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# HPLC analysis and activity to determine the effect of drying and extraction on *Ficus benghalensis* leaves

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ABSTRACT

*Ficus benghalensis* is one of those taboo plants in India, which was claimed to be possessed and have weird effects on human health. Apart from this ficus species has a great variety of chemical constituents and an abundant amount of antioxidants. Drying is the most critical stage of improving the activity or preventing the loss of chemical components from a drug. There is another stage of ensuring high chemical constituent content in the plant and that is the extraction procedure. So the point of focus in the current research is to find the effect of extraction method and drying on the anti-inflammatory potential of the plant. The result of the extraction method and drying method of the plant was investigated and found that the ultrasound-assisted extraction of the shade dried leaves was found to give the highest yield of flavonoids and activity.

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## INTRODUCTION

*Ficus benghalensis* is one of those taboo plants in India, which was claimed to be possessed and have weird effects on human health. Apart from this ficus species has a great variety of chemical constituents and an abundant amount of antioxidants. This plant leaves are traditionally used for diabetes, hepatic problems, and joint pains too. Since the plant is rich in flavonoids and polyphenols, the activity also revolves around these chemical constituents only [1].

Drying is the most critical stage of improving the activity or preventing the loss of chemical constituents from a drug. Drying is done in longer durations like shade drying, which is a slow and steady process wherein the moisture loss is determined by humidity and the partial pressure in the air. Direct sun-drying processes in which the drying is rapid and depends on the variations on the heat of the sun and another method of drying is the usage of machine for drying. Hot air oven is used to dry the plant parts. The mechanism by which drying happens is even but with high temperature. All the methods have the pros and cons in their manner. Process of drying will affect chemical constituents too, which will have an impact on the activity also [2].

There is another stage of ensuring high chemical constituent content in the plant and that is the extraction procedure. There is a lot of process for extraction and the commonest is Soxhlet apparatus. These days ultrasound method is also gained very much importance with an advantage of it being inclusive and efficient in extracting from hard drugs too. So the point of focus in the current research is to find the effect of extraction method and drying on the anti-inflammatory potential of the plant.

## EXPERIMENTAL DESIGN

### Herbs

The fresh leaves of ficus were collected from a native tree near the college and were shade dried for six days. After ensuring they are appropriately dried, they are finely powdered and stored. Another batch of leaves was dried in a hot air oven for two days and they are ground into a fine powder and then stored. The powder was extracted using two methods, Soxhlet and ultrasound-assisted extraction. The parameters used for ultrasound are 40KHz at 70% power and the temperature was maintained at $40^0$C and the system was allowed to run for about 35mins [3, 4]. The solvent used for extraction was methanol and the extracts were filtered and dried and named as USS-Ultrasound shade dried, USH-Ultrasound Oven-dried, SS-Soxhlet shade dried and SH-Soxhlet oven-dried, where their percentage yields were calculated as 18.63, 15.91, 18.55 and 12.74 respectively.

### HPLC Analysis

The extracts were subjected to HPLC analysis to determine the effect of the drying and extraction method on the chemical constituents. As we know, the flavonoids are the essential constituents in ficus, and the analysis was concentrated on the estimation of flavonoid using HPLC and comparing with standard quercetin(Table 1).

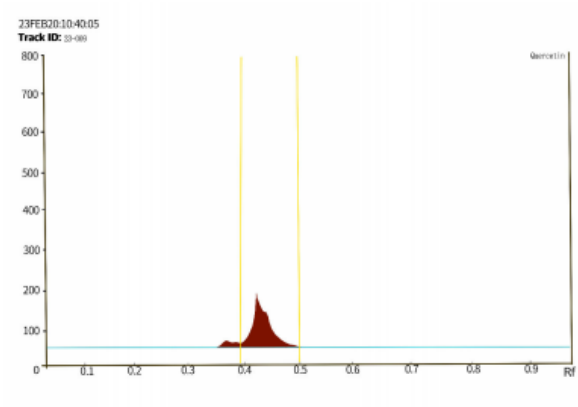
HPLC parameters are enlisted as the model of HPLC is alignment HP-1100 series for G1310A model with isocratic quarternary pump set up. The detector used was PDAD and FLD detectors. The column used was Zorbax eclipse XDB-C8 column with dimensions of 150x4.6mm with a constant flow rate of 1.5ml/min. The solvent system used was acetonitrile and water in a ratio of 70:30. Ambient temperature was maintained and the injection volume of the extract was 2micro litre. The analysis was made and measured under FLD at 254nm (Figures 1 and 2).

## COMPARISION OF ACTIVITY

The anti-inflammatory activity was compared between the extracts invitro in three methods as follows

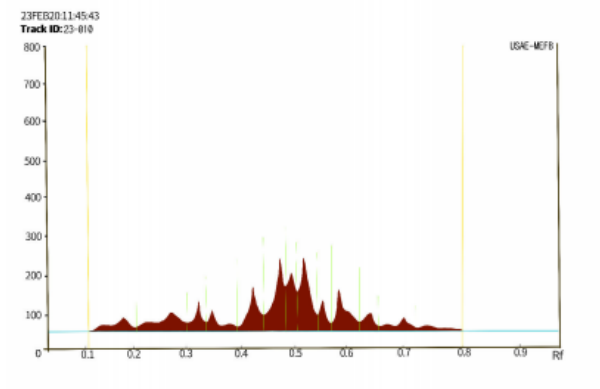
### Proteinase inhibitory action

This method was used as per procedure given by Paina et al. the solution was prepared using 0.06mg of trypsin and 1ml of tris HCl buffer solution and the extracts at different concentrations. The 0.8% of casein was added and the mixture was incubated in the oven for 15min and additionally added perchloric acid to seize the reaction. The absorbance was measured in UV at 210 nm [5](Figure 3).




| Peak | Start point | Maximum Rf | End point | Peak height (AU) | Area (AU) | Percentage area (%) |
|---|---|---|---|---|---|---|
| 1 | 0.35 | 0.36 | 0.38 | 75.8 | 227.4 | 9.79 |
| 2 | 0.39 | 0.43 | 0.5 | 190.6 | 2096.6 | 90.21 |

**Figure 1: HPLC report of Quercetin**




| Peak | Start point | Maximum Rf | End point | Peak height (AU) | Area (AU) | Percentage area (%) |
|---|---|---|---|---|---|---|
| 1 | 0.15 | 0.18 | 0.21 | 91.5 | 549 | 7.81 |
| 2 | 0.26 | 0.27 | 0.3 | 109.3 | 437.2 | 6.22 |
| 3 | 0.3 | 0.32 | 0.33 | 130.7 | 392.1 | 5.58 |
| 4 | 0.33 | 0.35 | 0.36 | 112.4 | 337.2 | 4.8 |
| 5 | 0.39 | 0.42 | 0.44 | 184.2 | 921 | 13.11 |
| 6 | 0.44 | 0.47 | 0.48 | 243.5 | 974 | 13.86 |
| 7 | 0.48 | 0.49 | 0.5 | 207.1 | 414.2 | 5.89 |
| 8 | 0.5 | 0.51 | 0.54 | 249.6 | 998.4 | 14.21 |
| 9 | 0.54 | 0.55 | 0.57 | 129.8 | 389.4 | 5.54 |
| 10 | 0.57 | 0.59 | 0.62 | 181.2 | 906 | 12.89 |
| 11 | 0.62 | 0.64 | 0.66 | 102.4 | 409.6 | 5.83 |
| 12 | 0.69 | 0.7 | 0.72 | 98.5 | 295.5 | 4.2 |

**Figure 2: HPLC report of ultrasound-assisted extraction of shade driedleaves**

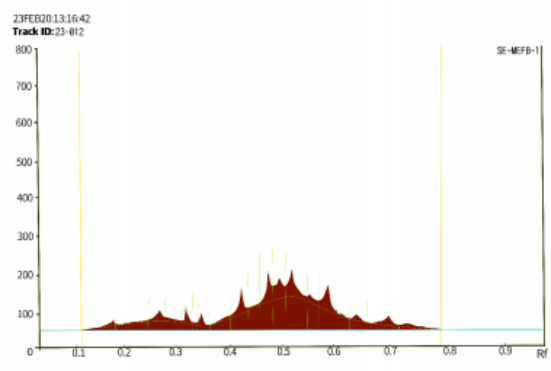
### Prevention of Denaturation of Proteins

The effect of extraction and drying on the anti-inflammatory activity by analysing the inhibition potential of the denaturation of proteins. By heat application in an acid medium using a concentrated acid. This was analysed following the procedure by Mariana et al. the solution was prepared using 1% aqueous solution of extract and protein solution (Albumin) and the medium was made into acidic

**Table 1: Anti-inflammatory activity of Ficus extracts**

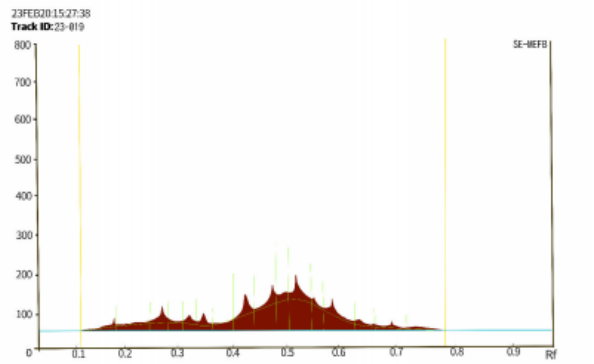
| Treatment | Protein Denaturation Assay | | Proteinase inhibition Assay | |
|---|---|---|---|---|
| | Absorbance at 660nm | % Activity | Absorbance at 210nm | % Activity |
| Control | 0.39±0.06 | - | 0.40±0.10 | - |
| DME 100$\mu$g/ml | 0.27±0.03** | 34.71 | 0.31±0.06** | 23.69 |
| DME 100$\mu$g/ml | 0.21±0.04** | 49.93 | 0.29±0.03** | 29.12 |
| DME 100$\mu$g/ml | 0.17±0.05** | 58.69 | 0.25±0.08** | 38.93 |
| DME 100$\mu$g/ml | 0.15±0.02** | 66.24 | 0.23±0.02** | 43.07 |
| DME 100$\mu$g/ml | 0.12±0.08** | 73.03 | 0.20±0.05** | 55.28 |
| Standard | 0.14±0.03** | 69.86 | 0.18±0.04** | 57.01 |

using HCl. The solutions were incubated at ambient temperature for 25min and then heated at $50^0$C for 20min. The mixture is cooled and UV absorbance was measured at 660nm (Figures 4 and 5) [4].




| Peak | Start point | Maximum Rf | End point | Peak height (AU) | Area (AU) | Percentage area (%) |
|---|---|---|---|---|---|---|
| 1 | 0.14 | 0.17 | 0.18 | 91.2 | 364.8 | 6.81 |
| 2 | 0.24 | 0.26 | 0.28 | 108.6 | 434.4 | 8.11 |
| 3 | 0.31 | 0.32 | 0.33 | 117.4 | 234.8 | 4.38 |
| 4 | 0.34 | 0.35 | 0.36 | 101.2 | 202.4 | 3.78 |
| 5 | 0.4 | 0.42 | 0.43 | 177.1 | 531.3 | 9.92 |
| 6 | 0.46 | 0.47 | 0.48 | 209.8 | 419.6 | 7.84 |
| 7 | 0.48 | 0.49 | 0.5 | 193.9 | 387.8 | 7.24 |
| 8 | 0.5 | 0.51 | 0.54 | 212.4 | 849.6 | 15.87 |
| 9 | 0.54 | 0.55 | 0.57 | 156.2 | 468.6 | 8.75 |
| 10 | 0.57 | 0.59 | 0.6 | 181.6 | 544.8 | 10.17 |
| 11 | 0.62 | 0.63 | 0.66 | 100.1 | 400.4 | 7.48 |
| 12 | 0.67 | 0.7 | 0.72 | 103.3 | 516.5 | 9.65 |

**Figure 3: HPLC report of soxhlet extraction of shade dried leaves**

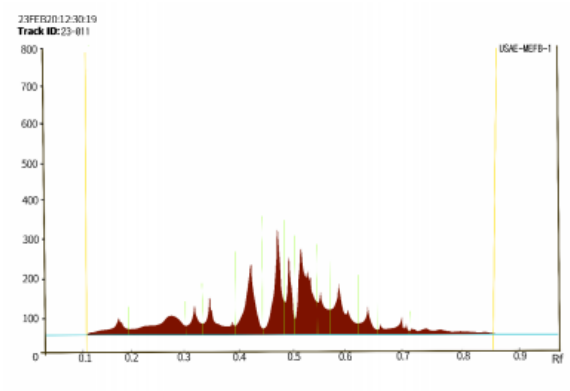



| Peak | Start point | Maximum Rf | End point | Peak height (AU) | Area (AU) | Percentage area (%) |
|---|---|---|---|---|---|---|
| 1 | 0.16 | 0.17 | 0.18 | 95.7 | 191.4 | 4.60 |
| 2 | 0.25 | 0.27 | 0.28 | 114.4 | 343.2 | 8.25 |
| 3 | 0.31 | 0.32 | 0.33 | 98.2 | 196.4 | 4.72 |
| 4 | 0.34 | 0.35 | 0.37 | 101.6 | 304.8 | 7.33 |
| 5 | 0.4 | 0.42 | 0.44 | 150.4 | 601.6 | 14.47 |
| 6 | 0.45 | 0.47 | 0.48 | 178.2 | 534.6 | 12.86 |
| 7 | 0.5 | 0.52 | 0.54 | 199.4 | 797.6 | 19.18 |
| 8 | 0.54 | 0.55 | 0.57 | 132.2 | 396.6 | 9.54 |
| 9 | 0.58 | 0.59 | 0.6 | 129.3 | 258.6 | 6.22 |
| 10 | 0.62 | 0.63 | 0.66 | 90.3 | 361.2 | 8.69 |
| 11 | 0.69 | 0.7 | 0.71 | 85.8 | 171.6 | 4.13 |

**Figure 4: HPLC report of soxhlet extraction of oven-dried leaves**

## DISCUSSION

The HPLC analysis of the extracts was performed and the results were given in figure 1-6. It is compared with quercetin. The amount of flavonoid was highest in the shade dried extract of ultrasound-assisted extraction. The activity was also dependent on the extract with the highest quercetin concentration.




| Peak | Start point | Maximum Rf | End point | Peak height (AU) | Area (AU) | Percentage area (%) |
|---|---|---|---|---|---|---|
| 1 | 0.17 | 0.18 | 0.2 | 100.5 | 301.5 | 3.902 |
| 2 | 0.26 | 0.28 | 0.3 | 109.8 | 439.2 | 5.68 |
| 3 | 0.3 | 0.2 | 0.33 | 124.1 | 372.3 | 4.81 |
| 4 | 0.33 | 0.35 | 0.36 | 153.7 | 461.1 | 5.96 |
| 5 | 0.38 | 0.39 | 0.39 | 92.3 | 92.3 | 1.19 |
| 6 | 0.39 | 0.42 | 0.44 | 238.4 | 1192 | 15.42 |
| 7 | 0.45 | 0.47 | 0.48 | 316.2 | 948.6 | 12.27 |
| 8 | 0.48 | 0.49 | 0.5 | 256.9 | 513.8 | 6.65 |
| 9 | 0.5 | 0.52 | 0.55 | 287.5 | 1437.5 | 18.6 |
| 10 | 0.55 | 0.56 | 0.57 | 179.2 | 359 | 4.64 |
| 11 | 0.57 | 0.59 | 0.6 | 191.6 | 574.8 | 7.44 |
| 12 | 0.6 | 0.61 | 0.62 | 118.7 | 237.4 | 3.07 |
| 13 | 0.62 | 0.64 | 0.65 | 135.3 | 405.9 | 5.25 |
| 14 | 0.65 | 0.66 | 0.67 | 88.2 | 176.4 | 2.28 |
| 15 | 0.69 | 0.7 | 0.71 | 106.9 | 213.8 | 2.76 |

**Figure 5: HPLC extraction of Ultrasound-assisted extraction ofoven-dried leaves**

In both the activities, the results showed that the extract with the highest concentration of flavonoids that is ultrasound-assisted extraction of shade dried leaves gave the highest activity and the results were tabulated below. The activity is totally dependent on the drying method and extraction method. So it can be concluded that the extract that is extracted with ultrasound-assisted and shade drying yielded in higher flavonoids. Least yield was achieved in oven-dried leaves extracted with Soxhlet extraction.

## CONCLUSION

The effect of extraction method and drying method of the plant was investigated and found that the ultrasound-assisted extraction of the shade dried leaves was found to give the highest yield of flavonoids and activity.

## CONFLICT OF INTEREST

Authors declared no conflict of interest.

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## ABOUT AUTHORS

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