Indian J. Plant Genet. Resour. 33(2): 187–191 (2020)
DOI 10.5958/0976-1926.2020.00027.3

RESEARCH ARTICLE

African Bitter Leaf [*Vernonia amygdalina* Delile]: Study on Seasonal Variations in Total Phenols and Seed Germination in India

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(Received: 21 January, 2019; Revised: 18 April, 2019; Accepted: 25 November, 2019)

Owing to reported medicinal value and seasonal variations in leaf bitterness, African bitter leaf (*Vernonia amygdalena* Delile) was studied for total phenol contents, as an indicator for accumulation of secondary metabolites. The total phenol content in the leaves were found variable [range from $0.158^{a}\pm0.01$ to $0.675^{f}\pm0.02$] in different months. Organoleptic assessment was done to correlate the results to assess leaf bitterness during different months of the consucutive year. Besides, seed germination study was also undertaken to record data on the viability of seeds for conservation in NGB.

Key Words: African bitter leaf, India, Leafy vegetable, Medicinal plant, Seed germination, *Vernonia amygdalina* Delile

Introduction

African bitter leaf or bitter leaf plant [*Vernonia amygdalina* Delile, *Gymnanthemum amygdalinum* (Delile) Sch. Bip. ex Walp.], a native to tropical Africa is a perennial plant shrub/tree of height between 1-6 m with dark green leaves and rough bark, this species has been cultivated in many parts of West Africa (Igile *et al*., 1994). *Vernonia amygdalina* Delile is a species under the genus *Vernonia* Schreb (Family Asteraceae) which has over 1,000 species (Swee Keong Yeap *et al*., 2010). Plant grows near water bodies, in forests margins, woodland and grassland up to 2800m altitude, in areas with mean annual rainfall 750-2000mm (Nwosu *et al*., 2013). Humid environment is more suitable for its growth (Ndaeyo, 2007). Ability of plant to thrive on wide ecological range and all types of soil has led it to perform better in adverse climate.

African bitter leaf plant produces large mass of forage for medicinal and vegetable use (Bonsi *et al*., 1995; Smith and Eyzaguirre, 2007). The species is widely cultivated in Yemen and Ethiopia, South Uganda, Kenya, Tanzania and Brazil (Robinson, 1999). Variations in plant growth, leaf size with degree of bitterness (less or more bitter types) are reported in areas of native regions. Leaves from less watered plant are bitter as compared to those under irrigated condition.

Literature review facilitated in identification of the neutraceutical potential. In view of its hardy nature and success of planting in extreme hot and cold condition in Delhi, it deserved to be assessed for health value.

It is a proven herbal medicine used in polyherbal therapy (combination of herbs or phytochemicals from several sources) to cure or manage many diseases (Ebong *et al*., 2008; Atangwho *et al*., 2011). The leaves are commonly used as a treatment against nematodes in humans and chimpanzees as well as for other intestinal worms (Huffman and Seifu, 1989). It is a well known traditional anti-diabetic plant known in Africa (Akah and Okafor, 1992; Atangwho *et al*., 2011).

In India this species may be a recent introduction from its native region. It is reported under sporadic cultivation in Bihar, Madhya Pradesh, Odisha and West Bengal for medicinal uses (Kumar and Verma, 2012; Bhattacharjee *et al*., 2013). This species has little known medicinal value and popularity in India (Pandey *et al*., 2014). Earlier publications have no mention of the species cultivation or its reported use from India (Ambasta *et al*., 1986; Rao *et al*., 1988; Uniyal, 1995; Karthikeyan *et al*., 2009). In view of its multiple uses as vegetable and in disease management, study was carried out to evaluate total phenols with respect to seasonal variation. Organoleptic assessment was also carried out to correlate

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the phenol contents in different seasons. Besides one of the thrust issues on propagation by seed was studied using seeds set under Delhi conditions with the objective to find out multiplication by seeds to facilitate conservation in National Gene Bank at ICAR-NBPGR, New Delhi.

Materials and Methods

Study was carried out during 2014-18 at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi. Ten mature leaf samples of African bitter leaf tree (third leaf from fifth node at tip) of 0-5 year's age, planted in the experimental garden, were harvested in the first week of each month. The leaves were cleaned using distilled water, surface water was removed by gently tapping on filter paper sheet and the samples were immediately extracted in 80% ethanol for estimating total phenols using folin ciocalteu reagent as mentioned in Arivalagan *et al.* (2018). For data on organoleptic use and medicinal value of the species was gathered from 10 respondents from Delhi and Bihar who were using the leaves for control of diabetes. Leaves of the plant were provides to 10 persons of different age in different months and intensity of bitterness was recorded in numeric scale.

For seed germination study, fully matured flowers were harvested during February-March from three year old plants. The filled seeds were separated using floatation test and were subjected to germination studies. Two replications of 50 seeds each were plated in petriplates with Whatman filter paper soaked in 5 ml of distilled water. The petriplates were kept in germinator at 25°C±2 in dark. In control after 21 days, no germination was observed and therefore seeds were subjected to the following treatments: 1) Co-application of Ethrel (100%); and 2) Presoaking in GA_3 (1000 ppm, 500 ppm, 250 ppm and 100 ppm) for 24 hours, then seeds were washed. After the treatment, 50 seeds in two replications were placed in petriplates at 25°C under dark conditions. Observations were taken on every alternate day for a period of 30 days. Statistical analysis for total phenol content and organoleptic value was done using *Duncan's multiple range Test* (DMRT) to ascertaining significant differences during study period.

Results and Discussion

Evaluation of total phenols

Leaves of *Vernonia amygdalina* have very bitter and astringent taste, which affects its intake when used as medicine or vegetable, etc. (Bonsi *et al*., 1995a). The phytochemical studies revealed the presence of saponins, flavonoids, alkaloids, terpenes, steroids, coumarins, phenolic acids, lignins, xanthones, anthraquinones, edotides and sesquiterpenes (Owoeye *et al*., 2010).

In this study plants of age above 2-3 years showed best phenol contents and there was no significant variation observed in leaves harvested from plants of different age groups. Total phenol content increased with high temperature and low relative humidity (RH) was evident from highly significant negative correlation (-0.716) at error confidence of 1%. Negative correlation was observed between bitters and high temperature with low RH. The phenol contents were assessed using organoleptic test involving 10 respondents in different seasons. Organoleptic value was minimum level in the month of November (2.875^{a}±0.83) and was maximum in the month of April (8.125^{e}±0.83) which is also associated to total phenol contents.

Table 1. Variation in organoleptic value and total phenol contents in different months

Month	Total Phenol (%)	Organoleptic value
January	0.289^{bc}±0.04	4.00^{b} ±0.75
February	0.253^{ab}±0.01	4.00^{b}±0.75
March	0.378^{cd}±0.03	5.00^{c}±0.75
April	0.675^{f}±0.02	8.125^{e}±0.83
May	0.564^{e}±0.05	7.75^{e}±1.03
June	0.384^{cd}±0.10	5.5^{cd}±0.92
July	0.300^{bc}±0.06	5.5^{cd}±0.92
August	0.475^{de}±0.11	6.375^{d}±0.74
September	0.278^{bc}±0.02	4.00^{b}±0.75
October	0.264^{b}±0.06	4.125^{b}±0.83
November	0.158^{a}±0.01	2.875^{a}±0.83
December	0.358^{bc}±0.03	3.625^{ab}±1.06

Correlation is significant at the 0.01 level (2-tailed)

A periodic cyclic increase and decrease in phenol content was observed. There was high phenol content in April (0.675^{f}±0.02) and May (0.564^{e}±0.05) when temperature was high, and rises in August, thereafter it showed continuous decline up to November and then the increased in December and January when temperatures are very low with corresponding values of August 2016-17. Leaves from 0.5-1 year old plants had high phenol contents during the month of May to August in comparison to other age group plants. Similarly 4-5 years old plants had high phenol contents from September to December. Similarly 2-3 years old plants had high phenol contents in the month of January, March and April. Overall results revealed that total phenol contents

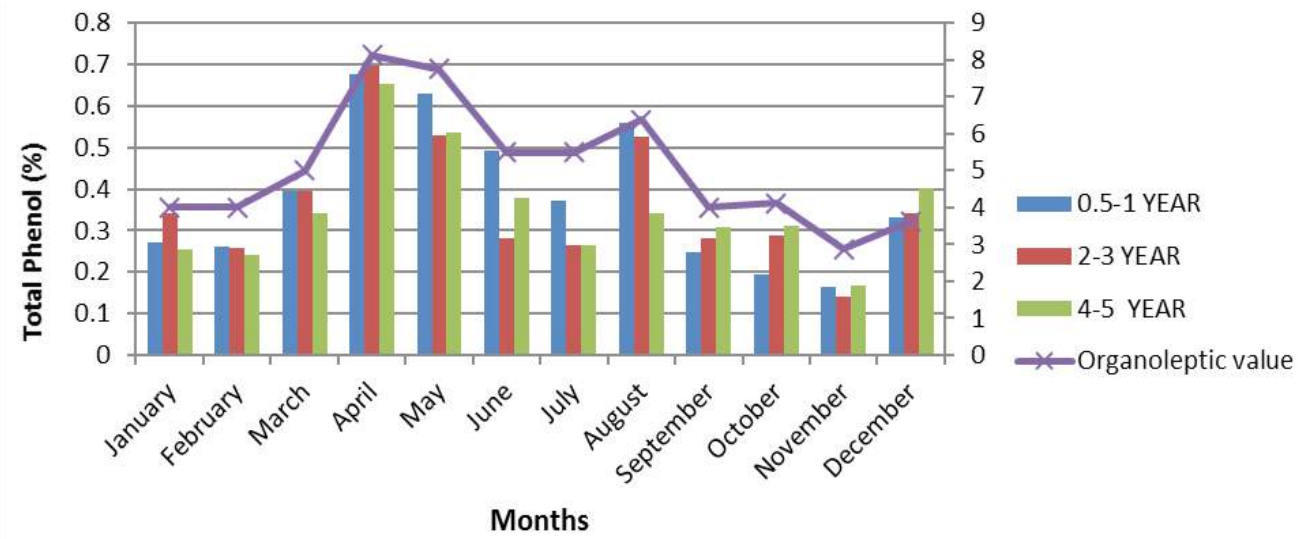

Fig. 1. Data on periodic variation in total phenol contents in plants of different age (2016 and 2017) on primary vertical axis and trends of bitterness in leaf of African bitter leaf based on organoleptic assessment (2016 and 2017) on secondary vertical axis

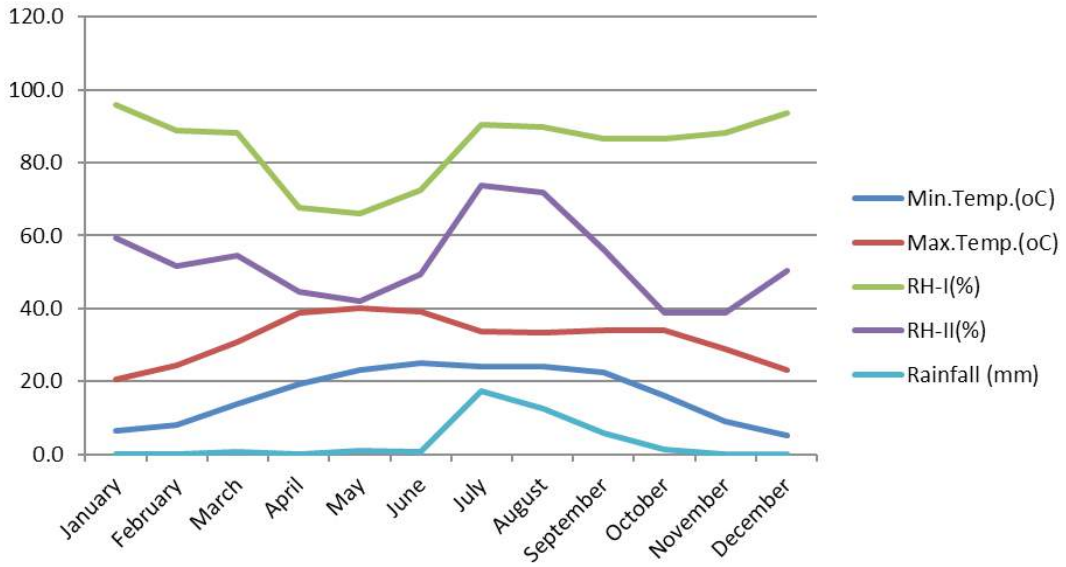

Fig. 2. Variation in climatic variables (rainfall, relative humidity and temperature) recorded during experimentation in Delhi (2016 and 2017)

were highest up to age of 2-3 years, later the phenol contents were decreased, indicating the efficacy of the leaves from plants under cultivation will be only up to three years.

Seed Germination

In planted cuttings in the experimental plots, flowering was reported after three years of age in Delhi. Seeds were harvested for seed germination study to check viability through seed floatation test. Two third of the seeds were found unfilled that was also confirmed through examining under the microscope and found poor or no germination. More routinely *Vernonia amygdalina* in its native area is propagated through stem cuttings although seed propagation is poorly known (Pandey *et al*., 2014). (Anonymous, 2000; http://www.fao.org/livestock/agap/frg/Visit/Ida/Vernonia%20amygdalina.htm accessed on 28-06-18). Pandey *et al*. (2014) have also confirmed that mature seed from dry flower heads raised in nursery in Delhi conditions showed poor seedling emergence.

Fig. 3. Propagation by vegetative cuttings at ICAR-NBPGR, New Delhi; flowering twig; seeds harvested from mature flowers

Its use as vegetable can be explored by selecting and introducing suitable types from native area. In dry months, 2-3 years old plants are best for medicinal use due to high phenol contents; for vegetable use high irrigation is needed to make it suitable for consumption as leafy vegetable. In Africa in highly irrigated conditions the bitters were found low which is in concurrence with our study undertaken in Delhi (Fig. 1).

Conclusions

Vernonia amygdalina has multiple economic uses, mainly as vegetable and for medicinal value (especially for anti-diabetic property) and therefore, deserves for further research in exploring its potential in Indian condition. Study highlights the preliminary work on analysis of total phenols during growth period. Maximum total phenols and organoleptic value was high in the month of April which is suitable for medicinal purpose. Similarly minimum value was recorded in the month of November which indicates lower bitterness and suitable for fresh leafy vegetable or dried powder for future use.

The following thrust areas were identified:

- Selection of types having suitability as vegetable or nutraceutical value.
- Developing agri-horticultural practices for best growth.
- Study on detailed phytochemical profile under Indian conditions and standardization of protocol for different use.
- Develop linkages for medicinal and health use with clinical trials.

Acknowledgements

Authors express their sincere thanks to the Director, ICAR-NBPGR, New Delhi for guidance as well as for providing all available help rendered during the course of study. Acknowledgements are also due to Sh Lalu Rai for providing material from Bihar and facilitating in study.

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