenone, an isoflavonoid, varied from 0.15 to 3.87 µg/g in the leaves and was within the safe limits for ruminant animals. The availability of this plant near coastal regions with high nutritive value and safe limits of rotenone content in leaves and stem, qualify *Derris trifoliata* as a potent legume fodder for coastal regions.

Keywords: *Derris trifoliata*, Fodder, Lenticels, Nutritive value, Rotenone, Salinity

Introduction

Coastal India endowed with abundant rains during monsoon, but scorching summers experience a shortage of green fodder, especially protein-rich legumes during dry summer months. Coastal regions account for 8% of the total cattle population of India. But the share of fodder crops in gross cropped area is only 1.23% in coastal regions (Kumar and Singh, 2008). In an extensive study conducted in the northern coastal zone of Andhra Pradesh including Srikakulam, Vizianagaram and Vishakapattanam recorded a total potential feed requirement of 4.83 million tonnes, whereas the total availability for ruminants is only 3.07 million tonnes (Raju *et al.,* 2017). A high-quality legume that is available throughout the year and that can tolerate the climate and soil salinity of coastal regions is essential for improving the health and productivity of animals in this region. An exploration was conducted along the coastal areas of Maharashtra to identify forage grasses or legume species ideal and locally available as cattle feed. During the investigation, it was observed that stray cattle were feeding on a creeping plant growing among the morning glory (*Ipomoea pes-caprae* L.R.Br), in Bhyte beach, Ratnagiri (Fig 1). On close observation, it was evident that animals were selectively feeding on the legume *Derris trifoliata* Lour.

Fig 1. Stray cattle feeding on *Derris trifoliata* creeping amongst morning glory (1) and ruminant animals in Dharwad feeding on *Derris trifoliata* foliages (2-4)

Derris trifoliata is a mangrove associated species and distributed abundantly in coastal regions of India. Its occurrence at ground level and climbing habit is vital for covering and stabilising the banks of creeks of brackish water (Aluri *et al.,* 2016). *D. trifoliata* is recommended for strengthening the mudflats and as a barrier to prevent sand erosion in the beaches (Chandran *et al*., 2012b). Orwa (2009) mentioned that leaves are used as fodder.

D. trifoliata is also widely used as alternative fodder in forest fringe areas of Sunderban, West Bengal during lean periods (Ghosh *et al*., 2015). Keeping this in view in the present investigation, the quality of *D. trifoliata* in terms of its acceptability, crude protein, rotenone content and a few morpho-physiological adaptations for growing in various grades of soil salinity were explored and discussed.

Materials and Methods

***Experimental site and designing*:** The plant samples for this study were collected from Bhyte beach in Ratnagiri district of Maharashtra (16° 58' 73.1" N latitude and 73° 17' 64.8" E longitude) and from Murudeshwar beach (14° 6' 12" N latitude and 74° 29' 14" E longitude) in Karnataka during 2016. The acceptability of this legume was measured simply by one time offering fresh forages collected from coastal mudflats of Karnataka to six cattle, and six small ruminants (goats) reared at Dharwad (15° 29' 20.7" N and 74° 59' 3.3" E) which was 315 km away from the natural habitat of *D. trifoliata*. The beach soil salinity was measured using EM 38 and calibrated following Kuligod *et al. (*2001). From a twig (length >1 m), the leaves were separated at 30 cm interval, and leaf area and leaf weight were measured. Leaf area was measured using graph paper method, and specific leaf area (SLA) was recorded. Specific leaf weight (SLW) was calculated as SLW = Leaf dry weight/ leaf area. Spots present on the stems were viewed under a stereo zoom microscope at 100 X magnification.

The samples were analysed on a dry weight basis (100 g) for estimating CP, OM and EE contents following AOAC (2005) and fibre contents (NDF, ADF, cellulose and lignin) as per Van Soest *et al.* (1991). Calcium (Ca) and magnesium (Mg) was estimated using atomic absorption spectrometry as per the standard procedure. Na and K content was estimated using flame photometry. The digestible dry matter (DDM) and total digestible nutrients (TDN), digestible energy (DE) and metabolisable energy (ME) values were calculated using the standard equations (Undersander *et al*. 1993; Fonnesbeck *et al*., 1984; Khalil et *al.,* 1986).

***Rotenone content analysis*:** The leaves and twigs from different positions of the vine were dried and analysed for rotenone using LC-MS at SAIF facility of ICAR-IIHR, Bengaluru. Dried samples were extracted in 50: 50 (v/v) n-hexane: dichloromethane and reconstituted in 300 µl acetonitrile. Electro Spray Ionisation source (ESI) operating in positive mode was used for all analytes. Liquid chromatography separation was achieved using Extend C18 column at 35 °C and a flow rate of 0.5 ml min^{-1}. Water with 0.1% formic acid (A) (w/v) and acetonitrile (B) were used as mobile phases under gradient conditions. Rotenone from Sigma chemicals was used as standard.

Results and Discussion

Distribution and availability: *Derris trifoliata* is a leguminous climber and found growing in eastern and western coastal regions of India (Table 1). The plant mainly grows in mudflats and as a mangrove associate plant. Total mangrove area along the Indian coast is 348710 ha. Out of the entire coastal region, 56.7% is present along the east coast, 23.5% along the west coast and the remaining 19.8% in Andaman Nicobar Islands (Singh *et al*., 2012). Eastern coast has more of mangroves due to its gradual slope in the east coast compared to the steep slopes on the west coast. The spotting in different areas (Table 1) suggested that this species is widespread in coastal regions of India and is readily available. Its growing pattern amongst mudflats in beaches and as a mangrove-associated species proved that it can grow in different grades of salinity. Raju and Kumar (2016) observed that it is a deciduous climber distributed from oligohaline to polyhaline zones in the mangroves. *D. trifoliata* was found as one of the Sundarban mangrove undergrowth species seen in all the 29 zones where salinity was in the range of 0-25 parts per trillion (Rashid *et al*., 2008).

***Morpho-physiological characteristics*:** The plant samples were collected from Bhyte beach in Ratnagiri, Maharashtra and Murudeshwar beach in Karnataka. Soil salinity of the beach soil at various depth were 1.20 dS/m (0-15 cm), 0.61dS/m(15-30 cm), 0.14 dS/m (30-60 cm), 0.034 dS/m (60-90 cm) during the month of February. *D. trifoliata*, a climber, had 3-5 meters long branches from the main stem. The average area of leaflets towards the first 30 cm of the twig was 8 cm^2, at 30-60 cm it was 12.5 cm^2 and > 60 cm it was 14.76 cm^2 (Table 2; Fig 2 a&b). This observation demonstrated that leaves were continued to grow (indeterminate habit) and being a climber, the smaller younger leaves occupied the top of the canopy for sunlight interception and the older leaves were braod for capturing maximum sunlight. The specific leaf weight (SLW) of the leaves also revealed special characteristics of sun and shade leaves in the canopy. The older leaves were showing the characteristics of shade leaves (Table 2).

Table 1. The distribution of *Derris trifoliata* in India

Region	State	Place of spotting	Reference
Western coast	Kerala	North Kerala coast of Kasaragod and Kanhangad	Bhagya and Sridhar (2009). Vaiga and Joseph (2016)
	Karnataka	Talapady, Someshwara, Mukka, Paubidri, Karwar Muredeshwar beach	Chandran *et al*. (2012a), Bhagya and Sridhar (2009) Exploration by authors
	Goa	Coastal saline estuaries	Bhagya and Sridhar (2009)
	Maharashtra	Navi Mumbai	Kale (2013)
		Ratnagiri Bhyte beach	Exploration by the authors
	Gujarat	Purna estuary	Shah and Bhat (2009) Bhatt *et al*. (2009)
East coast	West Bengal	Sundarban delta regions	Rashid *et al*. (2008) Ghosh *et al*. (2015)
	Odisha	Bitrakanika Devi estuary, Paradeep and Mahanadi delta, Subarna creek estuary, Budhabalanga estuaryBalasore	Pattanaik *et al*. (2008), Panda *et al*. (2013) Sujana *et al*. (2015)
	Andhra Pradesh	Coringa mangroves in Godavari mangrove	Raju and Kumar (2016), Aluri *et al*. (2016)
	Tamil Nadu	Rameshwaram mangroves	Suganya and Thangaraj, (2014b)
	Andaman and Nicobar Islands	Mangrove creeks in South Andaman, Havelock, middle Andaman, north Andaman, little Andaman and Car Nicobar	Raghavan *et al*. (2014)

Table 2. Leaf characteristics of *Derris trifoliate*

Position in twig (from the tip)	Number of leaves	SLA (cm^2)	LW (g)	SLW (g/cm^2)
0-30 cm	12	8.0±2.9	0.08±0.026	0.010±0.001
30-60 cm	10	12.1±4.	0.12±0.05	0.011±0.002
60-90 cm	>12	14.7±6.3	0.15±0.078	0.009±0.003

SLA: Specfic leaf area; LW: Lef weight; SLW: Specific leaf weight

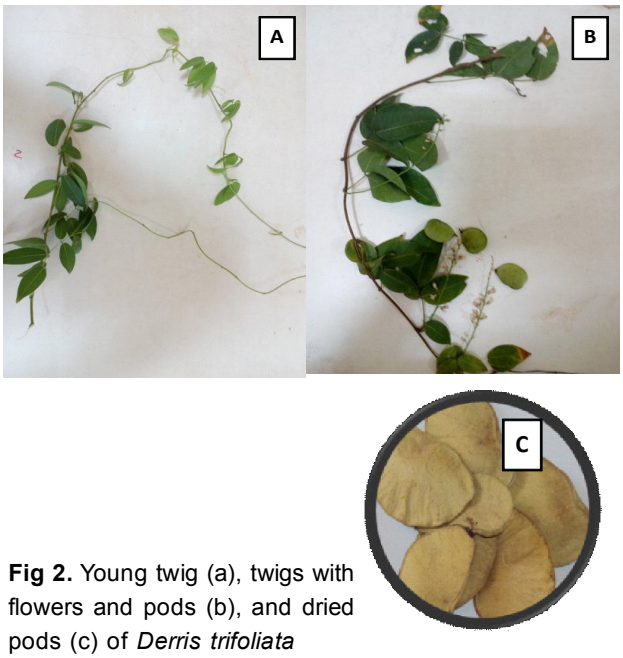

Fig 2. Young twig (a), twigs with flowers and pods (b), and dried pods (c) of *Derris trifoliata*

Flowers were borne in typical axillary racemes 8-15 cm (Fig 2b). Pollination was through tripping by external agents (Raju and Kumar, 2016) and un-tripped flowers did not set seed. The fruits resembled disc-like pods (Fig 2c) with one seed in the side and contained air-filled tissues and cavities, which is an essential feature for dispersal through the water. The seeds did not germinate while attached to the plant indicating dormancy. The seeds could germinate near the parent plant if the site was a mudflat or gets carried away in the tidal water to other favourable location. The movement of seeds suggested self planting and stranding strategies for seed dispersal and establishment (Raju and Kumar, 2016).

The young twigs and old stems of *D. trifoliata* were dotted with lenticels (Fig 3) which appeared as spots to naked eye. Perennials growing in stressful environments like semi-desert, desert and other seasonally dry/flooded environments have lenticels for non-foliar photosynthesis to acquire additional carbon (Aschan and Pfanz, 2003).

Methane emissions due to soil denitrification and methanogenesis are typical in mangrove ecosystems or anoxic wetlands (Krithika *et al*., 2008). Aerenchyma and pneumatophores are specialised adaptations for mangrove plants that function as a conduit for gas exchange. *D. trifoliata*, though associated with mangroves, did not have pneumatophores which can transport oxygen to roots. Instead of pneumatophores, lenticels could transport oxygen, promote methane oxidation, and considerably reduce the overall flux of CH_4 to the atmosphere to just over ambient levels (Krithika *et al*., 2008). Hence the presence of lenticels in *D. trifoliata* could be inferred to have functioned as stomata in leaves for gas exchange, promoting methane oxidation as well as non-foliar photosynthesis.

Fig 3. Lenticels in young twig (a) and old twig (b) as observed under stereo zoom microscope at 100X

***Acceptability*:** The selective feeding of stray cattle on *D. trifoliata* in Bhyte beach, growing amongst common weed (morning glory) provided the impression of its use as fodder. It is a climbing shrub and tends to remain short and creeps on mudflats until it finds support to climb. Being a climber, it is inaccessible to cattle due to their feeding habits, but when grown as a creeper, it is accessible. Cattle and small ruminants reared in Dharwad also readily consumed the foliage of *D. trifoliata* when fed, indicating its acceptability as a fodder (Fig 1). This observations were in confirmation with Ghosh *et al*. (2015) wherein it was reported that small ruminants extensively grazed on *D. trifoliata* in the estuarine belts of Sunderbans.

***Nutritive value*:** The nutritive value of *Derris trifoliata* in terms of protein, cell wall contents, energy, minerals and relative feed values was recorded (Table 3). The CP content (16.47%) of *D. trifoliata* was adequate to meet the protein requirement of maintenance and moderate to a high level of animal production and at par with several legume fodders like *Trifolium* and *Medicago* species (Kiraz, 2011) and shrubs/trees (Singh *et al.*, 2015; Nag *et al.*, 2017). However, Ghosh *et al*. (2015) observed higher CP contents (21%) in this legume, collected from the Indian Sundarban area. Average NDF, ADF and cellulose contents of *D. trifoliata* were relatively lower than most cereal fodders (sorghum, maize and grasses), but similar to mature/full bloom alfalfa (Lardy, 2018). ADF content of *D. trifoliata* was similar to legume hays harvested at flowering stage (Kiraz, 2011). The DDM, DMI, RFV and ME values recorded in the foliage of *D. trifoliata* were relatively lower than reported values of several legume hays (Kiraz, 2011). The variation in these values might be attributed to differences in their cell contents and their digestibility. Forages with RFV values greater than 100 are of high quality, but not considered excellent quality forage particularly for high yielding dairy animals which require fodder of RFV of 150 or more. In the present study RFV of *D. trifoliate* foliage was 108, which was higher than RFV of mature alfalfa (100), but similar to sorghum-sudan grass (vegetative stage). TDN content of *D. trifoliata* foliage (58.7%) was more or less similar to TDN values of full bloom alfalfa (55%).

Table 3. Nutritive value of *Derris trifoliata* foliage

Attributes	Mean values (%)
Crude protein (CP)	16.47±0.162
Ash	7.42±0.283
Organic matter (OM)	92.58±0.283
Ether extract (EE)	4.89±0.035
Neutral detergent fibre (NDF)	52.75±0.688
Acid detergent fibre (ADF)	35.49±0.410
Cellulose	23.06±0.743
Lignin	13.73±0.035
Total carbohydrate (TCHO)	71.22±0.156
Total digestible nutrients (TDN)	58.76±0.534
Digestible energy (DE; Kcal/g))	2.59±0.024
Metabolisable energy (ME; Kcal/g)	2.13±0.019
Dry matter intake (DMI)	2.28±0.030
Digestible dry matter (DDM)	61.25±0.320
Relative feed value (RFV)	108.1±1.973
Ca	0.186±0.009
Mg	0.038 ± 0.01
Na	0.079 ± 0.008
K	0.045± 0.017

***Rotenone content*:** The presence of the active ingredient rotenone qualifies the leaves and bark extracts of *Derris* as a piscicide as well as a botanical insecticide. The rotenoid (6aα,12aα-12a-hydroxyelliptone) found in *Derris trifoliata* stems is a broad-spectrum insecticide and piscicide (Bhagya and Sridhar, 2009). Toxicity of rotenone varied from 2.2 to 360 mg/kg based on the type and species of fish (Ling, 2002). However, Mammals are relatively resistant to rotenone. LD_{50} value for humans

ranges from 300-500 mg/kg (Ling, 2002). In this study, young leaves contained significantly less rotenone compared to old leaves and vice versa in twigs (Table 4). When the leaves and stems were bulked and analysed, rotenone content was only 0.266 µg/g of plant dry weight. However, all the values were in safe limits for ruminant animals. The LD-50 value of rotenone for rabbits was reported as 600-2,000 mg/kg body weight (Hartley *et al*., 1987). Hence, feeding leaves and young twigs to animals are safe despite the reports on its usage as insecticide and pesticide. In higher animals, rotenone is not easily absorbed and accumulated in the body. Absorption is slow and incomplete, but once absorbed, it is broken down by the liver to less toxic metabolites, and around 80% is excreted within 24 hours, as water-soluble products (Ling, 2002). Rotenones are isoflavonoid group of compounds and. *D. trifoliata* leaves contained different kinds of plant secondary metabolites like alkaloids, flavonoids, phenol, tannins, glycoside, terpenoids, and saponins (Ghosh *et al*., 2015). Seasonal variations in such plant secondary metabolites were reported in perennial fodder shrubs/ trees (Katoch, 2019) and therefore, there is a scope for future studies in this line in *D. trifoliata* also to make foliages more safer to ruminant animals.

Moreover, the rotenone content in *D. trifoliata* is used as herbal medicine in India, as a stimulant, antispasmodic and counter-irritant, and against rheumatism, chronic paralysis and dysmenorrhoea (Suganya and Thangaraj, 2014a). The rotenoid (6aα, 12aα-12a-hydroxyelliptone), along with other rotenone, was found to have antitumor properties without any cytotoxicity (Ito *et al*., 2004). *D. trifoliata* leaf extract was found to have antimicrobial activity against several microorganisms but lacked in antifungal properties (Khan *et al*., 2006). The methanol extracts of *D. trifoliata* showed the highest bactericidal activity against the *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) but not against gram-negative *Klebsiella pneumonia* (Suganya and Thangaraj, 2014b). Hence, there is substantial potential to exploit this plant as fodder for ruminant animals as well as ethnoveterinary medicine.

Table 4. Rotenone content in *Derris trifoliata*

Plant part	Rotenone content (µg/g)
Young leaves (0-60 cm from tip)	0.154±.005
Old leaves (> 60 cm from tip)	3.875±0.035
Young twigs (0-60 cm from tip)	3.75±0.055
Old twigs (> 60 cm from tip)	0.21±0.014
Leaves (bulked)	0.266±0.025

Conclusion

The nutritive value and animal acceptability of *D. trifoliata* revealed its suitability as a potential fodder. Besides, its wide distribution in coastal regions ensures its availability as fodder throughout the year. Due to its morpho-physiological adaptations, it survives in a wide range of saline waterlogged soils. The rotenone content was also significantly less. Its availability, nutritive value, adaptability and less rotenone content qualifies *Derris trifoliata* as an appropriate legume fodder for coastal regions to meet the nutrient requirement of animals. But studies to understand the effect of *Derris trifoliata* on physiology and its performance in animals need to be conducted in future to have a complete knowledge on plant-animal nutrition and its interactions.

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