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Regional and Irrigation-Induced Variations in Secondary Metabolites of *Calotropis procera* Flowers in Different Agro-Climatic Zones of Rajasthan

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ABSTRACT: Calotropis procera, a species of family Asclepiadaceae is known in traditional system of medicine from centuries due to its property of curing a number of ailments. It is a xerophytic shrub found in tropical regions of Asia and Africa. It's flower is useful in anorexia, indigestion and intestinal worm infestation, dysentry, abdominal disorders etc. It is used in many Ayurvedic, Unani and Siddha formulations. Hence investigation on the regional and irrigation-induced variations in secondary metabolites of Calotropis procera flowers across diverse agro-climatic zones in Rajasthan, India, was taken up to find best region for their collection. The study shows that ACZ IIIA has the highest petroleum ether extract content and ACZ V displays elevated methanol extract yields. Within ACZ IB, involving Hanumangarh and Suratgarh, contrasting results in petroleum ether extract and methanol extract yields are observed, with petroleum ether extract higher in the irrigated area and methanol higher in the non-irrigated region. ACZ V demonstrates the maximum total extractive yield, while ACZ IB shows minimum values. Flowers from the irrigated area in Hanumangarh (ACZ IB) exhibit lower values for sterols and alkaloids compared to those from Suratgarh, the non-irrigated region, indicating a complex interplay of environmental factors. The observed variations provide insights into the environmental influences on secondary metabolite content of Calotropis procera flowers, contributing to a deeper understanding from ecological and agricultural perspectives.

KEYWORDS: Agroclimatic Zones, Extractives, Medicinal, Secondary metabolites, Variation.

INTRODUCTION

Calotropis procera (*Aak, Aakda*) belonging to family *Asclepiadaceae*, is a soft wooded evergreen perennial shrub with conspicuous purple white flowers found commonly in the sandy desert soils of Rajasthan. The plant grows well up to 2 meters, and its roots are said to reach down to a depth of even 3 meters (Shetty & Singh , 1987; Bhandari, 1990). The flowers are available throughout the year and have medicinal importance. They are bitter, digestive, astringent, anthelmintic, tonic, anti-inflammatory, spasmolytic, stomachic, hepatoprotective & antioxidant and useful in cold, asthma, catarrh, anorexia, inflammation and tumours. The medicinal properties may be attributed to various secondary metabolites present in the flowers of *Calotropis procera* (Parihar & Balekar, 2016; Altayeb & Khalid, 2023**).** *Calotropis* flowers are used in many Ayurvedic, Unani and Siddha formulations. Through local sources it has been known that thses are collected from wild and sold in market at the rate of Rs. 10-15/-per kg on basis of their morphological appearance. The annual collection *Calotropis procera* flowers is about 500-1000 kg per year which is mainly done from Rajasthan as per demand from the market**.**

Rajasthan has been categorized into nine agro-climatic zones (ACZ) based on its climatic conditions and agricultural output, each exhibiting distinct characteristics. *Calotropis procera* is widely spread in almost all the districts of Rajasthan. Given the widespread distribution of this species present study aimed to explore regional variations in two types of secondary metabolites namely sterols and alkaloids. Also since one of the ACZ involving Hanumangarh and Suratgarh is divided into irrigated and non irrigated areas , variation in secondary metabolite content of flowers collected from these areas was also studied to see the effect of irrigation. Thus, this investigation seeks to identify the optimal locations for collecting *Calotropis procera* flowers of best quality.

MATERIALS & METHODOLOGY

All the chemicals used were of Analytical grade and were procured from Merck.

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Reconnaissance surveys were undertaken in various districts under nine different agroclimatic zones of Rajasthan (Table 1) for collection of flowers of *Calotropis procera*. The flowers were collected in single season (winter) so as to nullify the effect of seasonal temperature. The collected flowers were shade dried and their dry weights determined. The flowers were pulverized and extracted with petroleum ether 60-80 followed by extraction with methanol and the total extractive (Petroleum ether extractives (PEE), and Methanol extractives (ME) yield was determined. Sterols and Alkaloids were determined by the methods described below.

Quantitative estimation of total sterols (Goad, 1997)
Powdered flowers of *Calotropis procera* were extracted with acetone for 48 hours in soxhlet apparatus. The acetone extract was concentrated by distillation and then refluxed with 10% NaOH. The saponified extract was reduced to half the volume, then diluted with water and extracted with ether and dried. Evaporation of the solvent yielded sterols which were weighed and yield recorded.

Quantitative estimation of total alkaloids (Akihisa *et al*, 1990 ; Higuchi & Bodin, 1961**)**
The methanol extract of the flowers was neutralized with citric acid so as to extract the alkaloids. The extract was then neutralized when the alkaloids precipitated out. These were extracted in chloroform and then dried over anhydrous sodium sulphate. The solvent was evaporated to yield the alkaloids which were weighed and the yield recorded.

Table 1: Districts Covered in different Agro-climatic Zones

Agro-climatic Zone	District	Agro-climatic Zone	District
Zone IA	Bikaner	Zone IIIB	Alwar
	Jodhpur		Bharatpur
	Jaisalmer		Dhaulpur
	Barmer		Sawaimadhopur
			Karauli
Zone IB	Ganganagar		
	Hanumangarh	Zone IVA	Sirohi
			Udaipur
Zone IIA	Sikar		Rajsamand
	Jhunjhunu		Chittorgarh
	Churu		Bhilwara
	Nagaur		
		Zone IVB	Dungarpur
Zone IIB	Pali		Banswara
	Jalore		Chittorgarh
	Sirohi		
		Zone V	Jhalawar
Zone IIIA	Jaipur		Kota
	Ajmer		Bundi
	Tonk		Baran
	Dausa		Sawai madhopur

RESULTS & DISCUSSION

Variation of dry weight with temperature, rainfall and ACZ

The dry weights of the flowers collected from different agroclimatic zones was recorded (Table 2). *Calotropis procera* collected from different zones showed variation in dry weights from 7.3-15.2%. Higher dry weights were recorded in ACZ IA (14.4%), ACZ IB (12.5%), ACZ IIB (15.2%) and ACZ V (11.6%). Most of these regions are falling in arid zone except ACZ V. Lowest dry weight was found in flowers from ACZ IV B (7.3%).

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The dry weight of the flowers appears to be inversely proportional to the rainfall and temperature (Table 2). However, as seen in variation with different ACZ , other enviromental factors viz. soil etc also appear to play a role in distribution of total biomass of a plant.

Table 2: Variation of Dry weight with Temperature and Rainfall in winter

Agro-Climatic Zone	Max.Temp (degree C)	Average Rainfall (cm)	Dry Wt (%)
IA	23.6	23.5	14.38
IB	20.5	30.0	11.43
IIA	22.0	46.5	10.37
IIB	19.3	41.0	10.50
IIIA	22.0	52.5	15.20
IIIB	22.7	53.5	8.45
IVA	24.2	70.0	8.90
IVB	33.0	94	10.40
V	25.5	84.5	7.30

Variation of Petroleum ether and Methanol extractives in different ACZ of Rajasthan

The yield of the total extractives of *Calotropis procera* flowers in **Petroleum ether** and methanol are given in Table 3. The findings indicate that ACZ IIIA, characterized by slightly higher rainfall, exhibited the maximum content of Petroleum Ether Extract (PEE). This region on the western side is bordered by the low Aravalli hills, extending from the south-west to the north-east, with an annual rainfall ranging from 50 to 60 cms and an increasing trend towards the east. In contrast, Zones IA and IIA, situated in arid and transitional plains, respectively, experienced lower rainfall (10-40 cm). The analysis revealed that ACZ IA and ACZ IIA displayed the minimum content of Petroleum Ether Extract (PEE). The results align with previous studies indicating that moister and cooler conditions lead to higher fat storage (Tripathi *et al*, 1997).

The methanol extract (ME) yield was highest in ACZ V (16.16%) and ACZ IA (12.93%), while ACZ IIIA (7.05%) and IB (7.65%) showed the lowest ME yields. Except for ACZ V, the amounts of PEE and ME demonstrated an inverse relationship. ACZ V, characterized by generally high relative humidity and an annual rainfall varying from 60 to 85 cm, benefited from a developed canal irrigation system with dams and barrages, contributing to increased agricultural production. The higher values in ACZ V are attributed to leaching/dilution effects. The comprehensive analysis provides insights into the regional variations in *Calotropis procera* extractives based on climatic conditions across different zones in Rajasthan. Total extractive yield varies in accordance with methanol extract (Table 3). The maximum yield of total extractives was found in ACZ V (17.71%), while the minimum was observed in ACZ IB.

Table 3: Yield of Petroleum ether, Methanol and total extractives of *Calotropis procera* flowers in different ACZ

ACZ	Petroleum ether extract (%)	Methanol extract (%)	Total Extractives (%)	Total Sterols (%)	Total alkaloids (%)
IA	1.42	12.93	14.35	**2.65**	**8.03**
IB*	2.04	4.57	6.61	2.54	6.65
IB**	1.78	7.65	9.43	2.31	3.63
IIA	1.41	11.38	12.79	2.63	3.05
II B	1.61	12.09	13.70	2.17	5.26
IIIA	1.98	7.05	9.03	1.95	3.63
III B	1.51	13	14.51	0.83	3.08
IVB	1.74	9.96	11.70	1.92	4.09

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IVA	1.67	9.85	11.52	**0.82**	**2.95**
V	1.55	16.16	17.71	1.19	3.97

*****Irrigated ** Non-irrigated

Variation of total sterols and alkaloids in different ACZ of Rajasthan

Following the screening of the Methanol Extract (ME), the presence of alkaloids and sterols was identified. Subsequently, a quantitative estimation of sterols and alkaloids was conducted. ACZ IA emerged with the highest content of both sterols and alkaloids. The maximum total sterols recorded were 2.65%, while the total alkaloids reached 8.03% in this zone. These findings affirm the hypothesis that the elevated extractives observed in ACZ V are a result of the leaching effect of water.

Effect of Irrigation on secondary metabolite contents

Exploration of the impact of irrigation on the secondary metabolite contents of *Calotropis* flowers was carried out. In our explored areas two areas within the same Agro-Climatic Zone (ACZ IB) but in different districts were selected to minimize variations due to soil and other factors. The chosen locations were an irrigated area in Hanumangarh district and a non-irrigated area in Suratgarh of Ganganagar district. Hanumangarh district primarily relies on irrigation from the IGNP canal, with a gross irrigated area of 504161 ha and a net irrigated area of 311782 ha during 2002-03, resulting in an irrigation intensity of 162% (Anon, 2007). In Sri Ganganagar district, irrigation is divided between the northern region, served by the Gang canal, and the southern region, irrigated by the Anoopgarh branch of the IGNP canal (encompassing Anoopgarh & Gharsana tehsils). The irrigation intensity in Ganganagar is 139%. Suratgarh, the third region, remains un-irrigated due to its sandy dunes and desert conditions.

The analysis of PEE indicated a higher yield in the irrigated area (1.78%) compared to the non-irrigated area (2.04%). Conversely, in the case of ME, a higher yield was observed in the non-irrigated area (7.65%) . *Calotropis* flowers collected from irrigated region of Hanumangarh in ACZ IB gave lower values for both sterols and alkaloids as comparison with the flowers from Suratgarh, the non-irrigated region. However, the dry weight of flowers was more in irrigated region (12.4%) as compared to that in non-irrigated region (10.3%). This study sheds light on the intriguing impact of irrigation on the secondary metabolite contents of *Calotropis* flowers across different regions within the same Agro-Climatic Zone. The results of our study aligns with the reported literature that cultivated crops have lower levels of active constituents (Ku et al, 2020; Li et al, 2023). This also suggests that irrigation practices may influence the biosynthesis or accumulation of sterols and alkaloids in *Calotropis* flowers, highlighting the potential impact of environmental factors on the secondary metabolite composition.

CONCLUSION

This comprehensive study underscores the intricate relationship between climatic conditions, irrigation practices, and the secondary metabolite content of *Calotropis procera* flowers. The variations observed across different zones and under irrigation highlight the need for a nuanced understanding of environmental influences on secondary metabolites, contributing valuable insights for both ecological and agricultural perspectives. The study will help the flower gatherers in gaining more revenue by collection of flowers containing maximum metabolites from identified areas.

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