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COMPARISON OF *IN VITRO* ANTIOXIDANT ACTIVITY OF *CORIANDRUM SATIVUM* LINN FRUIT BY SOXHLATION AND MICROWAVE EXTRACTION TECHNIQUE

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ABSTRACT

The aim of the present study is to compare the in vitro antioxidant activity of Coriandrum sativum fruit by two different technique of extraction viz., Soxhlation and Microwave extraction and correlate the antioxidant activity with the amount of flavonoids and phenol content present in the extract. *Coriandrum sativum* fruits were extracted separately in soxhlet and microwave extractor with distilled water, 25% ethanol, 50% ethanol, 75% ethanol and ethanol respectively. The study were carried out with all 5 extracts by both methods using different *in vitro* antioxidant model viz., Phosphomolybdenum antioxidant assay, Reducing power assay and DPPH radical scavenging assay. Total flavonoid content and phenol content were also determined. The study exhibited strong antioxidant activity in different *in vitro* systems. The 75% ethanol extract showed better results than all other 4 extracts followed by 50% ethanol and 25% ethanol extract on evaluation with the different *in vitro* antioxidant methods. Flavonoid content and phenol content also correlated well the in vitro antioxidant activity. Microwave extraction values were better when compared to soxhlet technique. This experiment has concluded the strong *in vitro* antioxidant properties of *C. sativum.* 75 % ethanol showed the highest antioxidant activity when compared all other four extracts. Microwave extraction technique is better than soxhlet technique of extraction. The total flavonoid content and phenol content were also more when microwave extraction technique was used. Further, investigation on *in vivo* antioxidant activity has to be carried out

to understand its mode of action and to discover the main constituent of *C.sativum* fruit responsible for this antioxidant effect.

KEYWORDS: *Coriandrum sativum*, antioxidant activity, soxhlet extraction, microwave extraction, phosphomolybednum antioxidant assay, reducing power assay, DPPH assay.

1. INTRODUCTION

Many diseases are caused due to oxidative stress. It has been implicated in the pathology of diseases for inflammatory conditions, cancer, atherosclerosis, Parkinsonism, diabetes and aging. Cardiovascular diseases, tumor growth, wrinkled skin, cancer, Alzheimer's disease are contributed by accelerated cell oxidation rendering a decline in energy and endurance.[1] Oxygen is normally used in regular body processes like respiration and some cell mediated immune functions which tend to produce free radicals. The oxidation of cellular oxidizable substrates can be prevented by substances called antioxidants. The antioxidants act by scavenging reactive oxygen species, activating a number of detoxifying proteins or by preventing the generation of reactive oxygen species.[2,3]

Many a times the body is unprotected from deleterious effects of free radicals as the antioxidation activity normally carried out by the body is unable to sufficiently quench or scavenge the free radicals.

In order, to protect the human body from free radicals and retard the progress of many chronic diseases there has been a recent increase in interest in finding natural antioxidants.[4] α-tocopherol and ascorbic acid are the widely used natural antioxidants because they are regarded as safer and cause fewer adverse reactions. Therefore, there is a considerable interest to explore new natural sources that are safe thereby replacing the synthetic antioxidants.[5]

A number of studies have been carried out on various plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids which prevent free radical damage thereby reducing risk of chronic diseases.[6] This beneficial role of plants to provide natural antioxidants has led to increase in the search for newer plant based sources.

Most of the methods used in these studies are based on classical Soxhlet extraction. Nevertheless, this traditional sample extraction technique often uses large quantities of

organic solvents and is usually time-consuming. In the last few years, established methods, such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) and microwave-assisted extraction (MAE), were used to reduce the volume of solvents required, to improve the precision of analyte recoveries and to reduce extraction time. Of these techniques, SFE is the most selective extraction method, but its use has been limited by the strong matrix dependence of the extraction process. Most of the time, extraction conditions need to be optimised for each new matrix MAE technique offer advantages over the SFE and PLE methods as the extraction can be completed within minutes with less solvent.[7]

Hence, the present study has been undertaken to investigate the two different technique of extraction viz., traditional and microwave assisted extraction technique and compare *in vitro* antioxidant properties of different extracts of the fruits of *C. sativum* by various methods including Phosphomolybednum antioxidant assay, reducing power and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Antioxidant activity was correlated with the content of flavonoid and phenol content present in the extract.

2. MATERIALS AND METHODS

2.1 Plant material

The *Coriandrum sativum* were collected from local market in Bangalore, Karnataka, India and it was identified and authenticated by Botanist, Natural Remedies Pvt Ltd., Bangalore. A voucher specimen was deposited in The Oxford College of Pharmacy, Bangalore. T The fruits were dried in shade and powdered coarsely, passed through sieve no. 40 and stored in air tight container for further use.

2.2.1 Preparation of extract by soxhlet technique

Coarsely powdered fruits of *C.sativum* 20 g, each were subjected to extraction in soxhlet extractor with 25% ethanol, 50% ethanol, 75% ethanol and ethanol [150 ml] respectively. 20g fruits were extracted separately with distilled water on water bath. All the five extracts were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 16.25, 21.35, 33.75, 12.7 and 14 % w/w respectively with reference to the air dried plant material.

2.2.2 Preparation of extract by Microwave assisted extraction technique

Coarsely powdered fruits of *C.sativum* 20 g, each were subjected to extraction in Microwave extractor [M/s RAGATECH SYNTHEZIER with Power 3, set at temperature

50^0C for 30 minutes with continuous stirring] with 25% ethanol, 50% ethanol, 75% ethanol, ethanol and water [50 ml] respectively. All the five extracts were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 9.8,8.8,8.2,7 and 8.2 % w/w respectively with reference to the air dried plant material.

2.3 Drugs and Chemicals

The compounds, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 100% ethanol, ascorbic acid, potassium hydrogen phosphate, potassium di-hydrogen phosphate, sodium phosphate, ammonium molybedate, sulphuric acid were purchased from SD Fine chemicals ltd, Mumbai. Potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride, ascorbic acid, rutin, gallic acid and Other reagents were procured from Loba chemicals, Mumbai. All chemicals and reagents used in this study were at least of analytical grade.

2.4. Determination of total flavonoid content

The total flavonoid content in the extracts were determined by method modified by Zhishen's method.[8] Different concentration of extracts in methanol[3 ml] was mixed with 0.1 ml of 10 % aluminum chloride followed by 0.1 ml of 1 M potassium acetate solution. Add 2.8 ml of water and kept for incubation at room temperature for 30 min. The absorbance was measured at 415 nm. The total flavonoid content are expressed as Rutin equivalent [mg/100 g] of the dried weight.

2.5 Determination of Total Phenol Content

The total phenol content was determined by Folin-Ciocalteu assay.[9] Different concentration of extracts were made up to 3.5 ml, then 0.5 ml of Folin-Ciocalteu reagent followed by 2 ml of 7.5 % sodium carbonate solution. The above solution is incubated at room temperature for 10 min and absorbance was measured at 650 nm. Total phenolic content are expressed as gallic acid equivalent [mg/g] of the dried weight.

2.6 In vitro Antioxidant Activity

2.6.1 Determination of total antioxidant capacity (phosphomolybednum antioxidant assay)

The total antioxidant capacity of the extracts were evaluated by the phosphomolybednum assay method of Prieto et al.[10] which is based on the reduction of Mo (VI) to Mo (V) by the compounds and subsequent formation of a green phosphate - Mo (V) complex in acidic condition. 0.3ml (100-500 µg/ml) of different extracts were combined with 3ml of reagent

solution (600 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and the reaction mixture in the tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 3 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions. The antioxidant capacity was expressed as the number of gram equivalents of ascorbic acid (25-100 µg/ml) which was also processed and incubated under the same conditions.

2.6.2 Reducing power assay

The reducing power was determined by the Fe^{3+} and Fe^{2+} transformation in the presence of fractions described by Oyaizu.[11] The Fe^{2+}can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. One ml of the different extracts (100-500 µg/ml), 2.5ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide was incubated at 50^0C for 30 min and 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. About 2.5 ml of supernatant was diluted with 2.5 ml of water and shaken with 0.5 ml of freshly prepared 0.1% ferric chloride solution. The absorbance was measured at 700 nm. Ascorbic acid (100-500 µg/ml) was used as standard.

2.6.3 DPPH radical scavenging assay

The free radical scavenging activity of the different extracts were measured *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.[12] About 0.3 mM solution of DPPH in 100% Ethanol was prepared and 1ml of this solution was added to 3 ml of different extracts dissolved in ethanol at different concentrations (100-500 µg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance of the solution was measured at 517 nm against a blank using spectrophotometer. The % scavenging activity at different concentrations was determined and the IC_{50} value of the fractions was compared with that of ascorbic acid, which was used as the standard. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging ability. DPPH radical scavenging activity was calculated according to the following equation:

Scavenging effect (%) = $[(A_0-A_1)/A_0 \times 100]$

The results were expressed as mean values ± standard deviation. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of scavenging effect

percentage against the extract concentration. Ascorbic acid (0.125-1 µg/ml) was used as standard.

3. RESULTS AND DISCUSSION

3.1 Total flavonoid content

Fig.1 shows the flavonoid content of different extracts by soxhlation and microwave assisted extraction technique. It is very clear that the amount of flavonoid by microwave assisted extraction was found to highest in 75% ethanol followed by 25% ethanol, 50% ethanol and water. The flavonoid content in ethanol extract was found to be the least.By soxhlet extraction, the content of flavanoid was found to be highest in 75% ethanol, 50% ethanol, 25% ethanol, water and least in the ethanol extract.

3.2 Total phenol content

Fig.2 shows the phenol content of different extracts by soxhlation and microwave assisted extraction technique. It is very clear that the amount of phenol by microwave assisted extraction was found to highest in 75% ethanol followed by water, 50 % ethanol, 25% ethanol and ethanol. The phenol content in ethanol extract was found to be the least. By soxhlet extraction, the content of phenol was found to be highest in 25% ethanol, water, 50% ethanol, 75% ethanol and least in the ethanol extract.

3.3 Total antioxidant capacity (phosphomolybednum antioxidant assay)

The data for the antioxidant capacity by phosphomolybednum method was given in the Fig 3.The antioxidant capacity of the fractions was measured spectrophotometrically through Phosphomolybdenum method, which was based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of the extracts of *C. sativum* was found to decrease in this order in microwave assisted extraction as 75% ethanol, 25% ethanol, 50% ethanol and aqueous extract. The antioxidant capacity of the extracts of *C. sativum* was found to decrease in this order in soxhlet extraction as 75% ethanol, 50% ethanol, 25% ethanol and aqueous extract. In both method, ethanol extract did not show any activity.

3.4 Reducing power assay

Fig 4 shows the reducing power of the *C. sativum* extract by both the method. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers causes the reduction of

the Fe 3+/ferricyanide complex to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, we can monitor the Fe2+ concentration. The reducing properties are generally associated with the presence of reductones[13], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom.[14] However, as anticipated, the reduction power of ascorbic acid was relatively more pronounced than that of *C.sativum* . The reducing powers for the different extracts in the microwave assisted extraction were in the following order: 75% ethanol> water > 50% ethanol> 25% ethanol extract. The reducing powers for the different extracts in the soxhlet extraction were in the following order: 25% ethanol> water > 75 % ethanol> 50 % ethanol extract.

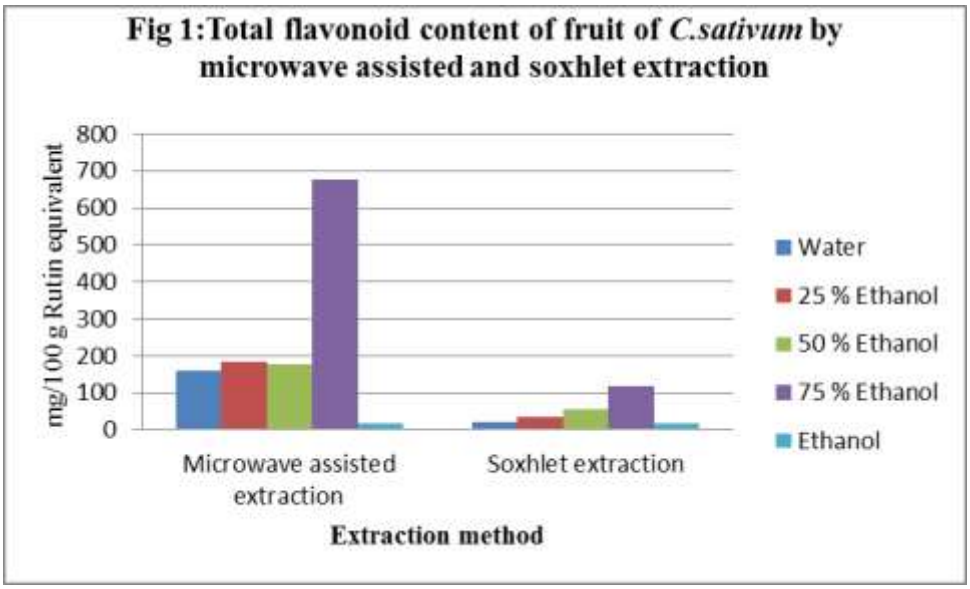
3.5 DPPH radical scavenging activity (RSA)

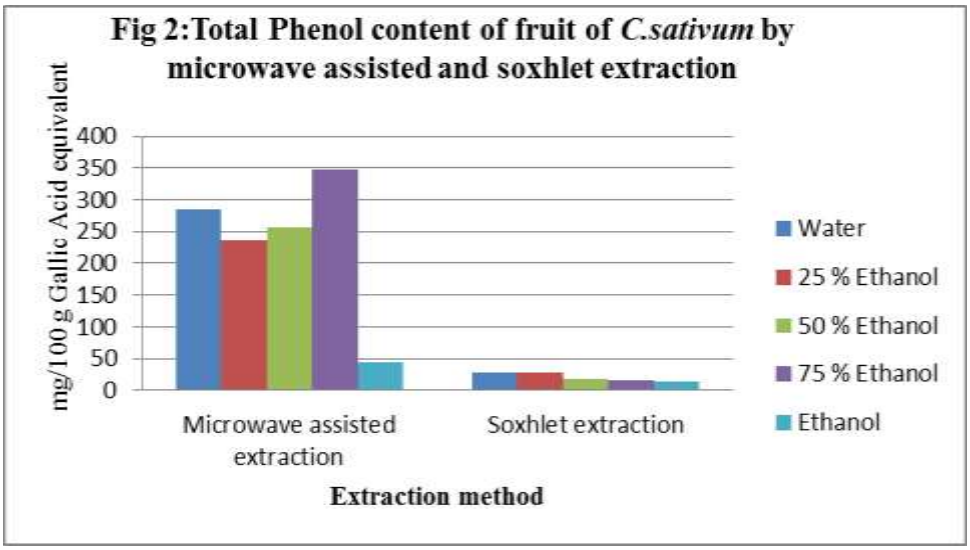
The RSA of the *C.sativum* extracts were evaluated using an ethanolic solution of the stable free radical, DPPH. The model for scavenging the stable DPPH radical is widely used model to evaluate antioxidant activities in a relatively short time to compare with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH is a stable free radical and accept an electron or hydrogen radical to become a stable diamagnetic molecule and therefore inhibit the propagation phase of lipid peroxide.[15,16] The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants.

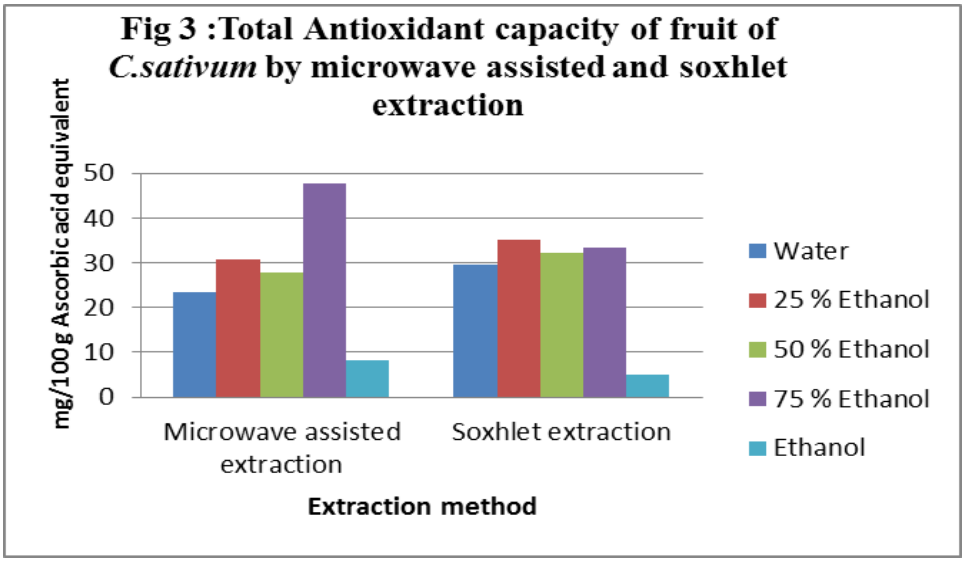
The RSA values of chloroform, ethanol and aqueous fractions are presented in Table 1. IC_{50} values (concentration of sample required to scavenge 50% of free radicals) of *C. sativum* extracts, ascorbic acid are indicated in Table 3. The antioxidant capacity of the extracts of *C. sativum* was found to decrease in this order in microwave assisted extraction as 75% ethanol, 50% ethanol, 25% ethanol and aqueous extract. The antioxidant capacity of the extracts of *C. sativum* was found to decrease in this order in soxhlet extraction as 25% ethanol, water, 50% ethanol and 75% ethanol extract. In both method, ethanol extract did not show significant activity.

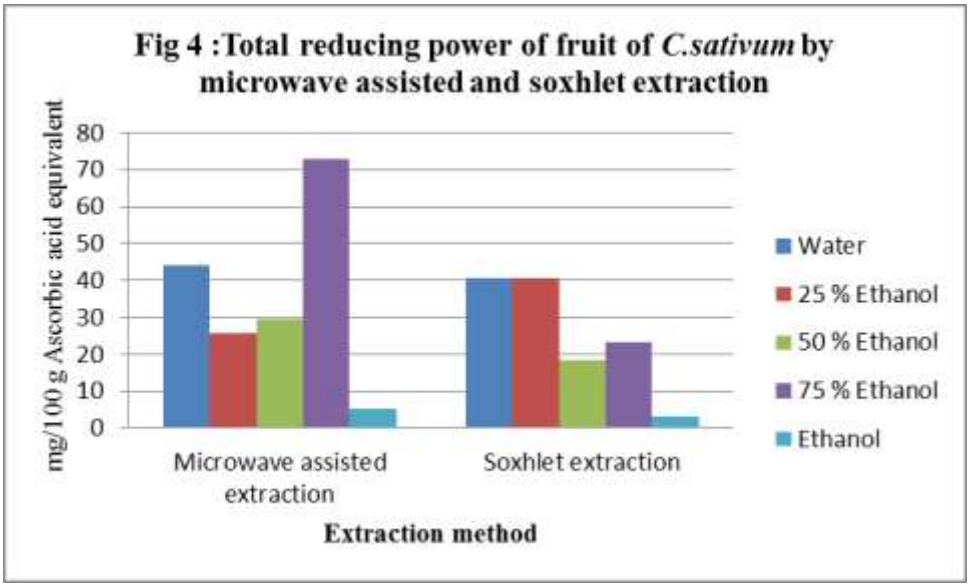
Table 1: DPPH Scavenging activity of fruit of *C.sativum* by microwave assisted and soxhlet extraction

Sl no.	Solvent used	IC_{50} value	
		Microwave assisted extraction [µg/ml]	Soxhlet extraction [µg/ml]
1	Water	158.77 ± 0.31	184.09 ± 0.36
2	25 % Ethanol	148.08 ± 0.29	151.40 ± 0.19
3	50 % Ethanol	92.77 ± 0.56	229.25 ± 0.85
4	75 % Ethanol	75.49 ± 0.63	337.50 ± 0.92
5	Ethanol	not significant	not significant
6	Ascorbic acid	5 ± 0.09	









4 .CONCLUSION

This experiment has concluded the strong *in vitro* antioxidant properties of *C. sativum.* 75 % ethanol showed the highest antioxidant activity when compared to all other four extracts. However, its activity was lower when compared to the standards in each test. Microwave extraction technique is better than soxhlet technique of extraction. The total flavonoid content and phenol content were also more when microwave extraction technique was used. Further, investigation on *in vivo* antioxidant activity has to be carried out to understand its mode of action and to discover the main constituent of *C.sativum* fruit responsible for this antioxidant effect.

5. ACKNOWLEDGEMENT

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6. FUNDING

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7. ETHICAL ISSUES

There is none to be applied.

8. CONFLICT OF INTEREST

None to be declared.

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