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Variation of total sugar, protein and EC of different parts of *Enicostema Littorale* and *Phyllanthus Niruri* on different days of Rewa Region

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Abstract
The basic principle of Ayurvedic & siddha medicine practices was based on the guna of plant parts sap tested on the properties of various taste i.e. Sweet, Sour, Salty, Bitter, Pungent and astringent. These are the main basic criteria to classify plants for various ailments of disease. And this ancient pathy was quite useful without no side effect. This successful sustainable pathy frequently used from the ancient time. New order of world economy imposed modern medicine pathy which is not cause based with various side effect, not affordable & not sustainable. There is urgent need for development of sustainable and affordable pathy for the rural poor. The scientific validation of locally available phyto resources for recovery of various disease is urgently required. The judicious management for conservation & cultivation of wild medicinal plants in specific climate increases the effective potential of the greater bio-molecules & their secondary path. Their is urgent need to asses the possibility of fitness of these bio-molecules with the inside protein of body system. Ecological modeling of greater bio-molecules is quite important to enhance the curability percentage of the disease.

Keywords: Ayurvedic, Siddha medicine, Plant parts, Disease, Modern medicine, Rural poor, Conservation & cultivation, Bio-molecules

1. Introduction

Plants have capability to synthesise various secondary compounds normally known secondary metabolite path, play vital role in the life cycle of plant species. According to one estimate 12000 type of various compounds have been isolated from the different wild plants reported by earlier worker. A number estimated to be quite less than 10% of the total. In many plant species these compounds play vital role for maintaing the plant life by creating defense against predation by variety of insects, microorganism & herbivores. Among these some chemical responsible for development of odour (Terpenoids) pigmentation (tannins and quinines) and flavour (capsacin) however, several of these compound possess various medicinal properties (Cowan, 1999; Charnock, *et al*. 2001) [7, 3]. Earlier many contribution on medicinal plants have already been proved the potentialing of these chemicals in wild medicinal plants (Taylor, *et al*. 2001; Ogunkunle and Tonia, 2006; Adeneye, *et al*. 2006; Parekh & Chandra, 2006, 2007; Mallikharjuna, *et al*. 2007) [24, 13, 1, 15, 16, 12]. Verpoorte, (1998) [26] emphasized the role of secondary metabolite in drug development.

Consumption of wild herbal medicine is popularising and increasing day by day, harvesting from the wild, the main source of raw materials, is causing enough loss of various genotype and destruction of natural habitat. Domestic cultivation of wild medicinal plants is a viable alternative and offers the opportunity to overcome the problems that are inherent in herbal extracts: misidentification, genetic and phenotypic variability; extract variability and instability, toxic components and contaminants. The various contributions on qualitative standarisation have been done to develop desired variation in phenotype for better production of bioactive greater molecules on controlled environmental conditions (Canter, *et al*. 2005) [2]. The beneficial medicinal effects of plant materials is due to result of the combinations of secondary product present inside the plant. The medicinal action of plants is unique to particular plant species or group is consistent with this concept as the combination of secondary products in a particular plant is often taxonomically distinct (Wink 1999) [28]. This is contrast to primary products viz carbohydrate, lipids, proteins, heme, chlorophyll and nucleic acids, which are common to all plants and are involved in the primary metabolic pathway processes of building and maintaining plant cells as suggested by Kaufman, *et al*. (1999) [11]. Although plant secondary products have historically been defined as chemicals that do not appear to have a vital biochemical role in the process of building and maintaining plant cells. Secondary products have both a defensive role against herbivory, pathogen attack, and inter-plant competition and an attractant role toward beneficial organisms such as pollinators, symbiotants (Kaufman, *et al*. 1999; Wink & Schimmer, 1999) [11, 27]. The great role of the secondary product have been reported by earlier workers. The plant secondary products also have protective action in relation to abiotic stresses, such as those associated with changes in temperature, water status, light level, UV exposure and mineral nutrients.

2. Material and Methods

Determination of fats and proteins

Crude fat in plant samples was determined by exhaustively extracting a known weight of powered plant parts material with petroleum ether using soxhlet apparatus. The ether is evaporated and the residue weighted. The extracted crude. Fat of plant samples represents, besides the true fat (triglyceides), phospholipids, sterols, essential oils, and fat soluble pigments etc. protein was determined by microkjeldhal methods by multiplying nitrogen with 6.25. This is based on the assumption that plant proteins consist 16% of nitrogen.

Determination of carbohydrates sugars cellulose Lignin

Carbohydrate content, other than sugars, for plant samples was obtained by the difference methods. The sum total of ash, acidity, crude fat, protein, sugars and crude fibers is subtracted from 100, represents primarily the carbohydrate content which also includes starch, protein, gums etc. the sugar content in the plant samples was estimated by determining the volume of unknown sugar solution required to completely reduce a measured volume of fehling's solution to red, insoluble curowsoxide. The reducing sugar in plant samples (Juice) was determined by mixing with lead acetate; kept overnight, mixed with potassium oxylate and titrated with fehling's solution A+B. For total sugar the overnight filtered juice of plant samples mixed with H_2SO_4, and again kept for another 24hrs thereafter neutralised with NaOH solution using phenolphthalein as an indicator. This solution is titrated with the fehling solution (A+B).

Acid detergent lignin (ADL) was determined using fibertec apparatus by de-fating a known weight of plant sample (w1) with acetone (cold extraction) and with acid detergent solution (hot extraction), and washed with hot water. The sample is mixed with H_2SO_4, for 3 hrs, again washed to free from acid. It is dried, weighted (w2) and ashed in muffle at 525°C for 3hrs and again weighted (w3). The ADL is calculated as per following formula

$$\text{ADL (\%)} \quad \frac{\text{W2–W3}}{\text{W1}} \times 100$$

Cellulose is determined by dezincification of plant samples, which yield the product consisting of cellulose plus various other polysaccharides, Mainly hemi-cellulose. Cellulose was determined by difference of acid-detergent fiber minus acid detergent lignin. Hemicellulose was determined as the difference 0 neutral detergent fiber and acid detergent fiber using fibertec apparatus.

Determination of minerals (Macro-Nutrients)

Nitrogen was determined through micro-kjeldahl method by digesting a known weight of plant sample and treating it with alkali. The liberated ammonia is collected in boric acid and titrated with HCl. Phosphorus was estimated calorimetrically by treating the digested sample. With ammonium molybdate and freshly prepared ascorbic acid. Spectrophotometer apparatus was used to measure the absorbance at 880nm. Potassium and sodium was determined through flamephotometer. The flame excited atoms of potassium and sodium emit radiation at different specific wavelengths, which is measured using different filters. Calcium and magnesium in plant samples was determined by EDT A (The disodium salt of ethylene diamine - terta acetic - acid) tiration method.

Determination of micro nutrients

The micro-nutrients (Fe, Zn, Cu, Pb, Mn) were determined through atomic absorption spectrophotometer method. The plant samples were digested in tri-acid solution of HCI04. $HN0_3$ and H_2SO_4 were passed. Through atomic absorption spectrophotometer using different lumps and values were recorded, which further calibrated for different micro-nutrients.

The ecology on stress habitat on different diverse community of vegetation of the habitat have been studied by Choudhari, *et al*. (1979) [6]; Varshney (1983) [25]. The study reflect the plasticity and adoptability of herbaceous vegetation of Rewa region. The grasses community have shown greater ecological amplitude and greater adoptability to adjust himself on specific microclimatic habitat. The phytosociological work done on the wild medicinal plants of region broaden the ecological concept for better cultivation & adoptations and increase the productive pattern of the community of vegetation of Rewa region.

3. Results

Table 1: Eco-Cultivation Growth Performance of *Enicostema littorale* on different types of soils.

S. No.	Growth Parameter	Mixed Soil		Garden Soil		Alluvial Soil		Black Cotton Soil		Murrum Soil	
		Control	Natural Site	Control	Natural Site	Control	Natural Site	Control	Natural Site	Control	Natural Site
1	No. of Roots	7 ±	9 ±	8 ±	10 ±	11 ±	14 ±	10	13	6 ±	8 ±
2	Average Length of Roots	25 ±	30 ±	27 ±	35 ±	29 ±	36 ±	28	35	20 ±	24 ±
3	No. of Branches	15 ±	17 ±	17 ±	21 ±	20 ±	22 ±	20	23	12 ±	15 ±
4	Average Length of Branches	18 ±	20 ±	19 ±	22 ±	20 ±	23 ±	19	22	15 ±	19 ±
5	Average No. of Leaves/Plants	16 ±	18 ±	19 ±	22 ±	21 ±	24 ±	20	22	14 ±	16 ±
6	Average No. of Seeds/Plants	55 ±	67 ±	65 ±	74 ±	68 ±	76 ±	65	72	52 ±	58 ±
7	Average Weight of Seeds (mg)	42 ±	46 ±	45 ±	52 ±	48 ±	54 ±	46	52	40 ±	43 ±

Table 2: Eco-Cultivation Growth Performance of *Phyllanthus niruri* on different types of soils.

S. No.	Growth Parameter	Mixed Soil		Garden Soil		Alluvial Soil		Black Cotton Soil		Murrum Soil	
		Control	Natural Site	Control	Natural Site	Control	Natural Site	Control	Natural Site	Control	Natural Site
1	No. of Roots	4 ±	5 ±	5 ±	7 ±	8 ±	10 ±	7	9	3 ±	5 ±
2	Average Length of Roots	15 ±	18 ±	16 ±	20 ±	20 ±	22 ±	18	20	12 ±	14 ±
3	No. of Branches	13 ±	15 ±	14 ±	18 ±	16 ±	19 ±	17	19	12 ±	14 ±
4	Average Length of Branches	14 ±	16 ±	15 ±	17 ±	18 ±	20 ±	19	21	12 ±	15 ±
5	Average No. of Leaves/Plants	10 ±	12 ±	12 ±	14 ±	14 ±	18 ±	14	17	8 ±	10 ±
6	Average No. of Seeds/Plants	65 ±	68 ±	66 ±	70 ±	70 ±	74 ±	65	72	55 ±	60 ±
7	Average Weight of Seeds (mg)	35 ±	38 ±	37 ±	40 ±	40 ±	45 ±	38	42	24 ±	31 ±

Table 3: Variation of Total Sugar, Protein and EC of different parts of Enicostema littorale on different days

S. No.	Plant Species	Plant Parts	No. of Days	Total Sugar %		Protein		EC (1:1)	
				Control Site	Natural Site	Control Site	Natural Site	Control Site	Natural Site
1	Enicostema littorale	Root	40	3.00	2.90	–	–	4.50	3.80
			80	2.65	2.55	–	–	4.30	3.40
			120	2.50	2.40	–	–	4.10	3.20
			160	2.20	2.10	–	–	4.00	2.90
			200	2.10	1.90	–	–	3.80	2.50
		Stem	40	2.80	2.65	–	–	4.20	3.70
			80	2.72	2.58	–	–	4.00	3.50
			120	2.62	2.50	–	–	3.90	3.30
			160	2.50	2.40	–	–	3.80	3.10
			200	2.30	2.20	–	–	3.00	2.80
		Leaf	40	2.60	2.50	–	–	4.00	3.50
			80	2.48	2.30	–	–	3.80	3.30
			120	2.35	2.10	–	–	3.70	3.10
			160	2.20	1.90	–	–	3.50	3.00
			200	2.10	1.80	–	–	3.00	2.70
		Seed	40	4.10	3.90	–	–	4.90	3.90
			80	3.85	3.60	–	–	4.60	3.50
			120	3.60	3.20	–	–	4.40	3.20
			160	3.20	2.80	–	–	4.20	3.10
			200	3.00	2.50	–	–	4.00	2.90

Table 4: Variation of Total Sugar, Protein and EC of different parts of *Phyllanthus niruri* on different days

S. No.	Plant Species	Plant Parts	No. of Days	Total Sugar %		Protein		EC (1:1)	
				Control Site	Natural Site	Control Site	Natural Site	Control Site	Natural Site
1	Phyllanthus niruri	Root	40	2.80	2.72			4.70	3.50
			80	2.60	2.48			4.40	3.20
			120	2.50	2.35			4.20	3.00
			160	2.20	2.00			4.00	2.90
			200	2.00	1.90			3.80	2.40
		Stem	40	2.70	2.38			4.00	3.30
			80	2.58	2.30			3.90	3.10
			120	2.40	2.10			3.60	3.00
			160	2.20	1.90			3.40	2.90
			200	1.92	1.68			3.20	2.60
		Leaf	40	2.35	2.00			3.90	3.20
			80	2.20	1.80			3.70	3.00
			120	2.10	1.75			3.40	2.90
			160	2.00	1.60			3.20	2.60
			200	1.80	1.50			3.10	2.48
		Seed	40	3.85	3.30			4.00	3.10
			80	3.60	3.10			3.90	3.00
			120	3.42	2.90			3.70	2.90
			160	3.20	2.75			3.50	2.70
			200	2.90	2.60			3.20	2.60

4. Discussion

Various contribution regarding the ecology of the various habitat of the world have been made by Duthie, (1903-29) [9]; Puri & Sharma, (1951) [18]; Prasad, (1966b) [17]; Chauhan, *et al*. (1967) [4]; Hooker, (1872-97) [10]. The vegetational pattern of Madhya Pradesh have been completed by Chauhan *et al*. (2008) [5]. The most valuable work on plant community has been done by Raymond & Graham, (2006) [20] and Sonja, *et al*. (2006) [23]. Modern aspect of Phytosociological work in the plant community of Europe have been reported by Direchke, (1998) [8]. Grassland communities are best known for reflecting the scenario of vegetation & climo-edaphic factors of whole region. The various work related with grassland ecology & phytosociology have been made by Sant, (1942, 55) [21]; Raman, (1966) [19] & Singh, (1967) [22].

This is evident from Table 3 & 4 that marked variation in level of sugar percent (basic primary metabolite) have been noted. Fluctuation in the values of sugar clearly indicative of the formation of secondary metabolite which is indeed cause of the greater bimolecules present inside the plant. These secondary products useful as antimicrobial medicine through cytotoxicity action while used as neurotoxin activity in many types of drug anti-depressant, sedatives, muscle, relaxants & anesthetics. The Secondary metabolite beneficial effect on human might be due to similarities in their potential target sites as noted by Kaufman, *et al.* 1999 [11].

Fluctuation in EC (1:1) values have been recorded (Table 3 & 4). Increase in secondary metabolite consequently decrease in primary metabolic pathway clearly indicative of the pathway of synthesis of bio-moleucles and their action on human might be due to resemblance of endogenous metabolite & their potential target sites. The research findings are agreement with the finding of so many earlier workers (Kaufman, *et. al.* 1999.) [11].

The decrease in EC (1:1) values consequently increase in secondary metabolite in 80 to 200 days noted for both the species as well as for both the sites. The work results are in conformity with the finding of the work of Wink (1999) [28]; Taylor, *et al.* (2001) [24]; Parekh & Chandra (2006, 2007) [15, 16].

The good growth parameters of both the species noted in alluvial & Black cotton soil whereas murrum does not shown promotion of vegetative growth of the plant. The value of growth parameters are already represented in table 1 & 2. The data clearly indicative of wild medicinal plant for better performance establishment & growth of plant species. The work is in agreement with the work of so many earlier workers.

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