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A Comparative Study on Effectiveness of Various Extraction Techniques Affecting the Anti-Oxidant Property of *Anethum graveolens*

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ABSTRACT

In this research, *Anethum graveolens* which is an aromatic plant that contains essential oils which have non-polar and polar components which are evaluated for their anti-oxidant property through *in-vitro* assays. Herbs have different chemical constituents that include the unique functions of ownership of different pharmacological activities. Extraction is the technique to extract the active constituents and phytochemical lead moiety from the crude plant. *Anethum graveolens* is an aromatic herb used in herbal medicine in India. An excess of flavonoids is known to have antioxidant properties in this herb. Traditionally has been used to cure and avoid illness, and the natural compounds in the plant have been used to treat other diseases. *Anethum graveolens* aerial parts of the plant have been extracted by using ethanol and chloroform by three separate extraction methods such as Soxhletion, Ultra-sound, and Microwave have been performed. Ultrasonic extraction was found to be the best extraction method, and ethanol was found to be the best solvent for flavonoids. Thus having a good antioxidant property.

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INTRODUCTION

The extraction method is an important step to separate the active phytochemical moieties from the crude drug, which is further used in the manufacturing of pharmacologically active drugs. Herbs are the roots of chemical elements responsible for diverse ownership processes, such as the action of anti-oxidants, anti-diabetic, anti-hypertensive, anti-ulcer, anti-inflammatory, etc., extraction is a crucial step that depends upon the nature of herbs and solvent used, which helps in the preservation of the crude extract and its phytochemical properties [1]. Both have their benefits and drawbacks regarding the efficiency of the extraction and the quantity of solvent used. Therefore, it is of vital importance to decide the type of extraction method required for the extraction of the given type of substance. When the process of processing is chosen by alteration or adjustment, changes in the chemical constituents exist. Herbs are rough and delicate, vulnerable and tender in such a way that a special method of extraction is needed, depending on the type of herb [2]. After the active constituent is removed, there is also a significant need to define and establish procedures for the safe handling of crude extracts. Finally, this determines the purity of the raw material based on the substance-derived chemical constituents. There are few other steps in the manufacture of medicines, and the discovery of the right solvent for extraction is one of the most relevant of these. To obtain the maximum yield

of the chemical constituents, rational solvents such as methanol, petroleum ether, chloroform, purified ethanol, ethyl acetate, hexane, etc., must be chosen from the different solvents available for extraction. The solvent preference must be determined based on the polarity and solubility of the chemical components present in the herbal plant material [3].

MATERIALS AND METHODS

Anethum graveolens, known to have several chemical constituents, is a medicinal plant in South India. An enormous volume of flavonoids is responsible for its antioxidant action. The presence of flavonoids and other chemicals responsible for the pharmacological activities of the plant is confirmed by the presence of numerous chemical elements derived from the plant. Therefore, the main focus of this inquiry was on the selection of the extraction method and the right solvent for extraction and to investigate the effect on the antioxidant ability of the plant of the change in the extraction solvent and the extraction technique (Comparative Study).

Methodology

Plant Profile

Anethum graveolens grow up to 90 cm tall, with slender stems and alternate leaves finely divided three or four times into pinnate sections slightly broader than similar leaves of fennel. The yellow flower develops into umbels [4]. The seeds are not true seeds. They are the halves of very small, dry fruits called schizocarps. Dill fruits are oval, compressed, winged about a one-tenth inch wide, with three longitudinal ridges on the back and three dark lines or oil cells (vittae) between them and two on the flat surface. The taste of the fruits somewhat resembles caraway. The seeds are smaller, flatter, and lighter than caraway, and have a pleasant aromatic odor [Figure 1].

Figure 1: Dill Seed Complete Iphym *Anethum graveolens L*

Medicinal Uses

Anethum is used as an ingredient in gripe water, given to relieve colic pain in babies and flatulence in young children. The seed is aromatic, carminative, mildly diuretic, galactagogue, stimulant, and stomachic. The essential oil in the seed relieves intestinal spasms and griping, helping to settle colic [5]. The carminative volatile oil improves appetite, relieves gas, and aids digestion. Chewing the seeds improves bad breath. *Anethum* stimulates milk flow in lactating mothers and is often given to cattle for this reason. It also cures urinary complaints, piles, and mental disorders.

Plant Collection

The *Anethum graveolens* plant's aerial components have been harvested and authenticated from the local farmland. Under shaded conditions, the aerial parts were sufficiently dried and powdered. They were grounded and sieved for fine powder and packed in an air-tight container for potential use [6]. Three methods of extraction were chosen for the same sample of Anethum graveolens and two solvents were selected based on higher and lower polarity (Chloroform and Ethanol).

Extraction Methods

Soxhletion Technique

About 500gm of whole plant powder was weighed and packed in a thimble and placed in a soxhlet apparatus. By using different solvents (Ethanol and Chloroform) plant was extracted for 8 hours. The extracts were extracted and screened through the filter paper after the process and condensed by using a rotary vacuum evaporator. A dense paste was collected called extract [2].

Ultrasound Technique

The plant's aerial components were measured at approximately 500 gm and were isolated using ultra-sound instruments. The variables for ultrasound are set with a pressure of 70% and a frequency of 40 kHz, the temperature for extraction in the bath was continuously held at 400C. For around 30 minutes, the machine was allowed to run. The resultant extract, resulting in a thick paste, was then cleaned out and evaporated [7].

Microwave Technique

In this process, the extraction was powdered through the microwave and the use of various solvents remained unchanged. In the refrigerator, the 500gm of powder was taken into beakers and the two solvents were added. For microwave efficiency, variables have been standardized. The energy is processed at a temperature of 400W and the temperature is set at 400C and the extraction process is carried out for approximately 30 minutes. The resulting extracts were purified and then evapo-

rated for dryness [8]. To assess the content of phenols and flavonoids and the ability of antioxidants, dry extracts were then collected and used.

Qualitative Chemical Analysis

Determination of Total Flavonoid Content

Total flavonoids content in the plant extract in brief 1000 μg/ml of sample were added to two different solvents ethanol and chloroform (comparative study) and 3ml followed by 200μl of 10% AlCl3 solution, 200μl of 1M potassium acetate solution, and 5.6ml of distilled water was added [9]. The absorbance at 420nm was monitored (biotech instrument, instrumenting Winooski, VT, USA) after 30min incubation at room temperature. Total flavonoids content was calculated concerning the standard curve of the flavonoid [Table 1]. Quercetin dehydrated quantification was performed concerning the standard curve of Quercetin result were expressed in micrograms (mg) of Quercetin dehydrated equivalent (QE) per ml of the extract[10]. All tests were performed for different extraction methods [Figure 2].

Determination of Total Phenolic content

The amount of total phenolic content in the extract was determined according to the Folin-Ciocalteu method. 0.2 μL of sample solution (1mg/mL) were introduced into a test tube containing 2 ml of Folin-Ciocalteu's reagent and 5 mL of Na2CO3 (7.5%) and methanol [10].

The final volume was brought up to 7 mL with deionized water. After 2hrs incubation at room temperature, the absorbance was measured at 765 nm with a spectrophotometer (Shimadzu, UV-1800).

The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram of extract (mg GAE/g extract) (Comparative study) [Table 1 & Figure 2].

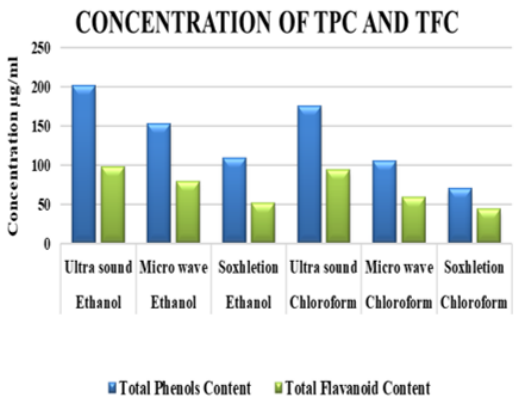

Figure 2: Effect of extraction estimation of TPC and TFC of *Anethum graveolens*

Screening Methods of Antioxidant Activity

Determination of 1,1- Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity

The DPPH assay is popular in natural product antioxidant studies. DPPH radical scavenging method is simple and sensitive.

Principle

The DPPH assay method is based on the reduction of alcoholic DPPH solution(dark blue) in the presence of a hydrogen donating antioxidant converted to the nonradical form of yellow-colored diphenylpicrylhydrazine.

Mechanism

DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay is used to predict antioxidant activities by a mechanism in which antioxidant acts to inhibit lipid oxidation. So scavenging of DPPH radicals and therefore to determine free radical scavenging capacity. DPPH is a synthesized nitrogen-centered radical [11].

It is stable based on a special chemical structure with 3 benzene rings, making the unpaired electrons of central nitrogen lose the pairing function [Figure 3]. Free radical DPPH solution displays typical dark purple color after dissolved in methanol, with strong absorption in 517nm.

After reacting with antioxidants, the absorption in 517 nm will decrease to yellow coloration, which can be detected by spectrophotometer. The antioxidant activity of the sample was evaluated by the inhibition percentage of the DPPH radical.

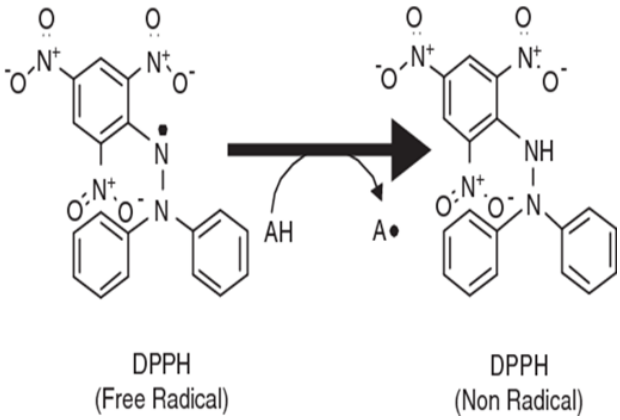

Figure 3: Reaction of Free Radical converted into Non-Radicals

Preparation of DPPH Solution

4 mg of DPPH was dissolved in 100 ml of (ethanol and chloroform) to make 0.004 % of DPPH solution and kept overnight in a dark place for the generation of DPPH radical [12].

Table 1: Effect of various extraction methods on determination of TPC and TFC

S.No	Solvent	Extract-type	% yield	Total Phenols Content	Total Flavanoid Content
1		Ultrasound	27.34	201.75±10.46	98.23±8.61
2	Ethanol	Microwave	24.27	152.91±11.80	79.62±5.56
3		Soxhletion	20.59	108.63±8.12	51.82±9.74
4		Ultrasound	28.83	175.8±12.6	93.57±6.01
5	Chloroform	Microwave	23.01	104.76±9.52	58.68±7.90
6		Soxhletion	19.82	70.28±79.41	43.94±8.28

Table 2: Effect of various extracts of *Anetheum graveolens on* DPPH radical scavenging activity

Solvent/ variable	Extract Type	Concentrations of extracts (units- μg/ml) and %RSD±SEM					IC_{50}
		20	40	60	80	100	
Ethanol	Ultrasound	44.39±0.93	55.24±3.06	78.51±1.02	94.46±1.06	103.59±4.51	58.22
	Microwave	40.53±0.94	54.40±5.57	74.7±2.05	88.73±2.09	96.8±5.62	58.163
	Soxhletion	32.56±2.01	43.81±0.79	64.92±4.8	77.02±7.51	81.24±1.12	58.41
Chloroform	Ultrasound	42.23±2.09	56.05±6.67	75.01±0.82	91.8±0.90	97.85±5.31	58.47
	Microwave	28.4±4.58	37.52±0.97	55.87±1.02	66.56±4.47	73.06±3.04	58.58
	Soxhletion	26.82±6.11	33.70±0.89	41.35±5.47	54.48±0.91	65.51±6.37	58.061
Standard	Ascorbic Acid	45.10±5.76	58.9±7.34	76.3±5.28	90.01±3.02	99.65±0.99	57.77

Preparation of Standard (ASCORBIC ACID) Solution

10 mg of Ascorbic acid was dissolved in 10 ml of Ethanol to make a stock solution with a concentration of 1000μg/ml then serial dilutions are performed to prepare different concentrations (20, 40, 60, 80, 100 μg/ml). To 0.1 ml of solutions add 3 ml of 0.004 % DPPH solution [13].

Preparation of Blank Solution

0.1ml of Ethanol add 3 ml of 0.004 % DPPH solution. (For ethanol extract)

0.1ml Chloroform add 3 ml of 0.004 % DPPH solution. (For chloroform extract)

Preparation of *Anethum graveolens* Stock Solution

Dill (ethanol and chloroform) extract stock solutions were prepared in ethanol and chloroform respectively with a concentration of 1000μg/ml. From the stock solution, various concentrations (20,40,60,80,100μg /ml) were prepared in (ethanol and chloroform) and to 0.1ml of solutions add 3 ml of 0.004 % DPPH solution.

Procedure

To 0.1 ml of different concentrations of Dill (ethanol and chloroform) extracts, 3 ml of DPPH 0.004% solution was added. The mixture was shaken vigorously and allowed to reach a steady-state at room temperature for 30 minutes. Decolorization of DPPH was determined by measuring the absorbance at 517nm. Ascorbic acid was used as standard control and the same procedure was followed [14].

The percentage radical scavenging activity was calculated using the following formula.

DPPH scavenging effect (%) = (A control - A sample/ A control) * 100

since, A control = Absorbance value of control / blank

A sample = Absorbance value of test sample

Y= mx+c

IC 50 = (0.5-b)/a

Since, b= value, a= value of m

RESULTS AND DISCUSSION

Various solvents and various methodologies were used to isolate various active constituents from the crude drug/whole plant. The values of the inquiries are seen in Table 1 demonstrates Qualitative chemical analysis of the Dill plant. This table demonstrates the percentage yield of various extraction methods by using various solvents and it provides Total phenolic compounds and Total flavonoid compounds. The polyphenols and flavonoids found in the extracts were analyzed by using Folin-Ciocalteu's reagent method. In comparison to the chloroform extract and the ethanol

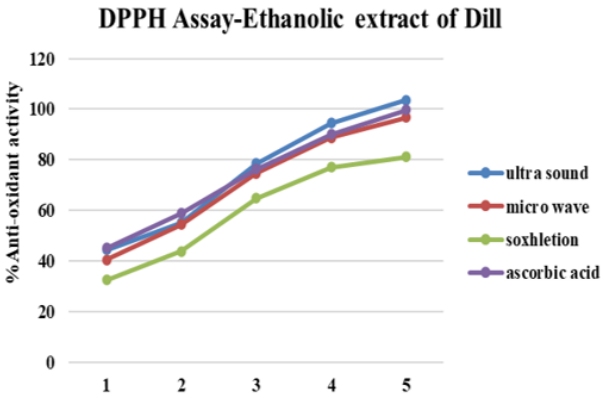

Figure 4: Effect of *Anethum graveolens* ethanolic extract on DPPH radical scavenging activity

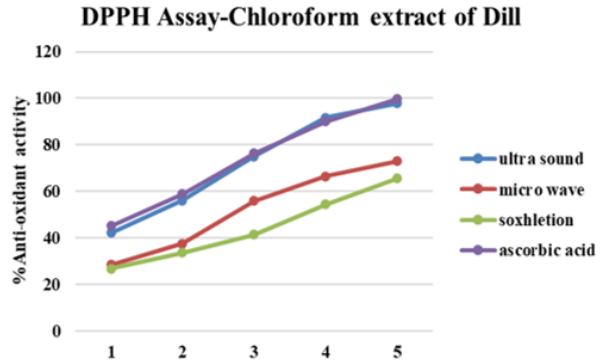

Figure 5: Effect of *Anethum graveolens* chloroform extract on DPPH radical scavenging activity

extract, the ethanol extracts produced a higher yield. This may be due to the solubility of phenols and flavonoids components in ethanol solvent. In comparison with all 3 extraction methods, ultrasound extraction yielded a higher yield compared to other extraction methods as shown in Figure 2.

Table 2 displays the percentage of free radical scavenging activity of the respective solvents and extraction processes. By using the DPPH assay technique, the antioxidant activity of the extracts was determined and the results were interesting and close to the percent yield of the extraction system and the extraction solvent as shown in Figure 4 and Figure 5.

The Ultrasound-assisted extraction system created more effective than the other two methods in the extraction process, accompanied by microwave-assisted extraction. In ultrasound extraction, the ethanol extract displayed a higher yield, followed by microwave-assisted extraction and soxhlet extraction. Overall ultrasound extraction resulted in improved activity in all solvents and ethanol extract obtained the best results in all three extraction methods.

CONCLUSION

Aerial parts of the Dill plant have been active in the treatment of many pathogens, and chemical components are proven to be useful in some situations. After preparing ethanol and chloroform and after using three methods of extraction, different sections of Dill were extracted. Ultrasound extraction was found to be the most efficient way to extract the flavonoids, while ethanol was the optimal solvent to extract the flavonoids.

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Conflict of Interest

The authors declared no conflict of interest.

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Contribution of Authors

Authors declare that the work done by the names mentioned in the article and all the liabilities and claims related to the content of the article will be borne by the authors.

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