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COMPARATIVE STUDY OF PHENOLICS AND ANTIOXIDANT ACTIVITY OF PHYTOCHEMICALS OF *T.CHEBULA* EXTRACTED USING MICROWAVE AND ULTRASONICATION

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ABSTRACT

The extraction of active compounds from plants is one of the most critical steps in the commercial development of natural products for medicinal and health benefits. In view of the large number of plant species potentially available for study, it is essential to have efficient systems for the rapid and efficient extraction of phytochemicals for further investigation on their properties. The present study involves the use of microwave and ultrasonication as methods to extract phytochemicals from the fruits of *Terminalia chebula*. The effect of the extraction methods were studied by determining the total phenolic content, tannin content and the antioxidant activity of the extracts. The total phenolic content, tannin content and the antioxidant activity were determined by the Folin Ciocalteau method, Indigo carmine method and the DPPH free radical scavenging assay method respectively. The study revealed a 17.6% increase in the yield of phenolics and a 14% increase in the tannin content of the microwave extracts. A 20.6% increase in the antioxidant activity of the microwave extract was also obtained. The sonication extracts showed an increase of 0.6%, 5% and 9.69% in the yield of phenolics, tannins and antioxidant activity respectively. On comparison with the typical aqueous extraction method the extraction efficiency was highest for microwave treatment followed by ultrasonication.

INTRODUCTION: Medicinal herbs are moving to mainstream use with greater number of people seeking to utilize plant-based products for the prevention and cure of different human diseases. Due to the adverse effects of synthetic drugs considerable attention has been paid to natural remedies which are safe and effective. This growing interest in plant secondary metabolites has prompted the need to review the traditional phytochemical extraction technologies and develop new economical, efficient and rapid extraction technologies for enhancing the concentration of phytochemicals.

The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat or/and agitation to increase the solubility of the desired compounds and improve the mass transfer. Usually the traditional technique requires longer extraction time thus running a severe risk of thermal degradation for most of the phytoconstituents [1].

The fact that one single plant can contain up to several thousand secondary metabolites, makes the need for the development of high performance and rapid extraction methods an absolute necessity [2].

Keeping in pace with such requirements recent times have witnessed the use and growth of new extraction techniques like the application of microwave and ultrasonication to increase the yield of phytochemicals.

Microwave Assisted Extraction (MAE): Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz [3]. Plant cells contain minute microscopic traces of moisture that serves as the target for microwave heating. The moisture when heated up inside the plant cell due to microwave effect evaporates and generates tremendous pressure on the cell wall leading to its rupture [4].

Ultrasonication Extraction: Ultrasonication Extraction (often called sonication) uses high frequency sound to liberate phytochemicals from plant materials. This type of extraction was used for the isolation of essential oils, polysaccharides and bioactive phytochemicals including menthol, cardiac glycosides, pyrethrins and camptothecin [5].

Terminalia chebula has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. *Terminalia chebula* is rich in tannin. The chief constituents of tannin are chebulic acid, chebulagic acid, corilagin and gallic acid [6, 7, 8]. *Terminalia chebula* has 32% tannin content and besides this, fructose, amino acids, succinic acid, β-sitosterol, resin and purgative principle of anthroquinone and sennoside is also present [9, 10]. Flavonol glycosides, triterpenoids, coumarin conjugated with gallic acids called chebulin as well as other phenolic compounds were also isolated [11]. This plant is known to exhibit potential antibacterial, antifungal and antioxidant activities[12].

The present study aims to investigate the effect of novel extraction methods like the application of microwave radiation and ultrasonication on the extraction of phytochemicals from *T. chebula* and to study its antioxidant activity.

MATERIALS AND METHODS:

Plant Material: The dried fruits of *T. chebula* were purchased from a local herbalist and identified by the Botany Department of the College. The dried fruits were powdered and the extracts were prepared.

Extraction Procedures:

Hot Aqueous Extract: Hot aqueous extract was prepared by boiling 10g of *T. chebula* fruit powder in 100ml of distilled water for 30 minutes and kept in a conical flask for 24 hrs undisturbed.

Microwave Extract: 10g of *T. chebula* fruit powder in 100ml of distilled water was microwave extracted (100% power) for a total of 5 minutes with a 2 minute pause and kept in a conical flask for 24 hrs undisturbed.

Sonication Extract: 10g of *T. chebula* fruit powder in 100ml of distilled water was ultrasonicated for 5 minutes and kept in a conical flask for 24 hrs undisturbed.

All extracts were filtered using Whatman filter paper no. 1 into a clean conical flask. The filtrates were then evaporated to dryness. The dry extract was used for estimation of phenolics and tannins and for determining its antioxidant activity.

Determination of Total Phenolics: The amount of total phenolics in extracts was estimated by the Folin-Ciocalteau method [13]. 3 mL aliquots of the diluted extracts were pipetted into different test tubes to which 0.5mL of Folin- Ciocalteau reagent and 2 mL of 20 % (w/v) Na_2CO_3 solution were added. The tubes placed in a boiling water bath for exactly 1 min and then were cooled under running tap water. The absorbance of the resulting blue solution was measured at 650 nm with a spectrophotometer. The amount of phenolics present in the sample was determined from a standard curve prepared with catechol and was expressed in mg per gram of the dry extract.

Tannin Assay: The amount of tannins in the plant extracts was determined by the Indigo carmine method. In 1 mL of sample, 2.5 mL of indigo carmine solution and 75 mL distilled water were added. This mixture was titrated against 0.04N $KMnO_4$ solution ("A" mL). To determine the volume of $KMnO_4$ ("B" mL) used for non tannin compound, each sample of 5 mL quantity was mixed with 2.5 mL of 2% gelatin solution and 5 mL of the acidic NaCl solution. After shaking the mixture for 15 minutes, it was filtered

through Whatman filter paper no.1. 2.5 mL of filtrate was mixed with same volume of the indigo carmine solution and 75mL H_2O. This mixture was again titrated against 0.04N KMnO4 solution. The percentage of tannin is calculated as:

%Tannin (as gallotannic acid) =

$$\frac{(A\text{-}B) \times 100 \times (\text{'g' of tannin / mL of } KMnO_4)}{\text{mL of sample solution}}$$

Where, A = Total tannin material; B = Non tannin material; A-B = True tannin material; 1 ml of $KMnO_4$ = 0.0042g of tannin (as gallotannic acid)

Determination of Antioxidant Activity by DPPH Radical Scavenging Assay: DPPH free radical scavenging assay was performed to determine the antioxidant activity of different extracts. DPPH (0.002%) was used as free radical. Equal volume of extracts and DPPH were mixed and the tubes were incubated at room temperature in dark for 30 minutes. The optical density was measured at 517nm using UV-Vis Spectrophotometer. The degree of stable DPPH decolourization to DPPHH (reduced form of DPPH) which is yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable DPPH was calculated using the following equation.

Scavenging activity (%) = A – B / A x 100

Where, A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

RESULTS AND DISCUSSION:

Total Phenolic Content: The total phenolic content of the hot aqueous extract, microwave extract and the ultrasonication extract was determined. The microwave treated extract had the highest phenolic content of 290mg/g followed by the ultrasonicated extract which yielded a phenolic content of 120mg/g. The hot aqueous extract yielded the lowest phenolic content of 114mg/g. The result from the present study indicates that the phenolic compounds are better extracted with microwave treatment than with ultrasonication or aqueous treatment (**Fig. 1**).

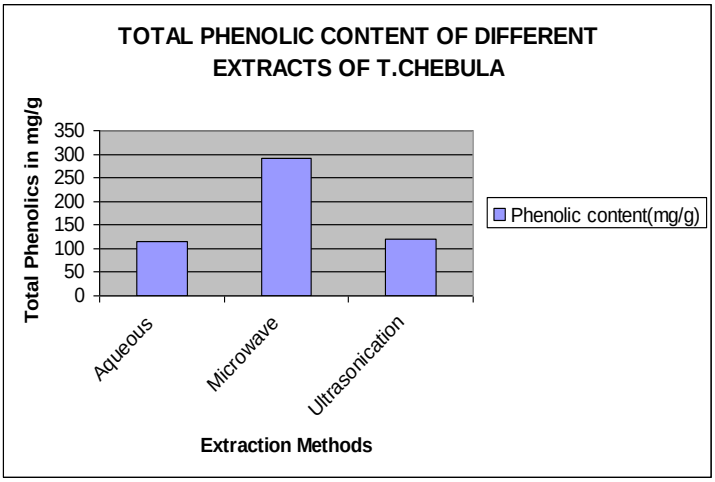

FIG. 1: TOTAL PHENOLIC CONTENT OF DIFFERENT EXTRACTS OF *T. CHEBULA*

Tannin Assay: Tannin content for each extract was estimated and the yield of tannin was 21%, 35% and 26% for the aqueous, microwave and sonication extracts respectively. The highest yield of tannin content was obtained in the microwave treated extract followed by ultrasonication and the least was obtained in the hot aqueous extract. This indicates that tannins are also better extracted with microwave treatment than the other methods of extraction (**Fig. 2**).

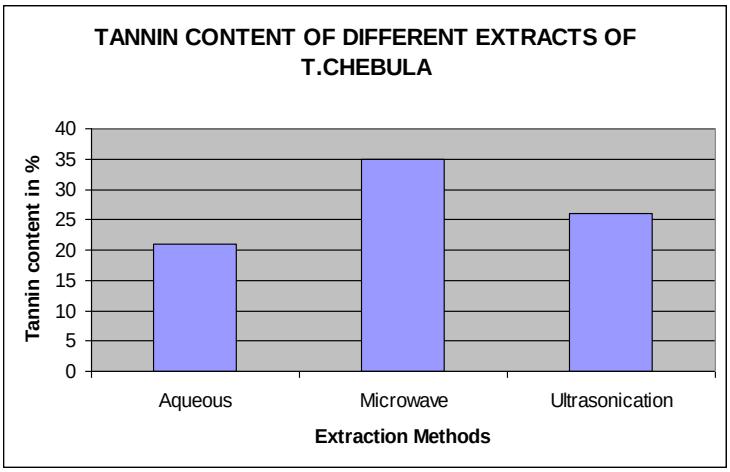

FIG. 2: TANNIN CONTENT OF DIFFERENT EXTRACTS OF *T. CHEBULA*

Antioxidant Activity by DPPH Radical Scavenging Assay: The DPPH radical scavenging activity of *T. chebula* extracts was determined. The microwave extract showed the highest scavenging ability of 82.2% as compared to the ultrasonication extract which showed 61.29% activity and the hot aqueous extract which showed an activity of 51.6% (**Fig. 3**).

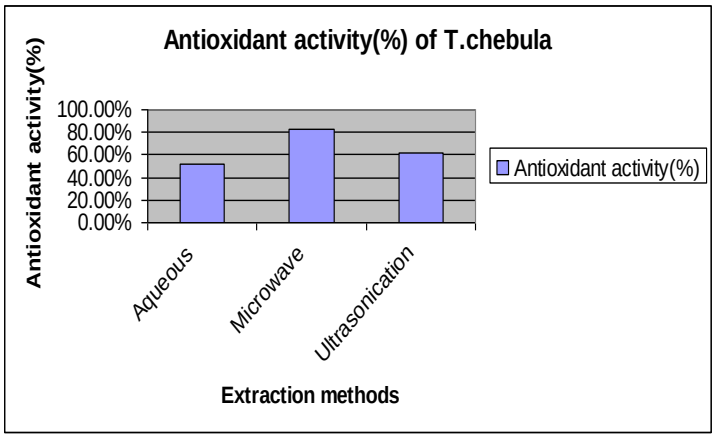

FIG. 3: ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS OF *T. CHEBULA*

The microwave extract recorded the highest phenolic and tannin content and also had the highest scavenging activity. There was a linear correlation between the antioxidant activity and total phenolic and tannin content of *T. chebula*

CONCLUSION: Microwave assisted extraction yielded a 7.6% increase in the phenolic content, 14% increase in the tannin content and a 20.6% increase in the antioxidant activity. However, ultrasonication treatment yielded only a 0.6%, 5% and 9.69% increase in the phenolic content, tannin content and antioxidant activity respectively. The present study reveals that the microwave extract of *T. chebula* yielded the highest phenolic and tannin content and showed highest antioxidant activity.

On a comparative study of the different methods of extraction using microwave and sonication treatment with the typical aqueous extraction method it can be concluded that both microwave and sonication assisted extraction proved to be effective in increasing the yield of phenolics and tannins and in increasing the potential of antioxidant activity . This could be because the cell wall of plant cells gets ruptured under the influence of this treatment. These procedures had the advantages of less time consumption and high efficiency of extraction and can be applied for the extraction of other plant materials.

Polyphenols and tannins are plant secondary metabolites and are very important by virtue of their antimicrobial and antioxidant activity. *T. chebula* extracts prepared using microwave would therefore have a higher therapeutic efficiency due to high phenolic and tannin content, thus increasing their application in the field of herbal medicine.

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