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Sumera Javad
Lahore College for Women University, Lahore, Pakistan, zif_4@yahoo.com

Maimoona Mubarik
Lahore College for Women University, Lahore, Pakistan

Arusa Aftab
Lahore College for Women University, Lahore, Pakistan

Amina Tariq
Lahore College for Women University, Lahore, Pakistan

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Microwave assisted extraction of Phenolics from *Trachyspermum ammi* (L.) Sprague

Sumera Javad*, Maimoona Mubarik, Arusa Aftab, Amina Tariq

Department of Botany, Lahore College for Women University, Lahore, Pakistan

*Corresponding Author E.mail: zif_4@yahoo.com

ABSTRACT

Trachyspermum ammi (L.) Sprague is an annual herbaceous plant belonging to the family Apiaceae and is mostly administered for stomach disorders. During the present research work, cold maceration and Microwave Assisted Extraction (MAE) of *T. ammi* Sprague was done by using methanol, dichloromethane and n-hexane separately as extracting solvents. For cold maceration, 2, 4, 6, 18 and 24 hours of time durations were used for each solvent. Maximum extract (50mg/g) was obtained at 18 hours of cold maceration with methanol, while maximum phenolics were also obtained from the same extract, which were calculated to be 170.89µg/g equivalent of Gallic acid. For Microwave Assisted Extraction, 30, 60, 90, 120, 150 and 180seconds of time durations for microwaves irradiations were used at power levels 200W, 500W, 800W and 1000W. Maximum extract (35mg/g) was obtained at 90 seconds with methanol at power level 800W, while maximum phenolics were obtained after 30 seconds of MAE in methanol (196.90µg/g). Hence, it is concluded that cold maceration has better extraction efficiency for extracts of *T. ammi* than MAE. It was also concluded that the amount of phenolics were obtained in a higher quantity by MAE of *T. ammi*, as compared to the cold maceration in the lesser time of extraction.

Keywords: Microwave assisted extraction, MAE, *Trachyspermum ammi,* microwaves, cold maceration, phenolics.

INTRODUCTION

Herbal products have played a marvelous role in history of treating infectious diseases. A number of plant families like Sapotaceae, Asteraceae, Liliaceae, Malvaceae, Solanaceae, Apocynaceae, Caesalpinaceae, Rutaceae, Piperaceae are reported to have pharmaceutical activity. Resistant strains of bacteria and fungi, against which synthetic drugs have failed, are being treated with plant-derived drugs. A number of studies are happening to develop new drugs from medicinal plants to combat these rapidly evolving resistant microbial strains (Verma *et al.,* 2008).

For pharmaceutical purposes, the very first step in the formulation of herbal drugs is extraction, which involves the separation of the bioactive components of the plant cells. It comes out in a semisolid or liquid form, which is further processed to isolate and formulate the drug. Common extraction methods in use are decoction, cold and hot maceration, etc., but nowadays there are some modern methods of extraction available which allow precise and rapid extraction. These modern methods are definitely advantageous over conventional methods. Modern methods include Microwave Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE), ultra-sonics assisted extraction, etc.(Gupta *et al.,* 2012).

Microwave Assisted Extraction (MAE), or simply extraction using microwaves, is a combination of microwaves and conventional solid-liquid extraction. In this method, microwaves are the working principle which enhances the kinetics of the reaction by heating the solvent and plant matrix, thus increasing the

extracting capability of the solvent. MAE has a number of advantages over customary methods of extraction using plant material as raw material, especially natural products, e.g., short timings of extraction, extraction of targeted molecules, reducing solvent utilization, elevated extraction rate, overall cost effectiveness of extraction etc. (Delazar *et al.,* 2012).

Trachyspermum ammi (L.), belonging to the family Apiaceae, is an annual herb with a grayish brown fruit or seed. The plant may be up to 90 cm tall with an erect, glabrous or branched stem and is cultivated throughout Asia (Chauhan *et al.,* 2012).*T. ammi* is a significantly valued medicinal seed, used as spice which possesses exceptional aphrodisiac properties. Ajwain oil, which is a brown colored oil, is extracted from its seeds (2–4% of weight) and is very well known for its health benefits. This oil includes thymol, which is used for the treatment of bronchial problems, appetite loss and gastro-intestinal ailments (Bairwa *et al.,* 2012). Ajwain seeds have also been reported to have anesthetic, stimulant, antiseptic, carminative, antiviral, diuretic, cytotoxic, antioxidant, antiulcer, antihypertensive, bronchodilatory, hepatoprotective and antihyperlipidemic effects. Roots of the plant also possess diuretic properties (Zarshenas *et al.,* 2014). These biological activities of *T. ammi* are attributed to the presence of various phytochemicals, mainly phenolic compounds, glycocosides, saponins, volatile oils (thymol, para-cymene, γ-terpinene, and α- β-pinene), and mineral components containing iron, phosphorous, calcium and nicotinic acid (Bairwa *et al.,* 2012).

In the present study, extraction of *T.ammi* was done by using cold maceration and microwave assisted extraction. Comparisons for the amount of extract and phenolics were then made for both methods of extraction.

METHODOLOGY

The present study was conducted in the biochemistry lab (Dept. of Botany) of the Lahore College for Women University Lahore. The plant material, *T. ammi* (Ajwain), was obtained from a local market. For cold maceration, 1 g of plant material was placed in vials with lids. The extracting solvent was added and placed at various times according to the experiment. After completing extraction procedures, samples were filtered in pre-weighed vials and were placed in the open for drying. Solvent quantity was optimized at first by macerating the samples in 5, 10, 20, 30 and 40 mL of solvent. Time of extraction was optimized for the maximum extraction of the plant material. It was extracted for 2, 4, 6, 18 and 24 hrs. Solvent and plant samples were placed in vials with lids to avoid evaporation. The amount of extraction solvent was optimized by using different solvents, i.e. methanol, hexane, and dichloromethane. The final extract thus obtained was evaporated in the open at room temperature and was placed in a fridge at 4^{o} C for future use.

For Microwave Assisted Extraction (MAE) of *T. ammi,* an exactly weighed (0.1g) amount of the Ajwain powder was taken in a beaker with 5mL of the solvent each time. The whole set up was placed in the lab and was covered with polythene bags with pores to avoid solvent evaporation caused by microwaves, as well as to control the boiling pressure of the solvent after microwave heating. For MAE extraction, the power levels selected were 200, 500, 800 and 1000W.The microwave irradiation time range used was 30, 60, 90, 120, 150 and 180 seconds and a cooling time of 30 seconds was used to cool the sample solution. Solvent quantity for MAE was optimized with 5, 10 and 15 ml. This was done by using three solvents, namely methanol, dichloromethane and hexane.

Spectrophotometric assay (Arabshahi and Urooj, 2007) was used to estimate the total phenolics from all types of extracts. According to this method, 1mL of sample + 1mL of Folin and Ciocalteu's reagent were placed for 3 minutes to react. After that, 1 mL of saturated sodium bicarbonate (Na_2CO_3) solution was added and the total volume was made up to 10 mL with distilled water. This reaction mixture was reserved in the dark for completion of the reaction. After 90 minutes, the absorbance of the mixture was checked at 725nm on a spectrophotometer. Results were expressed as µg of Gallic acid equivalents/mg of extract (GAEs). Gallic acid was used as a standard phenolics acid to construct the standard curve.

Different extraction parameters, like time and solvent volume, were compared for cold maceration and Microwave Assisted Extraction to determine the efficacy of the extraction method to extract phenolics from *Trachyspermum ammi*. The data generated were analyzed by using COSTAT Computer software (Anonymous, 2009). One way ANOVA was applied to compare the means and treatments for significance by applying Duncan's New Multiple Range test at 5% level of significance.

RESULTS AND DISCUSSION

Figure 1 indicates the extraction of *T. ammi* through cold maceration with a maximum extract quantity of 50mg/g of plant material in methanol. The maximum quantity of extract in dichloromethane and n-hexane was 42 and 20mg respectively. In comparison, Microwave Assisted Extraction produced 35mg (Figure 2), 25mg (Figure 3) and 30 mg (Figure 4) of extract from methanol (90 seconds), dichloromethane (120 seconds) and hexane (90 seconds). Results clearly indicate the fact that lesser amounts of extraction is achieved throughMicrowave Assisted Extraction, but the difference lies in the time of extraction, which is in hours for cold maceration and in seconds for MAE.

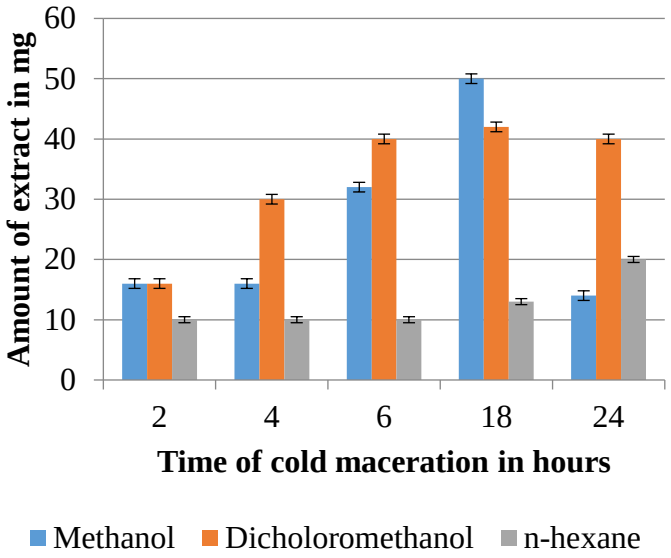

Figure 1: The yield (mg) of extract in different solvents for different time duration using cold maceration method.

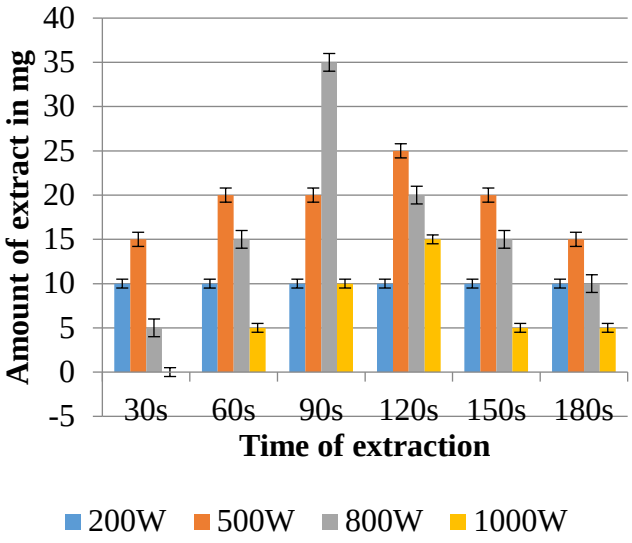

Figure 2: The yield (mg) of extract in methanol for different time duration and power levels of microwaves.

Rizvi *et al.*, 2012, performed a comparative study on the chemical composition of oil obtained from the whole

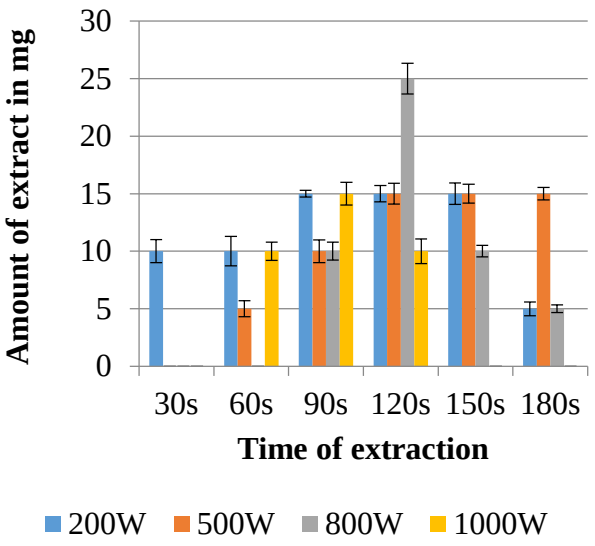

Figure 3: The yield (mg) of extract in Dichloromethane for different time duration and power levels of microwaves

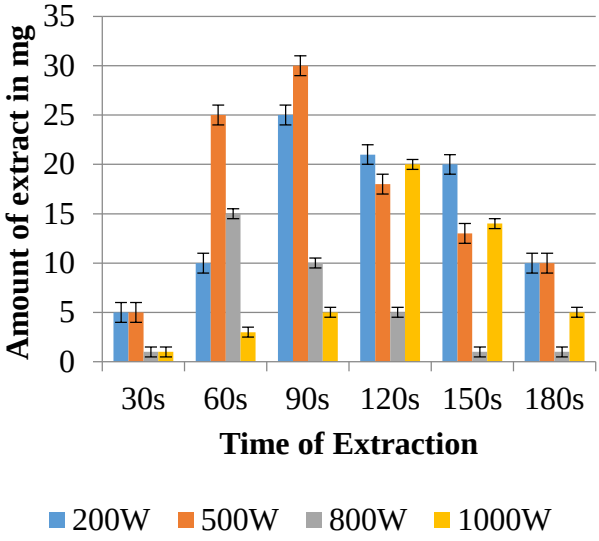

Figure 4: The yield (mg) of extract in n-hexane for different time duration and power levels of microwaves

seeds and crushed seeds of *Nigella sativa* and used the process of cold maceration for the purpose of extraction. They used dichloromethane as a solvent. Musa *et al.*, 2004, reported that 90% ethanol was the best solvent to produce the maximum extract of *Nigella sativa*, but it took longer times to get optimum extract. Mandal *et al.*, 2008, reported an elevated content of curcumin when they used a combination of lower powers and higher timings for MAE of *Curcuma longa* L.

In cold maceration, no external force or source of heating acted upon the sample, which means a lesser transfer of atoms takes place (much less kinetic energy).That's why it is solvent and time consuming. The major benefit of cold maceration is that it is distinct from other processes. Its cool and gentle nature as does not require heat and its extracts/products are never damaged by exposure to high temperatures (Handa *et al.*, 2008).

During Microwave Assisted Extraction, two important factors, such as time of irradiation and power level of microwaves, strongly affects the final yield of plant secondary metabolites. Time to power combination is used for optimizing the extraction of any compound using Microwave Assisted Extraction (Zhang *et al*, 2011). In recent years, MAE has proved its simplicity of work, cost effectiveness, reduced power and energy consumptions make it an inexpensive technique for the extraction of plants' bioactive compounds (Zhang *et al*., 2009).

Figure 5 indicates the estimation of phenolics to extract biomolecules from *Trachyspermum ammi* at different time periods of cold maceration with all the three solvents used, i.e. methanol, dichloromethane and n-hexane. At first, the amount of phenolics showed an increasing trend that then started to decrease. Figure 6 indicates the estimation of phenolics from all solvents from *Trachyspermum ammi* at different time periods of microwave assisted extraction. Results indicate that Microwave Assisted Extraction of *T. ammi* with methanol was best at 90 seconds of extraction (196.90µg eq of Gallic acid) as

compared to 18 hours of cold maceration (170.89µg eq of Gallic acid).

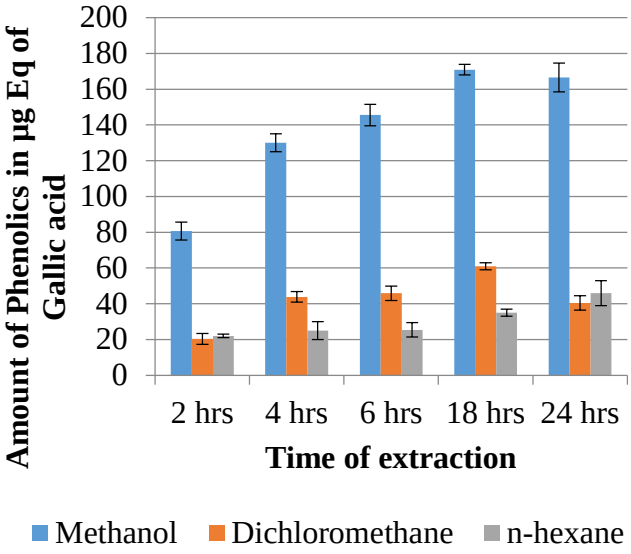

Figure 5: Graphical representation of phenolics from cold maceration of *Trachyspermum ammi*.

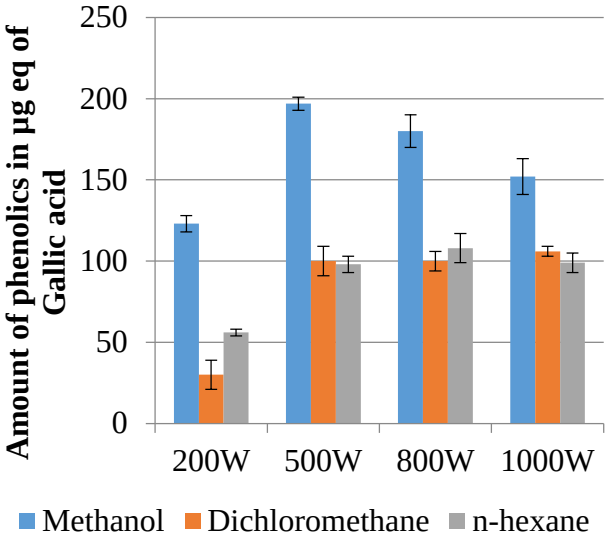

Figure 6:Graphical representation of phenolics from Microwave assisted extraction of *Trachyspermum ammi.*

Phenolics are the bioactive compounds which are isolated from plants using different extraction techniques, and are to be used in different industries. In the most recent few years, the detection and isolation of phenolic compounds of commercial interest, from different plant parts has become a major area of industry, health and medical linked research (Dai *et al.*, 2010).

CONCLUSION

In comparing cold maceration and Microwave Assisted Extraction, MAE proved to be a better analytical technique to get maximum extract per mg of plant material (35mg) with lesser solvent (10mL) and lesser time (90 seconds) as compared to 50 µg extract of 1g plant material in 18 hours of cold maceration. Microwave Assisted Extraction is a rapid method of extraction which may be applied to the extraction of herbal products with a lesser consumption of resources in this age of energy crises. We can save the resources for the next generations.

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