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The Impact of Geographical Factors on the Proximate Composition of *Rosmarinus Officinalis* L. leaves

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Abstract

In worldwide, the use of natural compounds has enormously improved the significance of persistent quality of plant materials. As a result, there is a rising scientific concern in the impact of geographical distributions of the plants on their chemical constituents, physical characteristics and biological activities. The present study was carried out to observe the effect of geographical distributions on the proximate composition of *Rosmarinus officinalis* L. (Rosemary) leaves collected from five different locations of Kodaikanal hills, Dindigul district, Tamil Nadu, India. The total crude protein content of five samples of rosemary leaves (RO1, RO2, RO3, RO4 and RO5) was 11.58, 13.38, 15.47, 9.3 and 7.29 % dry weight, respectively. The moisture content of five samples of rosemary leaves (RO1, RO2, RO3, RO4 and RO5) was 6.32, 5.4, 4.47, 7.8 and 8.93 % dry weight, respectively. The results revealed the considerable impact of geographical locations on the levels of proximate nutrient in the samples of *R .officinalis*. These variations must be taken into consideration while utilizing raw plant materials for industrial applications and traditional therapies.

***Keywords*:** Pharmacognostic, Extractive value, *Rosmarinus offcinalis,* crude fibre

1. INTRODUCTION

India has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in healthcare (Mukherje et al., 2006). According to a survey, 75-80% of the world's population relies over such plants as they are

famous for healing several diseases and are considered as a healthy source for life (Verpoorte, 2000; Alfawaz, 2006). But in recent years, the number of patients experiencing negative health consequences caused by the use of herbal medicine and its mainly due to poor quality of herbal drug and raw medicinal plant materials (Richardo, 2006). Authentication and standardization are requirement steps especially for herbal drugs and their formulations in conventional systems of medicine (Nagani *et al*., 2011).

In the present investigation some of the pharmacological properties such as moisture content, ash content, crude fibre content and solubility value of Rosemary plant were analyzed. Ash values are used to determine quality and purity of crude drug. It indicates the presence of various impurities, carbonate, oxalate and silicate. Ash is the inorganic residue remaining after water and organic matter have been removed by heating, which provides a measure of total amount of minerals within the drug (Apraj *et al*., 2011). Total ash may vary within wide limits for specimen of genuine drugs due to variable natural or physiological ash. Water soluble ash is used to estimate the amount of inorganic compound present in drug. The acid insoluble ash consist mainly silica which indicates contamination of sample. Moisture content of drug should be minimum level to describe the growth of bacteria and fungi during storage. Estimation of extractive value determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. Crude fibre content determined by standard procedure indicates that the plant is rich in fibre (Preetham *et al*., 2015). Nutritional composition data are an essential resource for food researchers and epidemiologists who investigate the relationship between food and disease in populations and required an accurate estimation of nutrient intake, and are also the basis for the development of dietary recommendations (Costa *et al*., 2010).

Rosmarinus officinalis L. is one of the important medicinal herbs belongs to Lamiaceae family. Traditionally, rosemary plant has been used medicinally to improve memory, relieve muscle pain and spasm, stimulate hair growth, and support the circulatory and nervous systems. It has been used to treat tumor, diabetic, inflammatory, ulcer and alzhimer diseases because it contains

number of antioxidants including carnosic acid and rosmarinic acid (Bruni *et al*., 2004; Frutos and Hernandez-Herrero, 2005).

According to the literature, bioactive compounds concentration being influenced by genetic factors, maturity stage, environmental and cultural practices, and postharvest conditions (Odriozola-Serrano, Soliva-Fortuny & Martín-Belloso, 2008). The chemical differences of *Radix Scrophulariae* among various production regions were demonstrated to different extents. The plants grown in Zhejiang Province has better medicinal effect and is recognized as geo-authentic (Wang and Wang, 2007). Several methods based on high performance liquid chromatography (HPLC) or combined with liquid chromatography-electrospray ionisation-mass spectrometry (LC-ESI-MS) were developed to quality and quantify the bioactive compounds in *S. ningpoensis* (Liu *et al*., 2007; Zhu *et al*., 2008). In the present investigation, samples of Rosemary plant were collected from five different localities with various altitude height of Kodaikanal region to understand influence of geographical variations on physicochemical properties.

2. Materials and Methods

2.1 Plant Collection

The leaves samples of Rosemary plant were collected from five different localities of Kodaikanal region with various altitude heights namely, Shenpaganur (2100m above the sea level), Kodaikanal town (2200m above the sea level), Observatory (2343m above the sea level), Attuvampatti (2000m above the sea level) and Adesarai (1900m above the sea level). The fresh leaf samples were dried in shade and then ground into fine powder. The powdered samples were used for further analysis.

2.2 Pharmacognostic Analysis

The powder of all samples of Rosemary plant leaves were subjected to evaluate its total ash, water soluble ash, acid insoluble ash, water soluble extractive value, methanol soluble extractive value, moisture content and crude fibre. Each determination was carried out three times and the average values were taken.

2.2.1. Moisture content

Two gm of the extract was weighed and placed in a crucible of constant weight. This was placed in an oven at 105° C then dried; the weight was measured carefully to get a constant weight. The loss in weight indicates the moisture content.

2.2.2 Total ash content

Two grams of air dried leaf powder of *R. officinalis* was taken in a silica crucible and incinerated at a temperature not exceeding 450°C until the sample turn into white color. After cooled down in desiccators for 30 min, total ash content was recorded.

2.2.3 Total Acid-insoluble ash content

The total ash obtained from 2g of leaf powder was gently boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on a Whatman filter paper. It was washed with hot water until the filtrate become neutral and then ignited at 450°C. Then the residue was allowed to cool in desiccators for 30 min. The percentage of acid insoluble ash was calculated.

2.2.4 Total Water soluble ash content

The total ash obtained from 2g of leaf powder was boiled for 5 minutes with 25 ml of water and the insoluble matter was collected on a Whatman filter paper and then washed with hot water and ignited for 15 min at 450 °C. Then the residue was allowed to cool in a desiccators for 30 min. The percentage of water soluble ash was calculated.

2.2.5 Crude fibre

This is organic residue which remains after the materials have been treated with standardized conditions with light petroleum, boiled diluted H_2SO_4, boiled diluted hydrochloric acid, alcohol and ether. The crude fibre consists largely of cellulose together with little lignin and it can extrapolated as: 100 – (Moisture % + ash % + lipid % + protein%).

2.3. Extractive value

Solubility values of crude drugs are useful for their evaluation especially when the constituents of a drug cannot be ready estimated by any other means. Further, these values indicate the nature

of the constituents present in a crude drug. The raw materials were dried and powdered and the powdered materials were used for analyzing different parameters.

2.3.1. Water soluble extractive (WSE)

Four gram of the air dried and coarsely powdered tissue was macerated with 100ml of (5%) chloroform water in a glass stopper conical flask for 24hrs, the contents were shaken frequently during the first 6 hrs. Thereafter the contents were filtered rapidly by decanting the water extract. 25ml of the filtrate was evaporated to dryness on a water bath in tarred flat bottomed petriplate/shallow dish. 2ml of alcohol was added to the dry residue and the contents were shaken and dried again on water bath. It was then dried at 105°C for 1hrs in the hot air oven and cooled in a desiccator for 30 mins and weighed. The process was repeated till the concordant weight is obtained. The % of WSE was calculated using the formula mentioned for calculation of alcohol soluble extractive value (Kokate, 2008).

2.3.2. Methanol soluble extractive

Four grams of coarsely powdered samples of leaf was separately refluxed with 100 ml of methanol for 1 hour. Then the mixture was filtered and total weight was re-adjusted by adding methanol. Then 25 ml of the filtrate was concentrated in a rotavapour (Buchi Rotavapour, Type-R-114A29 B-480, Switzerland) at 45 ◦C. The residue was dried at 105 ◦C for 6 h and allowed to cool for 30 min. The weight was recorded.

Statistical analysis

All determinations were based on triplicate measurements and the results were expressed as means ± standard error. The data were evaluated by one-way ANOVA and the significance of the difference between means was determined by Duncan's multiple range test. Differences at $P < 0.05$ were considered statistically significant. The SPSS 20.0 (Chicago, Illinois, USA) was used to perform statistical analysis.

3. Results and discussion

Geographic locations of the plants may enhance the types and levels of their phytocompounds production. The present study deals with the comparative analysis of proximate nutrients of *Rosmarinus officinalis* collected from five different locations of Kodaikanal hills, dindigul district. The proximate compositions of rosemary leaves collected from various geographic locations are given in table 1. Studies of physicochemical constants can serve as a valuable source of information and are usually used in judging the purity and quality of the drug (Gami and Parabia, 2010; Kunle *et al*., 2012).

Table 1. Physicochemical parameters of powdered leaves of *Rosmarinus officinalis* L.

S.No	Experimental Studies	RO1 % (w/w)	RO2 % (w/w	RO3 % (w/w)	RO4 % (w/w)	RO5 % (w/w)
1.	Total ash	11.85 ± 0.03^{b}	10.18 ± 0.13^{c}	9.28 ± 0.03^{d}	12.6 ± 0.08^{b}	13.51 ± 0.08^{a}
2.	Water soluble ash	3.06 ± 0.15^{c}	2.78 ± 0.16^{d}	2.57 ± 0.11^{d}	4.28 ± 0.17^{b}	5.03 ± 0.09^{a}
3.	Acid insoluble ash	2.68 ± 0.23^{b}	2.11 ± 0.12^{b}	1.87 ± 0.49^{c}	3.26 ± 0.04^{a}	3.97 ± 0.32^{a}
4.	Water soluble extractive value	11.42 ± 0.05^{b}	9.67 ± 0.13^{c}	14.51 ± 0.28^{a}	7.35 ± 0.26^{d}	6.25 ± 0.16^{e}
5.	Methanol soluble extractive value	13.41 ± 0.28^{c}	15.56 ± 0.27^{b}	19.40 ± 0.25^{a}	11.06 ± 0.29^{d}	9.63 ± 0.28^{e}
6.	Moisture content	6.32 ± 0.22^{c}	5.40 ± 0.27^{d}	4.47 ± 0.26^{e}	7.80 ± 0.23^{b}	8.93 ± 0.20^{a}
7.	Crude fibre	11.58 ± 0.19^{c}	13.38 ± 0.18^{b}	15.47 ± 0.27^{a}	9.30 ± 0.25^{d}	7.29 ± 0.12^{e}

Mean values ± standard error with the same letters within the same column are not significantly different a P> 0.05.

The highest moisture value was recorded for the sample RO5 (8.93% dry weight) and lowest value was recorded for the sample RO3 (4.47 % dry weight). Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. The ash values ranged between 9.28 % (RO3 sample) to 13.51 % (RO5 sample). Ash content is used to determine the mineral content of the original food (Onwuka, 2005). The concentrations of water insoluble ash between 6.25% (RO3 sample) to 14.51% dry weight (RO5 sample).The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The content of total acid insoluble ash in the range between 1.87 % (RO3 sample) and 2.11 % dry weight.

The acid insoluble ash consist mostly silica and indicate contamination with earthy material. The highest value of crude protein was found in RO3 sample (15.47 %) followed by RO2 (13.38 %) and RO1(11.58 %) and the lowest value was found in RO4 (9.3 %) and RO5 (7.29 %).Crude fibre is made up mainly of cellulose together with a little lignin and it aids absorption of glucose and fat, enhances digestibility. The water soluble extractive value of RO1, RO2, RO3, RO4 and RO5 samples were 11.42 %, 9.67 %, 14.51 %, 7.35 % and 6.25 %, respectively. The ethanol soluble extractive value of RO1, RO2, RO3, RO4 and RO5 samples were 13.41 %, 15.56 %, 19.40 %, 11.06 % and 9.63 %. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also in estimation of specific constituents soluble in a particular solvent (Thomas *et al*., 2008).

4. Conclusion

In the current study, the results showed significant variations in the concentration levels of proximate nutrients of *R. officinalis* collected from five different locations of Kodaikanal hills. This study could be used as a diagnostic tool for the standardization of this medicinal plant and will helpful in characterization of the crude drug. The pharmacognostic assessment of five different samples of this plant may helpful towards founding for quality, purity and sample identification and standardization.

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