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Development and optimization of advanced microwave assisted extraction for *Cucurbita pepo* oil: A Phytochemical and Physico-chemical screening perspectiv

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ABSTRACT

The objective of this study is to optimize the operational conditions of the microwave-assisted extraction (MWE) of seeds of *Cucurbita pepo* (*C. pepo*) and phytochemical, physicochemical screening of extract (fixed oil) which is responsible for the medicinal properties of the plant. Optimal conditions for microwave extractions proposed were 700 Watt microwave power, 70 ml solvent volume and 10 min extraction time. Total 14.57% extract was yielded using microwave assisted extraction method compared to 8.1% by traditional Soxhlet extraction method. The solvent selection was based on the extraction values. Extracted oil was subjected to characterization by using thin layer chromatography, phyto-chemical and physicochemical screening. The microwave assisted extraction was more advantageous, effective and economical to that of traditional extraction method.

Keywords: Soxhlet extraction, Microwave assisted extraction, *Cucurbita pepo*, Linoleic acid, Physic-chemical screening, Fixed oil.

INTRODUCTION

Herbal formulations have reached widespread acceptability as therapeutic agents like antimicrobial, antidiabetic, antifertility, antiageing, antiarthritic, sedative, antidepressant, antianxiety, antispasmodic, analgesic, anti-inflammatory, anti-HIV, vasodilatory, hepatoprotective, treatment of cirrhosis, asthma, acne, impotence, menopause, migraine, gall stones, chronic fatigue, Alzheimer's disease and memory enhancing activities [1]. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. The need of the hour is to evolve a systematic approach and to develop well-designed methodologies for the standardization of herbal raw materials and herbal formulations [2]. Selecting the right scientific and systematic approach to biological evaluation of plant products, based on their use in traditional medicine is the key to ideal development of new drugs from plants. One such plant is *Cucurbita pepo* (Family: Cucurbitaceae).

C. pepo becomes an increasing significance as a source of high quality edible oil. Besides, the seeds are used as an additive in the food industry. Pumpkin seed has been used in traditional medicine in North America and Mexico since long ago as anthelmintic and as a bladder disease agent. Its modern clinical uses are comparable to its traditional uses in Northern American aboriginal medicine. Pumpkin seeds are considered an alternative treatment for stage I and II benign prostatic hyperplasia (BPH) and for irritable bladder [3]. The entire plant is recognized to be beneficial in ethnic systems of medicine. The fruit is sweet, diuretic, antidiabetic, antidepressant, and anti inflammatory. The seeds are fattening, cooling, anthelmintic, and a brain tonic; they can cure cough, fever, scalding urine, and earache; they also reduce inflammation [4]. Pumpkin seeds appear to both reduce levels of substances that promote stone formation in the urine and increase levels of substances that inhibit stone formation.

In recent years, the use of microwave for extraction of active constituents from plant has shown tremendous research interest and potential. High resolution, effectiveness, and separation of active constituents have been obtained by microwave assisted extraction [5]. Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basis for developing a lead molecule. Herbs have provided us some of the very important life saving drugs used in the armamentarium of modern medicine. Among the estimated 400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phytochemicaly [6]. This shows a need of investigation of various chemical constituents, its activity and phyto-pharmacological evaluation

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of herbal drugs.

The basic parameters influencing the quality of an extract are the plant parts used as starting material, the solvent used for extraction, the manufacturing process (extraction technology) used with the type of equipment employed, and the crude-drug: extract ratio. The use of appropriate extraction technology, plant material, manufacturing equipment, extraction method and solvent and the adherence to good manufacturing practices certainly help to produce a good quality extract. From laboratory scale to pilot scale, all the conditions and parameters can be modelled using process simulation for successful industrial-scale production [7].

The present research work was aimed to develop and optimize the conditions for microwave extraction of fixed oil from *C. pepo* and characterize extracted oil using for its phytochemical and physico-chemical parameters as well as separation using thin layer chromatography.

MATERIALS AND METHODS

Collection of sample

Fresh fruits of *Cucurbita pepo (C. pepo)* were collected in the month of August from the local area of Sangli region, Maharashtra, India. The authenticity of plant (Fruit) was confirmed by Botany experts at Gopal Krishna Gokhale College, Kolhapur.

Preservation of sample

Seeds of *C. pepo* were sorted and collected carefully from the fruits and were washed thoroughly to remove dirt and debris. The seeds were spread out in thin layers on drying trays and kept in shade for drying. These seeds were dried naturally, powdered and weighed.

Determination of Ash value

Ashing involves an oxidation of the components of the product. A high ash value is indicative of contamination, adulteration and substitution. The total ash was determined by placing 2 g of *C. pepo* seed powder in an accurately weighed and previously ignited and tared silica crucible. The sample was ignited by gradually increasing the heat. The crucible was kept in desiccator and weighed. The content of total ash was calculated with reference to the amount of *C. pepo* seed powder.

Solvent Extractive value

Solvent extractive value is the amount of active constituent in a specified weight of medicinal plant material when extracted with specific solvent. The solvent extractive value can be determined by measuring the water soluble extractive, alcohol soluble extractive and hexane soluble extractive value using water, methanol and hexane as solvent for extraction, respectively.

Determination of extractive values

For the determination of water soluble extractive value, 5 g of *C. pepo* seed powder was weighed and taken into a conical flask containing 100 ml water and allowed to macerate for 24 h, shaking frequently for first 6 h and allowed to stand for 18 h which was further filtered. The 25 ml of the filtrate was allowed to evaporate to dryness in a tared 250 ml beaker; the difference in weight of the beaker is an indication of water soluble extractive value of that drug with respect to the amount of drug taken for extraction. Similarly, alcohol and hexane soluble extractive values were determined replacing water with alcohol and hexane, respectively, as solvents.

Extraction of plant material

Extraction of plant material was accomplished by conventional Soxhlet extraction and microwave assisted extraction.

Soxhlet extraction

Extraction was achieved by crushing dried seeds of *C. pepo* using mechanical grinder. The coarse powder of seeds weighing 50 g was taken in a thimble of filter paper. Initially the solvent was allowed to siphon once without the condenser by slowly adding solvent over the thimble to wet the powder. Extraction of oil was accomplished using different solvents to achieve highest percentage of oil. The solvent level was adjusted such that it completely fills the Soxhlet and partially the round bottom flask. After initialization the condenser was fixed. The heating process was started after addition of some porcelain pieces to avoid bumping and it was continued for 20 h. Solvents used for extraction were methanol, ethanol, chloroform, pet ether and n-hexane. Upon cooling, the extract was collected, filtered and allowed to evaporate to dryness. The percentage oil yield in "x" g of *C. pepo* seed powder was calculated based on volume of solvent used.

$$Percentage\ of\ yield = \frac{W2}{V2} + \frac{V1}{W1} \times 100 \quad \text{..... (1)}$$

Where, w_1 is initial weight (g) of seed powder, w_2 is the weight (g) of oil obtained, v_1 is total volume (ml) of filtered solvent and v_2 is fraction of solvent subjected to extraction.

Microwave assisted extraction

A primary goal of microwave-assisted extraction was to develop an efficient method to produce pure and highly representative extracts. The extractions were carried out in a fully instrumented and controlled CATALYST® Microwave System at 300 to 700 Watt power output. The system consisted of glass sample holders of 250 ml capacity. For monitoring of process temperature, a built-in optical fibre temperature sensor was used. An accurately weighed 10 g *C. pepo* seed powder was placed in the sample holders, with solvent n-hexane. This mixture was subjected to microwave treatment for different extraction times (2, 4, 6, 8, 10 and 12 min), at different volumes of solvent (60, 70 and 80 ml) and at different power (490, 560 and 700 Watt) outputs. After completion of extraction the solvent (n-hexane) was evaporated from the oil-solvent mixture and oil yield was determined.

Screening of extracted oil [8]

Different Phytochemical tests were carried out for *C. pepo* oil extracted by both Soxhlet and microwave extraction. The tests performed were;

a) Solubility test: The solubility of *C. pepo* seed oil was checked in ether, benzene and chloroform, 90% ethanol and water.

b) Filter paper test: The oil of *C. pepo* was applied on filter paper so as check the staining on the filter paper.

Organoleptic evaluation [8]

The oil samples were analyzed for morphological characteristics by visually analysis as well as small amounts of oil were smelled and tasted.

Specific gravity

Specific gravity, a physical constant, is the ratio of density of a substance compared to the density of fresh water. The specific gravity of *C. pepo* oil was determined using previously calibrated pycnometer. The weight of empty pycnometer was taken as w_1 g and the weight of pycnometer filled with *C. pepo* oil was also taken as w_2 g. The density

of *C. pepo* seed oil was calculated. The specific gravity of *C. pepo* oil was determined by following formula

$$\text{Specific gravity} = \frac{\text{Density of C.Pepo oil}}{\text{Density of water}} \quad \text{..... (2)}$$

Acid value

The acidity of fats and fixed oils is expressed as the number of ml of 0.1N alkali required to neutralize the free acids in 10 g of substance. Acidity is frequently expressed as the acid value, which is the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of the substance. An accurately weighed 10 g of oil was dissolved in 50 ml of a mixture of equal volumes of alcohol and ether (which had been neutralized to phenolphthalein with 0.1N sodium hydroxide) contained in a flask. Few drops of phenolphthalein were added and titrate with 0.1N sodium hydroxide until the solution remains faintly pink after shaking for 30 sec. The acid value was calculated using equation given below.

$$\text{Acid value} = \frac{A \times N \times 56.1}{W} \quad \text{..... (3)}$$

Where, A is ml of 0.1N NaOH, N is normality of NaOH and W is weight of sample (g).

Saponification value

The saponification value is the number of mg of potassium hydroxide required to neutralize the free acids and saponify the esters contained in 1.0 g of the substance. An accurately weighed 2 g of the oil was taken in a tared 250 ml flask, weighed accurately followed by addition of 25 ml 0.5N alcoholic potassium hydroxide. Flask was heated on a steam bath, under a suitable condenser to maintain reflux for 30 min, frequently rotating the contents. Few drops of phenolphthalein were added and titrated the excess potassium hydroxide with 0.5N hydrochloric acid. A blank determination was performed under the same conditions. The difference between the volumes, in ml, of 0.5N hydrochloric acid consumed in the actual test and in the blank test, multiplied by 56.1 and the exact normality of the 0.5N hydrochloric acid and divided by the weight in g of specimen taken, is the saponification value.

$$\text{Saponification value} = \frac{(Vs - Vb) \times 56.1 \times N}{N} \quad \text{..... (4)}$$

Where, Vs is volume of 0.5N HCl consumed by sample, Vb is volume of 0.5N HCl consumed by blank; N is normality of HCl and W is weight of sample.

Refractive Index

A refractometer measures the extent to which light is refracted when it moves from air into a sample and is typically used to determine the index of refraction of a liquid sample. The sample was introduced onto the prism surface. The prism was then closed slowly, allowing the excess to squeeze out. Scale was set properly and the borderline was brought near the crosshairs. The refractive index was read and recorded.

Separation by using thin layer chromatography

The n-hexane extract of *C. pepo* seed oil was subjected to the thin layer chromatography. The TLC plates of 15cm x 10cm size were used. Various solvent systems as mobile phase were tried to obtain the proper resolution. Linoleic acid was taken as standard, as it is one of the major constituent and was isolated from *C. pepo* seed oil and confirmed.

Solvent systems used for TLC

Chloroform: methanol: water (14:6:1), benzene: chloroform (16:4), methanol: water (7:3), (5.5:4.5), (9:1), benzene: methanol (18:2), chloroform: methanol (18:2), n-butanol: water (8:2), hexane: ethyl acetate (8:2), chloroform: methanol: acetic acid: water (8:0.9:1.2:0.2), chloroform: diethyl ether (8:2), toluene: ethyl acetate: glacial acetic acid (15:4:1), hexane: diethyl ether (7:3), (8.5:1.5), hexane: diethyl ether: acetic acid: water (90:20:2:3), hexane: diethyl ether: acetic acid (8:2:1.5), (8:2:1), (8:2:0.5), petroleum ether: diethyl ether (5:5), (6:4), (7:3), (8:2), (9:1), (9.5:0.5) and petroleum ether: diethyl ether: acetic acid (7:3:0.1), (7.5:2.5:0.1), (8:2:0.1), (8.2:1.8:0.1), (8.5:1.5:0.1), (9:1:0.1) and (9.5:0.5:0.1).

TLC of *C. pepo* seed oil

The separations of six distinct spots were obtained in iodine chamber and using spraying reagent, with the mobile phase petroleum ether: diethyl ether: acetic acid (8.2:1.8:0.1). The retention factor was calculated using equation 5.

$$\text{Rf Value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}} \quad \text{..... (5)}$$

RESULTS AND DISCUSSION

Study of various physical, phytochemical and physicochemical characteristics identifies the practical importance and provides bases for suitability and utility of various oils of plant origin in daily life. Physicochemical properties of oil like color, odor, density, specific gravity, refractive index, acid value, saponification value, etc. indirectly tell about the quality of fixed oils.

In the present study fixed oil of *C. pepo* extracted with microwave extraction method was evaluated for physical, phytochemical and physicochemical characteristics. During physical evaluation of *C. pepo* seeds, the ash value was found to be 7.3 % w/w which is comparatively very low and was indicative of very less contamination, adulteration and substitution. Solvent extractive value reveals amount of active constituents in a specified weight of plant material when extracted with suitable solvent. The water soluble, alcohol soluble and hexane soluble extractive values of *C. pepo* seed was found to be 12 % w/v, 59.2 % w/v and 62 % w/v, respectively. Higher hexane soluble extractive value implies that hexane is a better solvent of extraction than water and alcohol.

The extraction of 50 g powdered seeds was for about 20-24 h cycle using n-hexane as a solvent (300 ml) showed 8.1 %w/v total yield (% of oil). The aim of present investigation was to develop an efficient method to produce pure and highly representative extracts. Modern microwave extraction method was optimized by changing process parameters viz. power, time and solvent consumption. Based on obtained yield the optimum conditions for microwave extraction were finalized. The optimization parameters for microwave extraction with highest oil yield were obtained at 70 ml solvent, 700 watt power and 10 min time, Table 1. The comparative results of soxhlet and microwave assisted extraction techniques are given in Table 2.

Phyto-chemical screening tests were performed on the *C. pepo* oil extracted by both the methods. The oil was soluble in ether, benzene and chloroform. Permanent staining of filter paper with oils revealed positive inference of filter paper test. The results of phyto-chemical tests on *C. pepo* oil are given in Table 3. The extracted oil was yellowish with irritating odour and acidic to taste. The specific gravity, acid value, saponification value and refractive index for *C. pepo* oil were 0.905, 0.61, 133.23 and 1.45, respectively.

Table 1: Optimization of parameters for microwave assisted extraction

S No	Solvent volume (ml)	Power (Watt)	Time (min)	% Yield
1.	70	490	10	10.78
2.	70	560	10	11.35
3.	70	700	10	14.21
4.	60	700	10	12.85
5.	50	700	10	10.64
6.	**70**	**700**	**10**	**14.57**
7.	70	700	6	14.14
8.	70	700	2	12.85

Table 2: Comparative results between extraction techniques

Parameters	Conventional Soxhlet extraction	Modern Microwave extraction
Sample weight (g)	50 g	10 g
Solvent	n-hexane	n-hexane
Solvent volume	300 ml	70 ml
Time	20-24 hour	10 minute
Yield (%)	8.1	14.57

Table 3: Results of Phytochemical tests for *C. pepo* seed oil

S No	Test	Observation	Inference
1	Solubility test	Soluble in ether, benzene and chloroform	Positive
2	Filter paper test	Filter paper gets permanently stained with oils	Positive

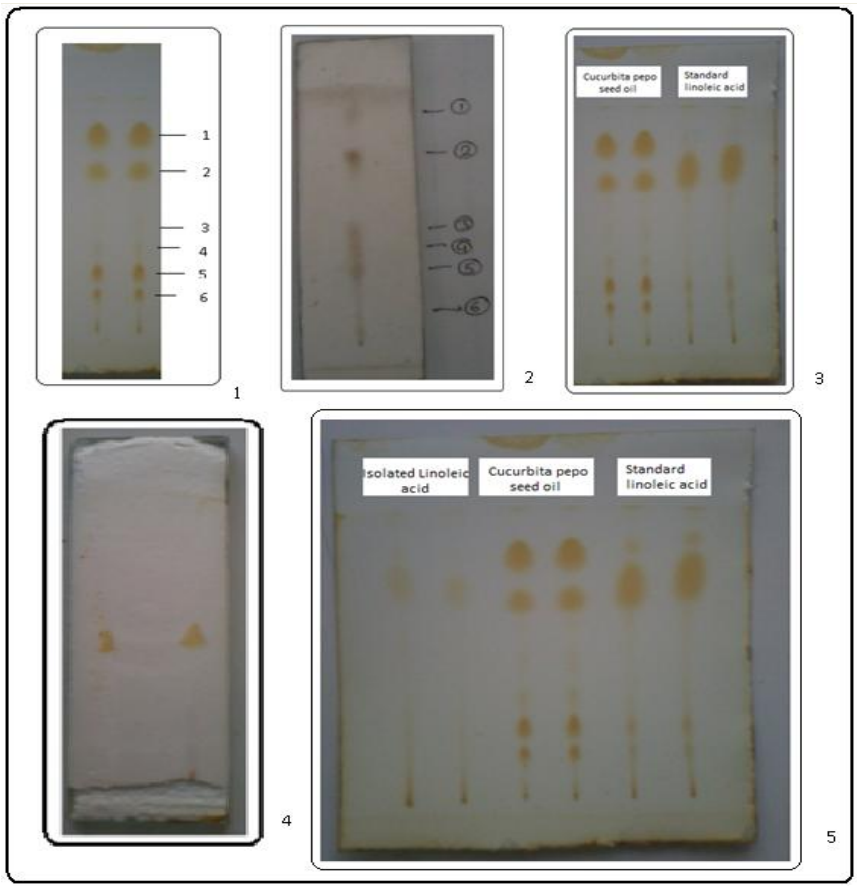

Figure 1: TLC of *C. pepo* oil, **2)** TLC of *C. pepo* oil using spraying reagent, **3)** TLC of *C. pepo* and standard Linoleic acid, **4)** TLC of isolated Linoleic acid and standard Linoleic acid, **5)** TLC of *C. pepo* oil, isolated Linoleic acid and standard Linoleic acid

Separation and isolation of different fractions of *C. pepo* oil was studied by using thin layer chromatography with various solvent systems as mobile phase to obtain better resolution. Linoleic acid being one of the major constituent of oil, as reported, was taken as standard. The linoleic acid isolated from *C. pepo* seed oil was compared with standard and confirmed. The separation of six distinct spots was obtained in iodine chamber as well as by using spraying reagent, with the optimised mobile phase petroleum ether: diethyl ether. The results of RF value determination revealed second spot of linoleic acid. The comparative results of TLC studies are shown in Fig. 1.

CONCLUSION

From the physical evaluation the extractive value of *C. pepo* seed powder was found to be highest in n-hexane than alcohol. The Ash value of *C. pepo* seed powder indicates purity of the substance. The optimised Microwave extraction is more advantageous, effective and economical than Soxhlet extraction with respect to time, yield, energy and solvent consumption. Phytochemical studies conclude the presence of Fatty oils and isolation and separation technique using thin layer chromatography reveals presence and confirmation of Linoleic acid in the extracted oil.

The results confirm the great potential of *Cucurbita pepo* fixed oil as medicinal oil beside its use as edible oil and corroborate the importance for screening plants as a source of bioactive compounds. Further work is needed for producing novel drugs from this natural source.

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Conflict of interest – None declared.

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