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# Variations in the Antioxidant Profile of *Aegle marmoles* with Drying and Solvent of Extraction

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ABSTRACT

There are various processes in the crude drug processing which affects the quality of the drug in many ways. The quality of the crude drug is the determining factor that is responsible for the imports and exports of the drug to international markets. Drying is a significant step in those post-harvesting stages of the crude drug. This alone determines the microorganism contamination in the drug. Other post-harvesting methods that affect the final extract of the crude drug is the selection f solvent for extraction. The solvent selection is to be based on the parameters like the partition coefficient, the solubility of the chemical constituents. *Aegle marmoles* is well known for its antioxidant activity and other potential activities, and the exports of the drug were in more massive scale. To ensure the quality of the drug, the post-harvest technology of the drug was essential to determine the quality of the crude drugs. The leaves were dried in different methods and extracted with different solvents, and the investigations were done for the antioxidant activity. The results showed that the extract of the shade dried drug and the methanol solvent extracted drug showed a better phenol content and also better antioxidant activity.

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## INTRODUCTION

There are various processes in the crude drug processing which affects the quality of the drug in many ways. The quality of the crude drug is the determining factor that is responsible for the imports and exports of the drug to international markets. Drying is a significant step in those post-harvesting stages of the crude drug. This alone determines the microorganism contamination in the drug. The various stages of drying prevent the drug from deterioration or the microbial contamination or most importantly, the loss of chemical constituents. There are various types of drying which are advantageous in many ways on their style and cases [1]. If the drying was not done properly, there might be a chance of attack by fungus on the drug. If the drying was overly done, there is a lot of chances of chemical constituent deterioration in the drug, which finally affects the low quality of the drug. This is the area where it calls for the scientific research for determining the post-harvesting technologies that make the crude drug rich with chemical constituents and ensure the drug is of exportable quality [2].

Other post-harvesting methods that affect the final extract of the crude drug is the selection f solvent for extraction. The solvent selection is to be based on the parameters like the partition coefficient, the solubility of the chemical constituents [3]. There are various solvents for extraction. They are ranging from high polar water to the highly non-polar benzene or N Hexane. Keeping in mind the simple concept the polar chemical constituents dissolves in the

polar solvents, the optimum choice is to be made to select the solvents out of all the available solvents.

*Aegle marmoles* is one of such drugs that is known for its potency against many diseases like hepatitis, inflammation and also it is used to treat wounds [4, 5]. The chemical constituents that are present in the plant are responsible for those activities are flavonoids, alkaloids etc. [6–8]. So in the current work, the effect of extraction and drying on the variation of the chemical constituents is to be determined.

## POST-HARVEST VARIABLES

### Drying method

The leaves of the plant were procured from the tree that was growing in the local area of Kapilatheetham Region of Tirupathi. The leaves were collected from the plant in December. The plant leaves were then torn into small pieces and divided into three batches wherein each batch goes into different drying methods like Shade, Sun and Oven.

Drying in the sun: the leaves were spread on a large table that is placed outside in the direct sunlight for about two days. The sunlight was about 35-40$^{O}$c during the day, and care was taken, the drug was brought into the shade while to avoid the exposure to the mist and snow in the night. After the leaves are dried for two days, the drug was powdered, and the fine powder was stored in an airtight container.

Drying in the oven: the leaves were first appropriately dried to remove any moisture on the parts or any droplet of water for about 30mins in the shade. Then they were spread on a drying tray and then placed inside an oven, and the temperature was adjusted for about 40$^{O}$c. The drug was dried in these conditions for about five days, and the contents are checked occasionally. They are then taken out powdered, and then the fine powder is stored in an airtight container for future use in the investigations.

Drying in the shade: the drug leaves were spread evenly on the table and dried under shade in an adequately ventilated room where there are indirect sunlight and easy spread of air into the room. The temperature inside the room was about 36$^{0}$c, which was constant according to the outside temperature. The drying was continued to 7days and then taken out and pulverized. This fine powder was stored in the airtight container for further use.

### Extraction Solvent

The powders that were stored were weighed to about 5g each, and then they are divided into two batches which then are extracted using the non-polar solvent pet ether and the polar solvent Methanol. The solvents that used for the extraction are analytical grade, and the soxhlet is used for the extraction procedure [9]. The plant powders were3 packed it the cylinder of the apparatus. The process is started by assembling the soxhlet setup. This was continued till the siphon ran clear solvent and the extract was collected off. This was filtered to remove any traces of the solid material, and the resultant filtrate was dried, and the dried extract was collected and named accordingly.

The total phenols content and Flavonoids content was estimated using the folins ciocalteau method, as described in the procedure [10, 11].

### Evaluation of the activity

A weighed amount of extract was dissolved in ethanol, and the specific concentrations of the solution at 20$\mu$g/ml, 40 $\mu$g/ml, 60 $\mu$g/ml, 80 $\mu$g/ml and 100 $\mu$g/ml were prepared. Hydrogen peroxide was mixed with the solutions at a concentration of 20%, and two drops of the indicator were added. This solution was allowed to rest for 5mins, and then the absorbance was measured at 230nm, and the values were recorded and noted [12]. The standard drug ascorbic acid was used as the reference standard, and the percentage of free radicals scavenging activity was calculated using the formula.

Percentage of scavenging = (Abs of the control solution - Abs of sample solution / Abs of control solution) X 100

## RESULT& DISCUSSION

The leaves of the plant *Aegle marmoles* were collected and dried using three methods as described in the procedure. The loss on drying was measured was around 25% w/w. the leaves are dried, and the moisture content in the leaves was estimated. The moisture content in the shade dried leaves was measured as 7.6%w/w, and the moisture content was measured was 5.4%w/w for the sun-dried leaves, 6.3%w/w for the leaves dried in the hot air oven. Overall, it can be said that the drying was conventional and similar in all the process as the moisture content was estimated to be similar in all the drying process. The crude drug weight and the textures were similar, and the powder density was constant.

The extraction of the crude drug yielded a crude extract that weighed, and the percentage yields were calculated. The results were tabulated in Table 1 . the solvent methanol yielded more extractive value than the pet ether. In all the drying methods the Pet ether showed a less value of the percentage yield.

**Table 1: Yield, Phenol and flavanoids in the leaf extract**

| Sl.no | Extract | % yield | TPC | TFC |
|---|---|---|---|---|
| 1 | Shade dried-Methanolic | 21.03 | 195.14±4.08 | 93.02±3.09 |
| 2 | Shade dried-Etherial | 15.17 | 103.18±3.64 | 46.71±4.53 |
| 3 | Sun dried-Methanolic | 19.5 | 147.43±5.36 | 74.24±0.98 |
| 4 | Sun-dried- Etherial | 14.36 | 66.02±3.21 | 39.03±3.02 |
| 5 | Oven-dried- Methanolic | 22.81 | 170.35±6.2 | 87.46±1.24 |
| 6 | Oven-dried- Etherial | 17.49 | 98.71±3.47 | 53.11±2.35 |

**Table 2: % Effect of drying and solvent on theantioxidant activity**

| Sl.no | Extract | Concentration ($\mu$g/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 20 | 40 | 60 | 80 | 100 |
| 1 | Shade dried-Methanolic | 38.04±0.46 | 50.01±0.72 | 73.15±0.51 | 88.42±0.63 | 97.03±0.84 |
| 2 | Shade dried-Etherial | 27.12±0.63 | 38.23±0.35 | 57.40±0.84 | 71.31±2.07 | 76.54±0.72 |
| 3 | Sun-dried-Methanolic | 36.19±0.52 | 49.52±0.93 | 68.6±0.65 | 83.24±0.84 | 91.08±0.69 |
| 4 | Sun-dried-Etherial | 21.34±1.04 | 28.41±0.47 | 36.72±0.95 | 49.02±0.36 | 60.13±1.23 |
| 5 | Oven-dried-Methanolic | 37.61±0.75 | 50.08±1.06 | 68.83±0.37 | 84.52±0.49 | 92.38±0.85 |
| 6 | Oven-dried-Etherial | 23.27±0.91 | 32.39±0.54 | 50.34±0.76 | 61.8±0.93 | 69.56±0.64 |
| 7 | Ascorbic Acid | 39.05±0.87 | 51.17±1.03 | 72.61±1.01 | 85.04±0.72 | 94.11±0.50 |

The total phenols and flavonoids were calculated using the formulae and standard method. The values were tabulated along with the percentage yields. The phenols and flavonoids were similar in the values. In Methanol solvent the extract showed a higher content of both the chemical constituents and the difference was about 30% with the solvents, and the drying method had a very less significance in the content of chemical constituents. The antioxidant activity of the extracts was also estimated, and the results showed that there was a significant relationship between the chemical constituent content and the activity. Similar activity was reported in line with the phenols. The methanol extract showed better activity in the crude drug that is dried in the shade. The sun-dried and oven-dried extracts showed a little less activity compared to the shade dried leaves. The results were tabulated in Table 2

## CONCLUSION

Aegle marmoles is well known for its antioxidant activity and other potential activities, and the exports of the drug were on a larger scale. To ensure the quality of the drug, the post-harvest technology of the drug was essential to determine the quality of the crude drugs. The leaves were dried in different methods and extracted with different solvents, and the investigations were done for the antioxidant activity. The results showed that the extract of the shade dried drug and the methanol solvent extracted drug showed a better phenol content and also better antioxidant activity.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest for this study.

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