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## Comparison of total flavanoid content of *Azadirachta indica* root bark extracts prepared by different methods of extraction

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### ABSTRACT

*Azadirachta indica* A. Juss. (AI) is a perinneeal plant of family Maleaceae. The total flavonoid content of AI was determined by using aluminium chloride colorimetric method. It was found that *Azadirachta indica* root bark extracts contain total flavonoids in a range of 0.198 - 0.512 % g quercetin equivalent. The decoction method gave the highest yield (20.2%, w/w) of crude extract, while maceration extract gave the highest total flavonoid content (0.512 % g). Qualitative chemical tests for flavonoids and thin layer chromatographic analysis were used to screen the extracts obtained from different methods. Extracts obtained from maceration, microwave and soxhlet showed spots of R<sub>f</sub> values 0.92, 0.93, 0.93 corresponding to quercetin flavonoid which is found to be the most potent flavonoid.

**Keywords:** *Azadirachta indica*, Quercetin, flavanoids, microwave.

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## INTRODUCTION

Medicinal plants, as source of remedies, are widely used as alternative therapeutic tools for the prevention or treatment of many diseases [1]. Herbal remedies are popular remedies for diseases used by a vast majority of the world's population [2]. Out of all the plants that have proved useful for humanity, a few are distinguished by their astonishing versatility. Among these, neem tree (commonly known as *Azadirachta Indica A Juss.*) is one of the most important ones found in arid regions of world. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. All parts of the neem tree- leaves, flowers, seeds, roots and bark have been used in traditional medicine as household remedies against various human ailments [3]. From its roots to its spreading crown, the tree contains a plethora of important compounds useful for animals, people and plants. Neem tree's virtues are, to a large extent, attributable to its chemical constituents [4]. Chemical constituents, biological activities and medicinal properties of different parts of neem were previously reviewed [5]. Root bark of neem contains tricyclic diterpenoids like margocin, margocinin, margocilin, gedunin and poly-saccharides. The root bark also yields an antitumor polysaccharide. Besides polysaccharides, several diterpenoids such as nimbinone, nimbolicin, margocin, nimbidiol, nimbione, etc. have been isolated from root bark [6]. Root bark was reported to act as astringent, tonic, antitumour and antioxidant [7].

Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity [8,9]. Therefore, the objective of our present study is to determine the total flavonoid content of different extracts of root bark of *Azadirachta Indica* using Aluminium Chloride colorimetric method.

## MATERIALS AND METHODS

### Plant material

The root bark of AI was collected from Deshmukhi village of Nalgonda, India. The authentication of plant material was done by a botanist at Osmania University and the Voucher no is **0125**.

### Chemicals

Quercetin, aluminium chloride

## **Preparation of root bark extracts by using different extracting methods [10,11]**

### **Decoction**

100g of shade dried root bark of AI was weighed and 1000 ml of distilled water was added to it. This mixture was taken in 1000 ml beaker and subjected to heating continuously for 6 hrs at a temperature of 100<sup>0</sup>C. Then the mixture was allowed to cool to room temperature and subjected to filtration by means of vacuum filter. The filtrate so obtained is concentrated so that all the excess solvent is evaporated in order to get concentrated extract.

### **Maceration**

In this method, 100g of AI root bark material was mixed with 1000ml of 80% ethanol thoroughly in a 1000 ml beaker. The beaker containing the mixture was well packed and kept for 7 days. On the 7<sup>th</sup> day, the macerated mixture was subjected to vacuum filtration. The filtrate obtained was concentrated.

### **Microwave Synthesis**

In this method for 100g of plant material, 3000 ml 70% ethanol was used. Specified amount of material was mixed with calculated amount of solvent and kept in microwave for 8 minutes. The resultant mixture was filtered at vacuum and the filtrate was concentrated.

### **Soxhlet Extraction**

100g of root bark material was taken in a soxhlet and 80% ethanol was added up to 2 siphons that is up to 500ml. The temperature is set to 70<sup>0</sup>C and the extraction was carried out up to 5 hours. Then the extract obtained is filtered and concentrated at 70<sup>0</sup>C.

## **Qualitative analysis and thin layer chromatography [12, 13]**

Qualitative chemical analysis for alkaloids, glycosides, tannins, saponins, flavonoids and terpenoids were conducted and results were produced in Table 2. TLC was conducted for all four extracts by using quercetin as biomarker and results were furnished in Table 1 and Figure 1.

## **Estimation of total flavonoid content Aluminium Chloride Colorimetric Method [14, 15]**

### **Principle:**

The basic principle of Aluminium chloride colorimetric method is that Aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids. Quercetin is reported to be suitable for

building the calibration curve. Therefore standard Quercetin solutions of various concentrations were used to build up the calibration curve.

### Procedure:

In this method, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm ( $\lambda_{\max}$  of quercetin) with a Shimadzu UV-1800 spectrophotometer. Aluminium chloride, 1% and potassium acetate, 1M solutions were prepared.

### Stock Solution of Extracts

100 mg of the each extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

### Preparation of Test Solutions

0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank of all four extracts were prepared and their absorbance was measured at 415 nm. All prepared solutions were filtered through whatmann filter paper before measuring.

## RESULTS

**Table 1: Comparison of different extracts with respect to volume of solvent consumed, time of extraction, percentage yield and  $R_f$  values**

S.NO	EXTRACTION	SOLVENT	TIME REQUIRED	% W/W YIELD	$R_f$ value
1.	SOXHLET	500ml 80% ethanol	4 hours	4.5%	0.93
2.	MACERATION	1000ml 70% ethanol	7 days	1.66%	0.92
3.	MICROWAVE	3000ml 70% ethanol	8 minutes	5.1%	0.93
4.	DECOCTION	1000ml distil water	12 hours	20.2%	0.91

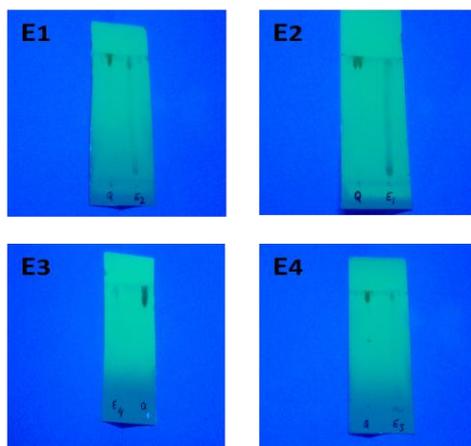
Solvent consumed is more for microwave extract and less for soxhlet extraction. Time consumed for extraction is more for maceration and very less for microwave extraction. Percentage yield is more for decoction extract and less for maceration extract.

Table 2: Qualitative chemical analysis

Name of the test	Soxhlet extract	Maceration extract	Microwave extract	Decoction extract
<b>Test for saponins</b>				
Foam test	++	++	++	++
Sodiumbicarbonate test	++	++	++	++
<b>Test for flavonoids</b>				
Shinoda's test	+++	+++	+	+
NaOH test	+++	++	++	++
Leadethanoate test	++	++	++	+
Fecl <sub>3</sub> test	++	++	++	++
<b>Test for terpenoids</b>				
	++	++	++	++

All four extracts were investigated for qualitative chemical analysis. All the extracts possess significant amounts of saponins, flavonoids and terpenoids and especially soxhlet and maceration extracts gave more significant results and we could not found positive results for alkaloids, glycosides and tannins.

### Thin Layer Chromatography



TLC of E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub> and E<sub>4</sub>

Figure 1: TLC of different extracts

TLC for all four extracts was performed by taking Ethyl acetate: Formic acid: Glacial acetic acid: Water (100: 11: 11: 26) as mobile phase solvent system and Quercetin as biomarker [16, 17]. In all the TLC plates, corresponding spot of quercetin (0.9) was found. The R<sub>f</sub> values of E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub> and E<sub>4</sub> are 0.93, 0.92, 0.93 and 0.91 respectively.

### Determination of Total flavonoid content

To perform the calculations of total flavonoid content in the studied plant using Chang et al method, a standard curve is needed which is obtained from a series of different quercetin concentrations.

### Standard calibration curve of quercetin

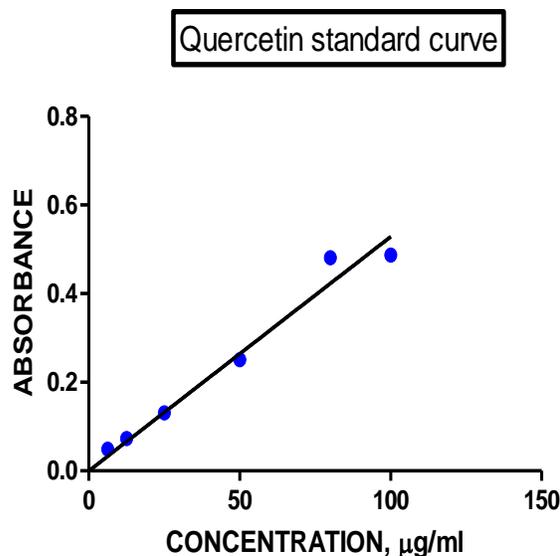


Table 4: Results of calibration curve

Name of the Extract	Absorbance	Concentration, µg/ml
Soxhlet	0.153	28.949
Maceration	0.271	51.276
Microwave	0.259	49.006
Decoction	0.105	19.861

Concentration values of all four extracts were obtained from Quercetin standard curve, by interpolating to the X- axis.

TFC was calculated by using the following formula [18]

$$TFC = \frac{R * D.F * V * 100}{W}$$

Where

- R - Result obtained from the standard curve
- D.F - Dilution factor
- V - Volume of stock Solution
- 100 - For 100 g dried plant
- W - Weight of plant used in the experiment

**Table 3: % yield and % total flavonoid content of different extracts**

Extract	%W/W	% TFC in grams
Soxhlet	4.5	0.289
Maceration	1.66	0.512
Microwave	5.10	0.490
Decoction	20	0.198

TFC = total flavonoid concentration was expressed in terms of quercetin equivalent per 100 grams of plant material.

The % total flavonoid content of the extracts are given in table 6. The decoction method gave the highest yield of crude extract (20% w/w) while the decoction method gave the low total flavonoid content.

Among all four extracts, maceration extract gave the less % yield (1.66% w/w) but the highest % total flavonoid content (0.512) in terms of quercetin equivalents. Microwave extract gave next highest yield to decoction while the % total flavonoid content of this extract is next highest to maceration extract.

**Order of % w/w yield of extracts:** Decoction > Microwave > Soxhlet > Maceration

**Order of % total flavonoid content in terms of quercetin equivalent for 100g of plant material:**  
Maceration > Microwave > Soxhlet > Decoction

## DISCUSSION

As flavonoids play vital role in scavenging the free radicals and these are the phyto constituents we should focus on for investigation of many biological activities, we have prepared the different extracts of root bark of AI and determined their total flavonoid content. The decoction method gave the highest yield but lowest total flavonoid concentration (TFC), while the maceration gave a low yield but high total flavonoid concentration, indicating that the active flavonoids in the root bark were better extracted by 80% ethanol than hot water.

Compared with other methods, decoction was simple, convenient and carried at low cost in terms of reagents and instrumentation.

Next to decoction, maceration method of extraction is simple and economical but time consuming is more (7 days) when compared to other extraction methods.

Soxhlet extraction method gave medium % yield and % total flavonoid concentration. This method is having advantage of solvent recovery and continuous percolation is possible and even this method took less time after microwave that is 4 hours.



Microwave extraction method gave second highest % yield and % total flavonoid concentration. This method promotes better yield and fast extraction (8 mins) of active constituents.

TLC of the extracts except decoction (insignificant) showed the spots of quercetin flavonoid which is the active flavonoid. This suggested that hydro-alcoholic extract is the appropriate method for extraction of flavonoids from root bark of AI.

### CONCLUSION

Decoction extract gave the highest yield. Maceration extract gave the highest % total flavonoid concentration. TLC of all extracts showed spot equal to quercetin. All four extracts showed significant amount of total flavonoid concentration. This research provides information which could trigger further research in the direction of partial or full isolation and characterization of the flavonoids of root bark and assessing their antioxidant activity

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