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In-Vitro Study Of Antioxidant, Anti Inflammatory And Anticancer Activity Of Eugenol From Ocimum Sanctum

Thenmozhi. M , Dhasarathan. P* and D. Priya

Department of Biotechnology, Prathyusha Engineering College, Tiruvallur- 602025, India

Abstract:

Introduction: India is endowed with a rich wealth of medicinal plants, being perhaps the largest producer and rightly acclaimed as the botanical garden of the world. Plants are the richest source of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates and chemical entities for synthetic drugs.

Materials and methods: The aqueous and ethyl acetate extract of Ocimum sanctumL. leaves were analyzed for phytochemicals. The properties of antioxidant, anti-inflammatory and anticancer activity were screened.

Results: The results showed the presence of phytochemicals such as flavonoids, phenols, alkaloids etc. phytochemicals were quantified and total flavonoid content was found to be 71.21µg/ml (aqueous extract) and the total phenol content was found to be 37.97µg/ml (ethyl acetate extract). The major constituents of O. sanctum were eugenol (25.56 mg/g) and eugenyl acetate (3.5 mg/g).Further the anti- oxidant, anti-inflammatory and anti-cancer effect of aqueous and ethyl acetate extract was studied. DPPH, Reducing power determination was used in identifying the antioxidant activity. and it was found that ethyl acetate extract have higher activity compared to aqueous extract. HRBC membrane stabilization was performed to study the anti-inflammatory activity and it was found that ethyl acetate extract have higher anti-inflammatory activity compared to aqueous extract. MTT assay was carried out to study the anticancer activity.
Conclusion: The eugenol content is present more in O. sanctum and it is used for treating anticancer and anti inflammatory activity. O. sanctum would be a potent weapon for fighting against cancer.

Keywords: Ocimum sanctum, Anti cancer, Anti oxidant, Anti inflammatory.

Introduction:

Plants are the richest source of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates and chemical entities for synthetic drugs [1]. India is endowed with a rich wealth of medicinal plants, being perhaps the largest producer and rightly acclaimed as the botanical garden of the world [2]. According to the world health organization(WHO), about 80% of the world's people depend on traditional indigenous medicines, since a large majority of rural people in the developing countries still use these medicines as the first defense in health care [3].

The leaves of Ocimum sanctum (Tulsi) contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterprine hydrocarbon caryophyllene [4]. Fresh leaves and stem of Ocimum sanctum extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid and appreciable quantities of eugenol [5]. In Ayurveda O. sanctum has been well documented for it's the rapeuticpotentials and described as DashemaniShwasaharni (antiasthmatic) and antikaphicdrugs (Kaphaghna) [6]. Tulsi has also been used in treatment of fever, bronchitis, arthritis, convulsions etc.Aqueous decoction of Tulsi leaves isgiven to patients suffering from gastric and hepatic disorders [7]. Herbal preparations containing Ocimum sanctum L. have been suggested to shorten the course of illness, clinical symptoms and biochemical parameters in patients suffering from viral hepatitis (O. sanctum). The Indian home remedy, Ocimum sanctum being powerful immune modulator, adaptogen/ anti- stress, antioxidant and anti-radiation agent can be used as a novel, safe and effective therapeutic agent in the treatment of human cancer as such or along with radiotherapy and chemotherapy where medicinal herb Ocimum sanctum reduces the ill effects of both and improve life span and life style [8]. Hence in the present investigation extract eugenol from O. sanctumfor anti-inflammatory, anti-oxidant activity, anti-cancer activity against lung carcinoma (A549)

Materials and methods:

The plant of O. Sanctum was collected from Egattur in Thiruvallur at sterile condition. The collected plants were placed in sterile polyethylene cover and transferred immediately to the laboratory. The collected plant was identified as O. sanctum [9]. Fresh leaves of those collected plants were separated and washed with distilled water. The washed leaves were dried under shade and ground into fine powder. The powder was stored in sterile air tight container and kept in cool, dark and dry place for further use. 15 grams of dried powder was measured and it was mixed with 100ml of water, ethyl acetate and hexane to prepare aqueous extract and solvent extract respectively. Those beakers were kept for 7 days at room temperature (28±2ºc) with occasional shaking. After 7 days the filtrate were collected from those extracts used for further analysis

Phytochemical analysis:

The preliminary screening of the bioactive components present in the aqueous, ethylacetate and hexane extract of the O. sanctum leaves were carried out through the qualitative phytochemical analysis. Qunatitative phytochemicals of O. sanctumwere anlaysed and recorded.

Total flavonoid content:

The total flavonoid content was determined by Aluminium chloride colorimetric method. Each plant extracts (0.5 ml of 1:10 g/ml) was separately mixed with 1.5 ml of methanol ,0.1 ml of 10 % aluminum chloride solution, 0.1 ml of the Potassium Acetate Solution (9.82 g of potassium acetate in 90ml of H_2O).Then 2.8 ml of distilled water (H_2O) was added to it. It was kept at room temperature for 30 min. The absorbance of the reaction mixtures were measured using spectrophotometer at 415 nm [10].

Total phenol content:

Standard Folin-Ciocalteu (FC) analytical methodology was used for the quantification of total phenolic components in the plant extracts. For the quantification, 1.0ml of the leaf extracts were

mixed with 2ml of distilled water. After that, 0.5ml of freshly prepared FC reagent was added and mixed thoroughly for three minutes for proper mixing. To that, freshly prepared 2% Na_2CO_3 solutions was added and incubate at room temperature for 2hrs.The change in the color was read at 760nm using spectrophotometer. Gallic acid was used as a standard phenolic compound. The amount of total phenolic compound in the extracts was determined as µg of Gallic acid Equivalent (GAE) per mg dry weight. Thin layer chromatography is performed to separate compounds by Ibrahim [8].

DPPH assay:

DPPH (1,1-diphenyl-2-picrylhydrazyl) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank. Add 100µl of BHT to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm.

Reducing power method:

Yen Schen method (reducing power determination) is used to determine the antioxidant activity [11].

Anticancer activity:

Cell line was obtained from NCCS, Pune. The cells were maintained in DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C [12]. In Vitro assay for Anti-Cancer activity (MTT assay) (Mosmann, 1983) Cells (1 × 105 /well) were plated in 24-well plates and incubated in 370C with 5% CO2 condition. After the cell reaches the confluence, the sample was added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5- dimethyl l-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells .The absorbance at 570nm was measured with UV Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula: % cell viability = A570 of treated cells / A570 of control cells × 100 Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

In-vitro anti-inflammatory activity:

Stabilization of human red blood cell membrane (HRBC) by hypo tonicity induced membrane lysis can be taken as an in vitro measure of anti-inflammatory activity of the drugs or plant extracts [13].

Results and discussion:

The preliminary phytochemical screening of aqueous, hexane and ethyl acetate extract of O. sanctum revealed the presence of phenols, flavonoids, tannins, and steroids in high amounts. Tannin was present in aqueous extract. Saponin was present in ethyl acetate extract. Flavonoids were

present in aqueous extract. Alkaloids were present in hexane extract. Steroids were present in aqueous and ethyl acetate extract. Phenol was present in aqueous and hexane extract (Table 1)

Qualitative phytochemical screening showed the presence of flavonoid, the total flavonoid content was quantified in both aqueous and ethyl acetate extracts [14]. The amount of flavonoid was found to be higher in aqueous extract which was about 71.21 µg/ml compared to ethyl acetate extract which was about 19.02 µg/ml (Table 2).

Qualitative phytochemical screening showed the presence of phenol, the total phenol content was quantified in both aqueous and ethyl acetate extract. The amount of phenol was found to be higher in ethyl acetate extract which was about 37.07 µg/ml compared to aqueous extract which was about 20.25µg/ml (Table 3).

The chromatogram developed with petroleum ether revealed the presence of three major compound at Rf value of 0.91,0.34 and 0.2 in case of ethyl acetate extract and presence of one major compound at Rf value of 091 in case of aqueous extract. The chromatogram developed with hexane revealed the presence of three major compound at Rf value of 0.92,0.41 and 0.2 in case of ethyl acetate extract and presence of one major compound at Rf value of 0.92 in case of aqueous extract (15).

From the dose dependent response curve of reducing power determination of leaf extract of Ocimum sanctum was observed that the ethyl acetate extract had higher scavenging activity at a concentration of 200µg/ml, the scavenging activity of ethyl acetate extract reached 50%, which was comparable to that of standard chemical (Fig 1) . The bioactive compound (eugenol) showed the higher scavenging activity at a concentration of 200µg/ml (Fig 2).

From the dose dependent response curve of reducing power determination of leaf extract of O. sanctum was observed that the ethyl acetate extract had higher scavenging activity at a concentration of 200µg/ml, the scavenging activity of ethyl acetate extract reached 50%, which was comparable to that of standard chemical (Fig 3).The bioactive compound (eugenol) showed the higher scavenging activity at a concentration of 200µg/ml, the scavenging activity of eugenol reached 50% (Fig 4).

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response [16]. Aqueous and ethyl acetate extracts exhibited anti-inflammatory activity in all the concentration, but the highest RBC membrane protection was observed in the ethyl acetate extract of O. sanctum at maximum protection showed in 200µg of plant extract. Diclofenac is used as standard (Fig 5).The bioactive compound (eugenol) showed the higher scavenging activity at a concentration of 200µg/ml (Fig 6)

The extract caused reduction of the cell number in both the cell viability and attachment assay suggesting the cumulative effect of cell killing and inhibition of cell attachment. The extract showed

IC50 value of 125µg/ml in case of aqueous extract, 15.6µg/ml in case of ethyl acetate extract and 7.8µg/ml in case of eugenol towards the cancer cells (Table 4).

Summary and conclusion:

Eugenol is a compound that is present more in phenol and flavonoid ethyl acetate crude extract. Eugenol is a compound that is used for treating cancer. The bioactive compound (eugenol) showed the higher scavenging activity at a concentration of 200µg/ml and the scavenging activity of eugenol reached 50%. In Anticancer activity the extract showed IC50 value of 125µg/ml in case of aqueous extract, 15.6µg/ml in case of ethyl acetate extract and 7.8µg/ml in case of eugenol towards the cancer cells. The eugenol content is present more in O. sanctum and it is used for treating anticancer and anti inflammatory activity.

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Table: 1. Qualitative phytochemical analysis obtained various extracts of Ocimum sanctum

Phytochemicals	Aqueous Extract	ethyl acetate Extract	hexane Extract
Tannins	+ve	-ve	-ve
Saponins	-ve	+ve	-ve
Flavonoids	+ve	-ve	-ve
Alkaloids	-ve	-ve	+ve
Proteins	-ve	-ve	-ve
Steroids	+ve	+ve	-ve
Anthroquinone	-ve	-ve	-ve
phenol	+ ve	-ve	+ve

Where +ve is present and -ve is absent

Table: 2. Quantitative flavonoid estimations in various extracts of Ocimum sanctum

Bioactive compounds	Aqueous (amount µg/ml)	Ethyl acetate (amount µg/ml)
Total flavonoid content	71.21	19.02

Table: 3. Quantitativephenol estimations in various extracts of Ocimum sanctum

Bioactive compounds	Aqueous (amount µg/ml)	Ethyl acetate (amount µg/ml)
Total phenol content	20.25	37.97

Table: 4. Determination of cell viability after exposure of various extracts of O. sanctum

S.No	Concentration (µg/ml)	Cell Viability(%)		
		Aqueous	Ethyl acetate	Eugenol
1	1000	34.76	12.53	15.23
2	500	41.48	19.82	19.59
3	250	46.80	25.90	25.60
4	125	52.81	32.42	32.25
5	62.5	61.74	38.69	36.74

6	31.2	69.79	45.66	40.59
7	15.6	78.84	51.23	46.84
8	7.8	89.99	63.90	50.40
9	Cell control	100	100	100

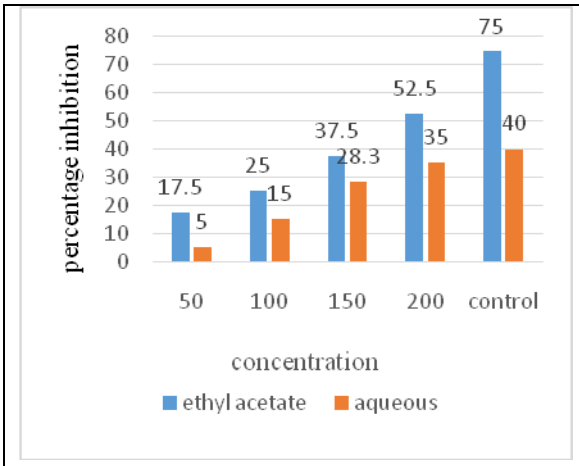

Fig: 1. Reducing power determination of O. sanctum Crude extract

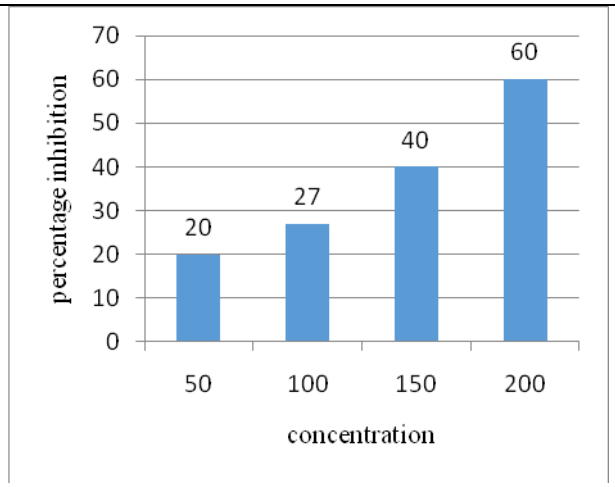

Fig: 2. Reducing power determination of Eugenol

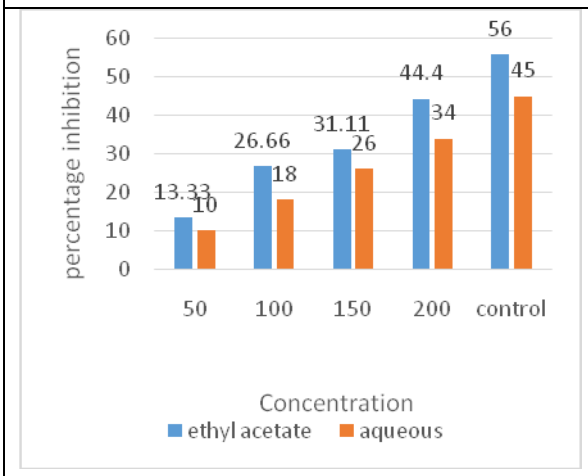

Fig: 3. DPPH activity of O. sanctum Crude extract

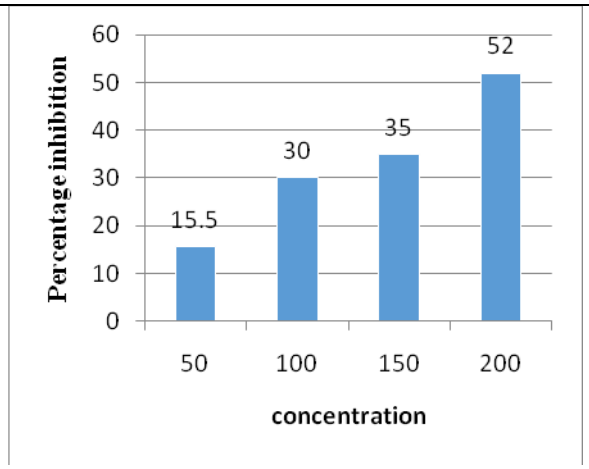

Fig: 4 DPPH activity of Eugenol

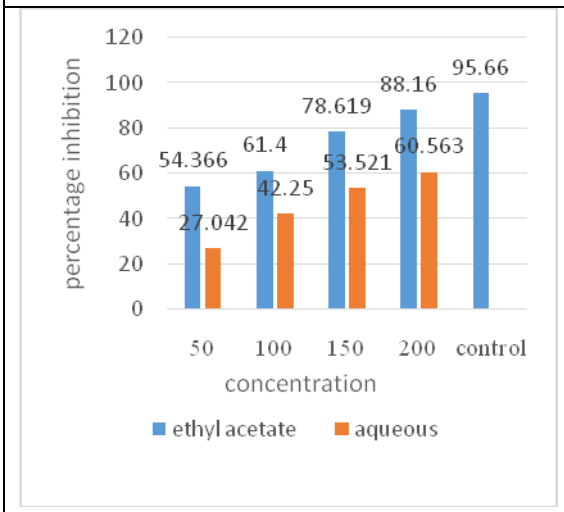

Fig: 5. Anti-inflammatory activity of O. sanctum Crude extract

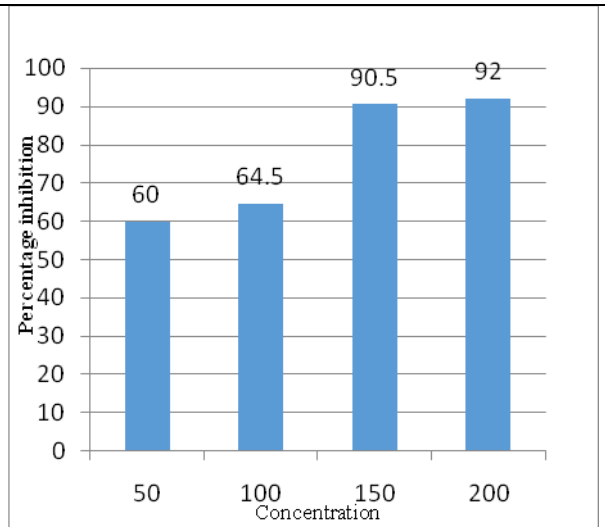

Fig: 6. Anti-inflammatory activity of Eugenol