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A STUDY OF SEASONAL VARIATION IN CYTOTOXIC AND ANTIOXIDANT ACTIVITY OF LEAF AND BARK EXTRACTS OF *TERMINALIA RACEMOSA* (FAMILY- COMBRETACEAE)

Dipti Ranjan Behera and Sunita Bhatnagar*

Medicinal and Aromatic Plants, Regional Plant Resource Centre, Nayapalli, Bhubaneswar.

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***Correspondence for Author**
Dr. Sunita Bhatnagar
Medicinal and Aromatic Plants, Regional Plant Resource Centre, Nayapalli, Bhubaneswar.

ABSTRACT

Terminalia racemosa belongs to family Combretaceae, well known for a large group of medicinal trees like *Terminalia arjuna*, *Terminalia chebula* and *Terminalia bellerica*. In the present study a less explored member of the family namely *Terminalia racemosa* was taken up for its medicinal potential. Seasonal variation plays a major role in the medicinal properties of the plant, so in the present study leaves and bark were collected in four different time periods namely in February, May, July and October from the botanical garden of Regional plant resource centre (RPRC), Solvent extracts were prepared and tested for their cytotoxic activity using brine shrimp mortality assay and antioxidant activity using thin layered chromatography(TLC) based 2, 2-diphenyl-1-picrylhydrazyl(DPPH) scavenging assay. In case of cytotoxic activity best results (>90%) were obtained in hexane, acetone and methanol extracts of the month of October for leaf samples and in bark samples all the extracts showed increase of cytotoxicity from February to October. In case of antioxidant activity samples collected in the month of February and July showed more number of antioxidant bands.

KEYWORDS: *Terminalia racemosa*, Brine shrimp, Antioxidant, TLC, DPPH.

INTRODUCTION

Combretaceae family is endowed with a number of medicinally important tree genera, one of the most important genus is Terminalia. *Terminalia arjuna* a well known medicinal plant have been used in folk medicines for hypolipidemic, cardiac stimulant, hypotensive, astringent and homeostatic potential.[1] Ayurvedic formulations such as Arjunarishta, Arjunghruta, Arjunakhsirpak etc have health benefits. The dried fruit of *Terminalia chebula*

(Haritaki), *Emblica officinalis* (Amla) and *Terminalia bellerica* (Vibhitaka) are used in a well known Ayurvedic formulation triphala.[2] *Terminalia chebula* Retz. has been reported for multiple pharmacological and medicinal activities, such as antioxidant, antimicrobial, antidiabetic, hepatoprotective, anti-inflammatory, cardio protective, arthritic, gastrointestinal motility and wound healing activity.[3,4] *Terminalia chebula* has also been tested on cancer cell lines and has shown significant anticancer activity. It was found that *T. chebula* extracts increases cell proliferation and decreases free radical production without affecting the normal cellular matrix.[5] Keeping in view of medicinal potential of all the Terminalia genus, a lesser explored species *Terminalia racemosa* was selected for its cytotoxic and antioxidant activity. As seasonal variation plays a major role in the secretion of secondary metabolites, hence activity was observed for the four months representing different seasons.

MATERIALS AND METHODS

Collection and Processing: - Leaves and bark of *Terminalia racemosa* were collected from Regional Plant Resource centre's botanical garden in four different months (February, May, July and October). Leaves and bark were thoroughly washed under running tap water and were dried in shade. After drying they were made into fine powder using a mechanical grinder.

Solvent extraction: Successive serial solvent extraction was conducted using the following solvents on the basis of polarity by soxhlet extraction method.[6] All the solvent extracts were screened for their cytotoxic and antioxidant potential.

Cytotoxic activity

Brine shrimp mortality assay: - The cytotoxic activity of plant extracts was done by brine shrimp (*Artemia salina*) mortality assay as per standard protocols.[7] A salt solution was prepared for hatching of brine shrimp larvae. 1.8g of KCl was dissolved in 100ml of distilled water and brine shrimp eggs were incubated for 48 hrs at 28 ± 2ºC. Activity of solvent extracts was assessed at doses of 50, 100 and 200µg/ml concentrations respectively. The brine shrimp motility was observed each hour up to 4 hrs to view the motility rate. The motility of experimental was compared with the respective controls and was graded as below; 4+ - Highly motile, 3+ - Motile, 2+ - Sluggish, 1+ - Non motile, Nil – Dead. After 24 hours all the samples were tested for live and dead larvae. Percentage inhibition was calculated by using following formula.

$$\% \text{ of Inhibition} = \frac{\text{Control} - \text{Experimental}}{\text{Control}} \times 100$$

Antixoxidant activity

TLC based antioxidant assay: - Thin layer chromatography based DPPH assay was performed for qualitative analysis of antioxidants. A stock solution of 2 mg plant extracts in 500µl of each solvent extract was prepared. TLC sheets 60 F_{254} (Merck Company) was used as stationary phase. Three different types of solvents were prepared as per the standard protocols.[7,8]

- Ethyl acetate/Methanol/Water (40:5.4:4) [EMW] (polar neutral)
- Chloroform/Ethyl acetate/Formic acid (5:4:1) [CEF] (intermediate polarity/acidic)
- Benzene/Ethanol/Ammonium hydroxide (90:10:1) [BEA] (Non-polar/basic)

0.2 % DPPH solution was used as spraying reagent to develop chromatogram. After drying of sheets DPPH solution was spread and the resulting bands were observed and Retardation factors (R_f) values were calculated by the formula,

$$\textbf{Retardation factor } (R_f) = \frac{\text{Distance travelled by the compound}}{\text{Total distance travelled by the solvent}}$$

Number of yellow bands on purple background was recorded for each extract.

Chemicals: All the chemicals used were of analytical grade and procured from Sisco Research Laboratory Pvt. Ltd. (Mumbai, India), Merck (Germany), Spectrochem Pvt. Ltd. (India), Hi-media (Mumbai, India).

RESULTS AND DISCUSSIONS

The cytotoxic study conducted on brine shrimp larvae showed a remarkable variation in activity of extracts from February to October month. As can be seen from Figure 1, hexane extract of leaf showed maximum activity in the samples of July month, where as DCM extract showed maximum activity in the samples of February, remaining three extracts which were more polar in nature exhibited maximum activity in the month of October. As per Figure 2, hexane and DCM extracts of bark exhibited maximum activity in January samples, where as other extracts of bark exhibited better activity in October samples. This clearly indicates the seasonal variation in the cytotoxic activity of extracts of *Terminalia racemosa*.

The leaf and bark extracts of four different months namely February, May, July and October were screened for antioxidant compounds using different mobile phases of varying polarity and the number of antioxidant bands were presented in the Table-3 & 4. This experiment proved that a significant change occurs in antioxidant pattern according to season and the maximum numbers of antioxidant bands were obtained in February and July month for both leaf and bark samples as compared with the other months.

Study is in confirmation with earlier studies in which secondary metabolite polyphenols which are found in polar solvents are more in the month of winters[9] here also samples of leaf as well bark extract maximum activity in the month of October which is onset of winters. Active principles like bacoside from *Bacopa monnieri* is also high in the month of September, similarly andrograholide content also varies depending upon the time of collection.[10] Thus, for maximum yield of the secondary metabolite and the biological activity of the medicinal plants it is essential to collect the sample at right time. *Terminalia racemosa*, just like other members[11] of family Combrataceae showed significant cytotoxic as well as antioxidant activities.

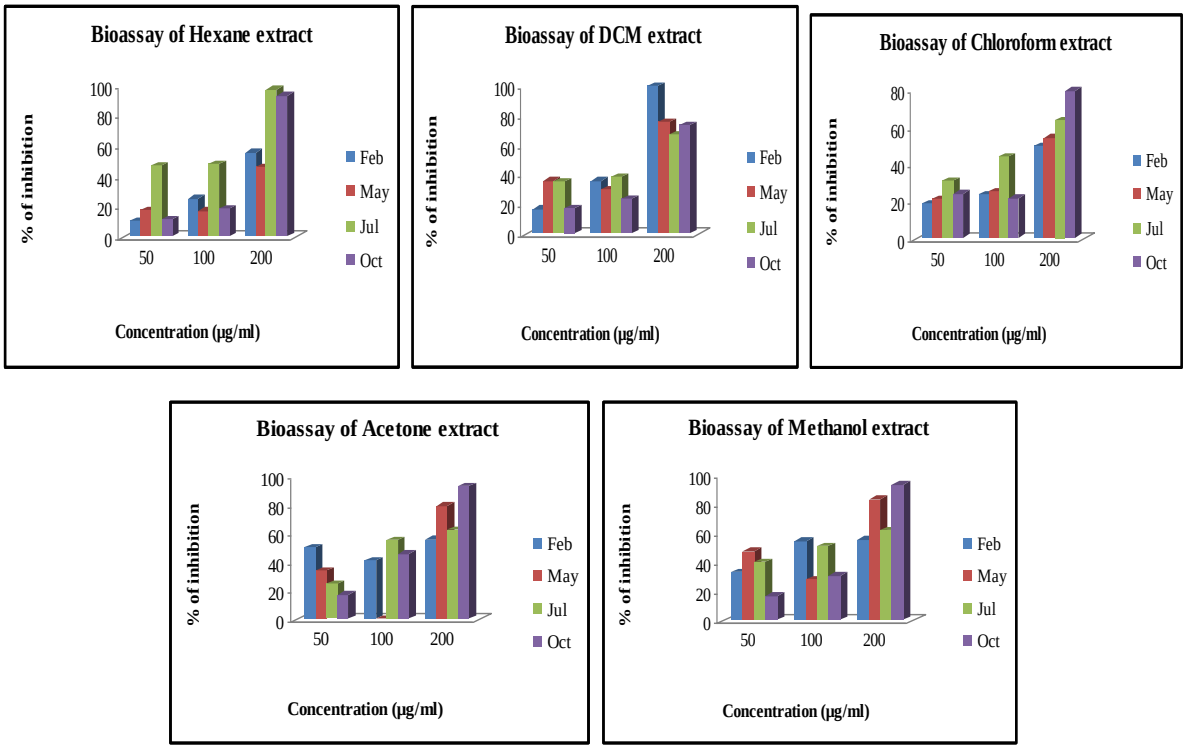

Figure 1: Cytotoxic activity of leaf extracts of *Terminalia racemosa*.

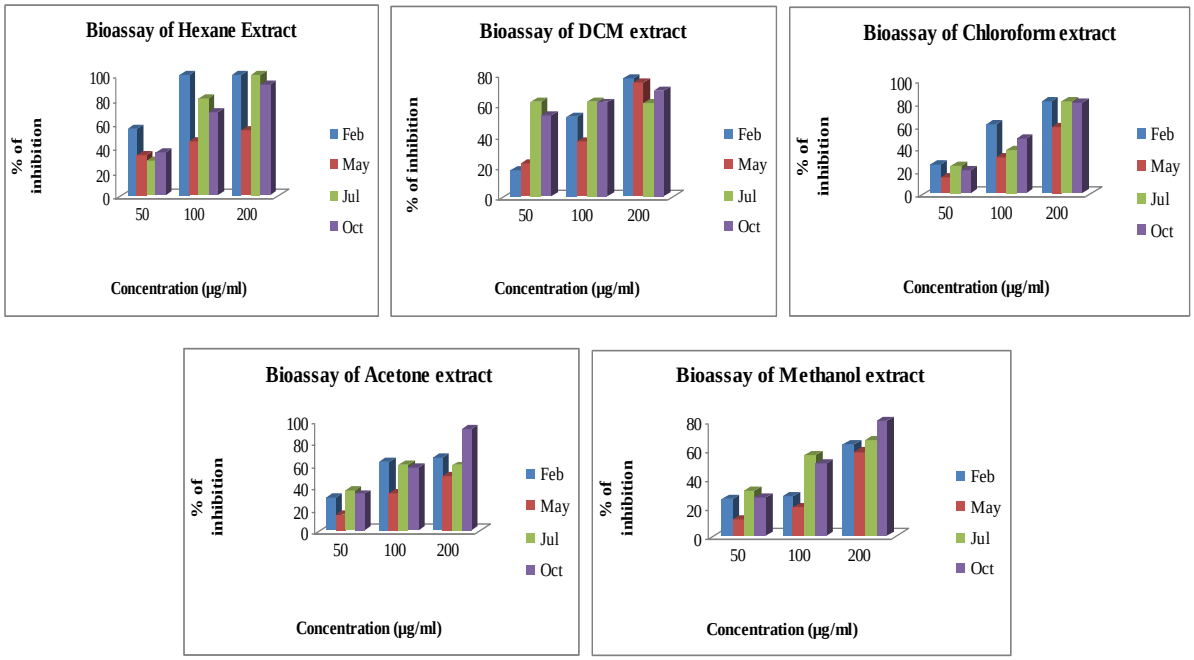

Figure 2: Cytotoxic activity of bark extracts of Terminalia racemosa

Table-1: Number of Antioxidant bands in the solvent extracts of *Terminalia racemosa* leaf extracts

EXTRACT	February			May			July			October		
	EMW	CEF	BEA	EMW	CEF	BEA	EMW	CEF	BEA	EMW	CEF	BEA
HEX	1	2	3	0	0	2	1	4	1	0	0	2
DCM	1	3	3	0	0	3	1	4	4	1	0	3
CHL	2	3	5	0	0	2	1	6	5	0	0	3
ACE	Infinite	1	0	Infinite	3	0	Infinite	1	0	1	2	0
MET	1	3	0	Infinite	3	0	Infinite	3	0	0	0	0

Table-2: Number of Antioxidant bands in the solvent extracts of *Terminalia racemosa* bark extracts

EXTRACT	February			May			July			October		
	EMW	CEF	BEA	EMW	CEF	BEA	EMW	CEF	BEA	EMW	CEF	BEA
HEX	0	2	3	0	0	2	1	4	1	0	0	2
DCM	2	1	3	0	0	3	1	4	4	1	0	3
CHL	1	2	2	0	0	2	1	6	5	0	0	3
ACE	1	Infinite	0	Infinite	3	0	Infinite	1	0	1	1	0
MET	1	Infinite	0	Infinite	3	0	Infinite	3	0	0	0	0

CONCLUSION

Terminalia racemosa has significant medicinal potential as shown by the cytotoxic and antioxidant activity in the study and seasonal variation has a major role in the accumulation of secondary metaboites in the medicinal plant resulting in variable results in the samples collected at different time period.

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