

A REVIEW ARTICLE ON RANDOM METHOD OF VEGETATION SAMPLING AND ANALYSIS OF VARIOUS QUANTITATIVE CHARACTERS OF MEDICINAL PLANTS OF REWA REGION

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ABSTRACT

Medicinal and aromatic plants are mostly found in forest wild areas throughout South Asia from the plains to the High altitude like Himalaya's and also in another peaks with the dense concentration in the tropical and subtropical habitat, even in the xeric environment. India recognizes more than 3,500 plant species having medicinal value distributed all over the zone according to the need of the micro-climatic suitability of plant species. Some wild medicinal plants have greater wide ecological amplitude and to adjust themselves into various ecological habitat. While others are localised into specific microclimatic conditions and growing only in specific habitat. Over harvesting, destructive collection technique and conservation, conversion of habitats are prime cause for extinction of some wild medicinal plants of tropical region. Recent study done on the various aspect of ecological & economic importance of wild medicinal plants of Rewa region which plays promising role for improving the physiological and morphological function of overall system of the body system. Modern pattern of living, eating and settlement on dirty places, destroy the basic immunity of the body due to the toxication and deposition of toxic substances inside the body system. Toxicated food, water and impure air consequently, reduce the physiological and functional aspect of body system. Subsequently, destroy the resistance of body consequently, a lot of chances to get infection of various new generation microbes, which are quite adoptable for new habitat causing various diseases and disorder in body system.

Keywords: Medicinal and aromatic plants, Wild, High altitude, Ecological Amplitude, Immunity, Toxicated food etc.

INTRODUCTION

After surveying the various localities of the region the floristic list was prepared by collecting and identifying the wild medicinal plants of Rewa region and herbarium was prepared as suggested by *Duthie, 1960; Maheshwari, 1963*. Random method of vegetation sampling and analysis of various qualitative and quantitative characters of vegetation analysed as formula given by *Misra, 1968*. Soil analysis done as methods suggested by *Munsull, 1905; Piper, 1944; Jackson, 1958; Watanable & Olson, 1962; Moodie, et. al. 1963 & Pandey, et. al. 1968*.

Standard methods have been used for identification of phytomedicinal properties, conservation and management strategies of wild plants grown on the habitat of Rewa region. Collection & Identification of the plants done with the help of various floras and important websites available on the internet (*Duthie 1960*).

Pal, et.al. (2002) has been reported a list of plant species used for treatment of hepatic disorder. Little research work has been taken to evaluate on wild plants for treatment of various disorder of body system. Some contributions have been made on wild plants & herbs to cure various disorder specially hepatitis without verifying or testing with serological test. These studies have not got proper importance due to lack of facilities for pharmacological testing and not classifying hepatitis-B, non A and non B hepatitis consequently, due to hepatitis-C. *Shiki, et.al.(1992)* has been studied antiviral properties of Glycyrrhizin a major component of the licorice root (*Glycyrrhiza glabra*) due to supply of endogenous interferon and their and multifarious action to betterise ruined hepatic cells, *Suzuki, et.al. (1983)* has been noted decrease in ALT Values and normalisation of hepatic cells and proved histological improvement. Glycyrrhizin has also been shown to inhibit RNA viruses

through a hitherto unknown mechanism (*Pompei, et.al.1979*). ICMR, CIMAP, CDRI and few medical laboratories conducting research on combinational therapy and their side effects, several other formulations also shown promising result in hepatitis-C & various other treatment. However, our concern is the side effects profiles of certain herbs like Sho-saiko develop pneumonitis and 55% cases occurred in anti HCV positive patients. Anecdotal evidence and several small and inadequately controlled studies of herbal preparation proved recovery or amelioration on the hepatic cellular system. There is great need to evaluate the potentialities of these bio-molecules of herbs & wild plants present on the used for treatment of liver disorder. These herbs and wild plants are frequently grown in the diverse microclimatic condition of Rewa region. Various wild plants of Rewa region is used as crude drugs for various disorders. Various noteworthy contributions on wild medicinal plant have been reported by *Jain, (1980)*; *Maheswari, (1984)* & *Prajapati, et. al.(2003)*. The description of various morphological parameters were verified directly from the plants. The extensive survey of Rewa region and surrounding area was done during the primary phase of study to know the distribution pattern of wild medicinal plants of Rewa region.

METHODOLOGY

Climate data: Month-wise climatic datas were noted such as minimum and maximum temperature, minimum and maximum relative humidity, rainfall Kuthulia office, Rewa. Average were calculated on the basis of data obtained.

Biometrical: After detail survey of many sites, four sites were selected for present investigation. Frequency classes of associated were assigned as per methods suggested by *Misra & Puri (1954)*, and *Pandeya, (1969)* frequency classes in relation to percentage of frequency is given below.

Frequency class	Frequency %
A	0-20
B	21-40
C	41-60
D	61-80
E	81-100

Standard methods and laboratories have been used for analysis of relevant parameters for nutraceutical value of plants as well as for their parts. Vegetation study site were analysed quantitatively by using quadrat method. The 01×01 M. size of quadrat was used as a unit of sampling of vegetation. Sampling was done by laying randomly 10 quadrat during the mid winter season. The presence and absence of each species in each quadrat was recorded. The basal area of the species was determined by measuring the diameter at the pointing of emergence for annuals while on one meter height for perennial. The formula for calculating frequency, density, abundance, basal area relative frequency relative density, relative dominance of each species determined with the help of following formula.

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which a species occurred}}{\text{Total numbers of quadrats sampled}} \times 100$$

$$\text{Density} = \frac{\text{Total number of individuals of species in all quadrats}}{\text{Total number of quadrat sampled}}$$

$$\text{Abundance} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Total number of quadrats in which the species occurred}}$$

$$\text{Basal Area} = \pi r^2$$

$$\pi = 3.14$$

r = radius of stem at the point of emergence.

Importance Value Index of each species was calculated by summing the percentage value of relative frequency, relative density and relative dominance expressed in per 300.

$$\text{Relative frequency(\%)} = \frac{\text{Number of occurrence of species}}{\text{Number of occurrence of all species}} \times 100$$

$$\text{Relative Density(\%)} = \frac{\text{Number of individuals of species in all quadrats}}{\text{Number of individuals of all species in all quadrat}} \times 100$$

$$\text{Relative dominance (\%)} = \frac{\text{Total Basal cover of a species in all quadrats}}{\text{Total basal cover of all species in all quadrats}} \times 100$$

$$\text{Importance Value Index (IVI)} = \% \text{ Relative frequency} + \% \text{ Relative density} + \% \text{ Relative dominance}$$

Composite Soil samples were collected from 4 different sites in middle of winter season. The first week of the month was used for soil collection. The physical chemical characteristics were analysed by using standard methods as suggested by earlier workers referred in the text.

Soil sampling was done by using wooden stakes and metal wire, 10 quadrat each of a square meter were laid randomly. Composite soil samples, each comprising ten sub samples taken from these quadrats up to 20 cm depth. composite soil samples were collected. Soil sampling was done systematically in the first week of month round the year from all four sites.

After collection, the samples were brought to the laboratory after crushing & mixing, the soil sample were passed through a 2mm sieves and then stored in labeled polythene bags.

Samples of various wild medicinal plants and their parts including all parts were collected from different areas of Rewa region. Which are frequently grown in the area and used by poor as food & medicinal material. All samples were washed thoroughly to remove off impurities, and blotted dry. After cleaning and drying plants and their usable parts were separated into usable and non-usable portions taken into small portions and non usable parts were removed before oven drying the samples. Five samples for different usable parts of wild species were oven dried at 100°C till constant weight. The dried parts crushed, sieved & packed as fine powder for analysis. For the urgent analysis the fresh samples were used (e.g. Crude fat, acidity, vitamin C; TSS). Nutrient examinations were done within one month of the sample collection.

Various standard for analyses of plant material were used as given by *Moerman, 1999, Loporatti, 2003*. The quantitative analysis of the plant parts samples was broadly done for analysis technique. Primary analysis for organic groups of the plant samples, such as fats, carbohydrate, proteins, sugars fibers, Ash, Acidity, etc. were done as per standard methods. It accounts for most of the organic dry matter of the food & medicinal materials. The other analysis refers to the determination of a particular element (Viz, N, P, K, La, Mg, Na, etc) or a compound present in the plant material. A brief analytical methods used as given below.

Analysis of Moisture, Fibers & Ash

Moisture in plant parts samples were determined by measuring loss in weight due to oven drying the plant samples till constant weight and calculating the difference between fresh weight and dry weight. Ash determination for plant materials refers to the residue left after combustion of dried samples and is a measure of the total mineral content in the plant parts. It was estimated by using 25g of the powdered plant samples in silica crucibles in a muffle furnace at 550°C for 3 hours. Crude fiber is essentially the residue left after sequential not digested with H₂SO₄ and NaOH. It mainly consists of cellulose together with a little lignin crude fiber was determined by acid and alkaline digestion methods using fibertec apparatus.

Determination of TSS, acidity and vitamin C:

Total soluble solids (TSS) content of plant parts samples was done through hand refractometer, which gives refractive index by placing a drop of usable parts, syrup on the prism and reading the corresponding value of substance by direct reading. Acidity of plant parts sap was determined by titrating a known weight of sample with 0.1 N NaOH using a few drops of 1% phenolphthalein solution as the indicator. The value calculated with reference to percent anhydrous citric acid. Fruits and vegetables are important source of ascorbic acid. The method used is based on the reduction of a dye (2, 6 - dichlorophenol) by ascorbic acid. The dye, which is blue in alkaline solution and red in acid solution, is reduced by ascorbic acid to a colorless form.

Determination of chlorophyll and anthocyanin

The colors of usable parts are very important as it is a primary determinant of the quality of the product. Chlorophyll and anthocyanin are frequently occurring as mixtures. Chlorophyll was determined by macerating plant sample in acetone in a pestle and mortar the filtered solution is tested for CO in a spectrophotometer at 663 nm and 645nm, anthocyanins are fundamentally responsible for all the colour differences. It was determined by mixing usable parts with ethanolic HCl using a mortar and pestle. Filtered extract is kept for 24 hrs and CO' is taken at 535 nm using the spectrophotometer.

Determination of fats and proteins:

Crude fat in plant samples was determined by exhaustively extracting a known weight of powdered 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 (triglycerides), 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 currowoxide. 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 H₂SO₄, 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 H₂SO₄, 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

$$\text{ADL (\%)} = \frac{W2-W3}{W1} \times 100$$

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 of 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 flame photometer. 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) titration 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 HClO₄, HNO₃ and H₂SO₄ were passed. Through atomic absorption spectrophotometer using different lamps and values were recorded, which further calibrated for different micro-nutrients.

CONCLUSION

Medicinal plants have great curative properties due to the presence of various complex chemical substance of different composition which are found as secondary plant metabolites in one or more parts of these plants (Kaufman, et.al.1999; Wink & Schimmer 1999; Lemar, et. al. 2002; Lindsey, et. al. 2005; Karthikeyan, et.al.2007). These plants metabolites are grouped as alkaloid, glycosides, cardiacosteroids and essential oil etc. Among alkaloids morphine and *codeine* (poppy), *strychine* and *brucine* (Nuxvomica), *quinine* (chin-chona), *ergotamine* (ergot), *hyocyanine* (belladonna), *scolapomine* (datura), *emetine* (ipecac), *cocaine* (coco), *ephedrine* (ephedra), *reserpine* (Rauwolfia), *caffèine* (tea dust), *aconitine* (aconite), *vascine* (vasaca), *santonin* (artemesia), *lobelin* (Lobelia) and large number of others, Glycosides form another important group represented by digoxin (foxglove), *Stropanthin* (strophanthus), *glycyrrhizin* (liquorice), *basbolin* (aloe), *sonnocides* (senna) etc. Cardicosteroids have come into sennocides (senna) etc. Cardicosteroids importance have come into light recently and diosgenin (Dioscorea), solasodin (solanum sp.) etc. are now in the large world demand. Some essential oils such as those of valerian kutch and peppermint also possess medicating properties and are used in pharmaceutical industries.

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