ABSTRACTS
Rauvolfia serpentina is also known as sarpgandha or snakeroot is one of the very important Ayurvedic herb. It is used in treating high BP lack of sheep (insomnia) etc. It, is belong to the Apocynaceae family. Here in the present study, Phyto Analysis and Comparative Estimation of TFC Rauvolfia Serpentina Quality Parameters in Marketed and Forest Samples. The herbal drugs and medicinal plant products have been widely used for thousands of years in many parts of the world. Medicinal plants constitute a source of raw material for both traditional and modern systems of medicine. The increase in demand of medicinal plants for the commercial herbal medicine sector led to the indiscriminate and unscientific collection without any consideration for the quality of the material collected. The ocular observations of the market sample of R. Serpentina indicate that mixing of old and diseased parts of same species and other adulterants is rampant in the local market. Laboratory analysis shows that in one kilogram of market sample, more than 20% raw material was found adulterated in all the selected species. Checking and testing the quality of proposed raw herbals traded in the markets and compared to the freshly collected raw material from forest.

KEYWORDS: R. Serpentina, Phyto Analysis, TFC, Marketed samples and Forest Samples.

INTRODUCTION
Indian Systems of Medicine (ISM) predominantly use plant material for the preparation of medicines. But Medicinal plant industry faces the problem of raw material supply and its quality. Adulteration and substitution of non-genuine plants is reported to be rampant in near
absence of assured supply of genuine medicinal plants as raw material. There is poor law enforcement system at the field level. In present study to evaluate the quality of medicinal plants namely *R. Serpentina* are evaluated comparatively for being used as a quality medicinal plants material resourced from local market and natural forest region on the basis of physical and phytochemical parameters. The safety and quality of raw medicinal plant materials and finished products depend on intrinsic (genetic) or external (environment, collection methods, cultivation, harvest, post-harvest care, transport and storage practices) factors. The WHO (2003) guidelines on good agricultural and collection practices (GACP) for medicinal plants are important initiative to ensure good quality of herbal medicines and environmentally sound cultivation practices for sustainable production and utilization of medicinal plants.

**MATERIAL AND METHOD**

**Sample Collection:** The collection of plant material which in general used for medicinal purposes in various forms was done for present study in two ways i.e. collection from forests and collection from traders. The forest regions of Raisen districts of Madhya Pradesh were chosen for the collection of medicinal plants directly from forest for the purpose of study. The most common plant species that are extensively used medicinal plants in India as well as other parts of the world are considered in the present research work with due respect to their high commercial demand as per the objective of present study. The selected plant species is *R. Serpentina* (Serpgandha).

![Map of Raisen District](image)

**RESULT AND DISCUSSION**

1. **Morphological and Physical Quality Evaluation:** The quality of raw material and herbal crude drug were assessed on of morphological and physical basis because each and
every plant depicts unique characteristics that could be helpful in identifying the purity of raw plant drug or presence of any substituted materials or adulterants.

1.1 Morphological Evaluation: - This involves the direct ocular analysis of raw material or samples with particular amount of dried material is analyzed using magnifying lens. The identification was done by the help of some taxonomist, consulting flora, Ayurvedic practitioners and experts (Mishra, et al., 2009).


2. Chemical Evaluation
To assess the quality of medicinal plants on a comparative basis among the samples collected from the market and forest or natural habitat, the medicinal plants were subjected to their phytochemical extraction with methanol and their methanolic extracts were investigated mainly on 3 parameters in terms of chemical evaluation.

The three parameters of chemical evaluations were percentage yield of extraction, screening of phytochemical groups and chromatographic analysis of extracted phytochemicals. Since, in present work the medicinal plant samples were collected from the Raisen District of Madhya Pradesh, thus for ease of understanding and content simplification, the results of the study for chemical evaluation are described average and most repressive facts of investigation in this part. The results of the experiments on chemical evaluation of medicinal plant considered under present work are discussed ahead:

2.1 Extraction of Phytochemicals
Methanolic extract by maceration process was prepared for investigation and quality evaluation since the alcoholic extraction generally extract out maximum number of phytoconstituents present in samples.

2.2 Determination of Percentage Yield
To assess the quality of medicinal plants on a comparative basis among the samples collected from the market and forest or natural habitat, determination of the percentage yield of extraction is one of the important parameter in present investigation.
Yield of Extraction: The crude extracts so obtained after extraction by maceration, extract were further concentrated on water bath to allow the complete evaporation of solvents so that we could obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from samples using methanolic as solvent is depicted in the table 1

Table 1: Percentage yield of methanolic extract of plant materials used in present study.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Plant</th>
<th>Marketed Percentage yield (W/W)</th>
<th>Control Percentage yield (W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.</td>
<td><em>Rauvolfia serpentine</em></td>
<td>14.6%</td>
<td>18.98%</td>
</tr>
</tbody>
</table>

From the results as depicted in table 1, it is very clear that the percentage yield of extraction are higher the sample which were directly collected from forest regions or from natural habitat. This indicates that may be adulteration or quality deterioration or the plant materials collected from market as well as from forest are of same year.

This parameter which is assessment of percentage yield of extraction of medicinal plant material may indicate the quality of medicinal plant but it is not possible to justify any kind of adulteration in the samples when compared to the fresh medicinal plant material of similar species that is investigated.

2.3 Phytochemical screening of extract

Phytochemical profiling of the methanolic extracts of plant materials considered in present study were subjected to phytochemical test for the presence or absence of alkaloids, glycosides, tannins, saponins, flavonoids and steroids. The phytochemical analysis of plant collected from market venders of Raisen district of Madhya Pradesh.

Since the extracts were prepared by the process of maceration, thus a partial phytochemical profile was generated in context to the present study as the aim of present work was to evaluate the quality of medicinal plant material collected from nature and from market out of the Raisen district of Madhya Pradesh; the sample volume of which was also huge, thus the extraction procedures like infusion, decoction, percolation or soxhlation were avoided in present work that might otherwise complicate the evaluation after experimental works. Thus maceration was considered which could extract out the chemical constituents present in the
samples necessarily present in specific plant material when extracted with the help of specific solvent system.

From the results of phytochemical profiling obtained by analyzing the representative sample clearly shows the differences in the samples when compared to the samples collected from the market and that of the natural habitat as control. In plant samples of *R. serpentina* the phytochemical those were reported positive in control samples but when the sample phytochemical tests were performed with marketed samples, some of the phytoconstituents were absent in them (refer table 2). For example, the control samples of *R. serpentina* contained alkaloid, flavonoid, diterpenes, amino acids, proteins, carbohydrates and saponins, but their representative marketed samples were positive for flavonoids and saponins only when analyzed for their phytochemicals.

**Table 2: phytochemical extraction of *E. officinales* (crud plants drug and Market samples).**

<table>
<thead>
<tr>
<th>Constituents</th>
<th><em>R. serpentina (crud plants drug)</em></th>
<th><em>R. serpentina (Marketed plants drug)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

[(+] means present and (-) means absent]

Such results could be inferred as the use of substandard crud drug quality but also the addition of unknown herbal components with similar morphological features that could not be detected by naked eyes but, when tested for their phytochemical profiles, they found to be different with phytochemical profiles of original, pure and fresh crud drug.

**Total flavonoids content estimation (TFC)**

Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve:

\[
Y = 0.040X + 0.009, \quad R^2 = 0.999.
\]

Where: \( X \) = absorbance and \( Y \) = quercetin equivalent (QE).
Table 4.22: Preparation of calibration curve of Quercetin AlCl₃ precipitation method.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Gallic acid Conc. (in µg)</th>
<th>Absorbance at 420 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>0.216</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.425</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.625</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0.815</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>1.021</td>
</tr>
</tbody>
</table>

Figure 1: Standard Curve of Quercetin for Estimation of Total Flavonoid Content at 420 nm by Aluminum Chloride Precipitation.

Table 3: Estimation of total flavonoidal content in control and marketed samples.

<table>
<thead>
<tr>
<th>S.N</th>
<th>Plant Samples</th>
<th>Total flavonoids Equivalent to Quercetin mg/100 mg of dried extract</th>
<th>Total flavonoids Equivalent to Quercetin mg/100 mg of dried extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Marketed</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td><em>R. serpentina</em></td>
<td>0.241</td>
<td>0.601</td>
</tr>
</tbody>
</table>

Again from the results as depicted in table 3 regarding the presence of total flavonoidal content in methanolic extracts of herbal medicinal plant samples investigated for quality assessment, it is noticed that the flavonoidal content in marketed samples are less compared to control plant samples freshly collected from forest. Among control samples *R. serpentina* TFC values as 0.7291mg/100 mg dry extract while its marketed samples were comparatively lesser in TFC ranging as 0.601mg/100 mg dry extract.
Selected parts of *R. Serpentina* (*roots*) collected from natural forest and local market of Raisen. Due to morphological and physical evaluation it is clear that, maximum proportion of good quality root was found in sample collected from natural forest on the contrary very less proportion of good quality root was found in sample collected from market. Adulteration is more in the samples collected from market whereas nil the samples collected from forest. The proportion of diseased root was more in market sample in contrast to nil in forest samples. From the result the TFC value is higher in control samples (natural forest samples) than the market samples.

**CONCLUSIONS**

There is a long history of the traditional Indian system of medicine which describes the importance of plants as a potential source of useful structures for the development of new drugs and chemotherapeutic agents (Tona et al., 1998). Substitution or intentional adulteration is not a common practice in the local market. The adulteration was found more intense only during less production of fruits form natural forests and high demand by Ayurvedic industries. More demand and less supply forces stakeholders to adopt malpractices like mixing of similar looking fruits, chaff matter etc. in genuine material (Mishra et al, 2010). Here in the present study Phyto Analysis and Comparative Estimation of TFC in *R. Serpentina* Quality Parameters in Marketed and Forest Samples, the forest samples are rich in total Flavonoid content as compare to market samples. Consequently from this study it can be concluded that the natural forest root xtracts of *R. Serpentina* are rich in TFC the potential of which could be utilized in many ways after further advance studies and proper data generation for the development of new chemotherapeutic agents.

**ACKNOWLEDGEMENTS**

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