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IN VITRO ANTIAGING ACTIVITY, GC MS ANALYSIS OF AQUEOUS PEEL EXTRACTS OF *RAPHANUS SATIVUS* AND *DAUCUS CAROTA*

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Abstract

The traditional medicines as culturally specific knowledge, abilities and practices used to either prevent or treat physical or mental disorders. The present study was aimed at determining the anti-aging activity and GC-MS analysis of aqueous peel extracts of *Raphanus sativus* and *Daucus Carota*. The results of antityrosinase and anticollagenase activities of aqueous peel extract of *Daucus carota* showed significantly increased when compared standard and *Raphanus sativus* were respectively. The GC- MS results of *Daucus carota* peel extract showed that seven bioactive compounds were identified. The Prevailing compounds are namely 9, 12-octadecadienoic acid (z z), n hexadecanoic acid, dodecanoic acid 3-hydroxy-, melezitose, L- Glucose, 5 hydroxymethylfurfural and 2,3-dihydro-3,5-dihydroxy-6-methyl were respectively. Therefore, the aqueous peel extract of *Daucus carota* the revealed the presence of bioactive compounds with important medicinal properties. Hence, the presence of these phytochemicals could be responsible for the therapeutic effects of the plant.

Keywords: Traditional medicines, *Raphanus Sativus, Daucus carota and GC- MS* analysis.

1. Introduction

All living things eventually experience the process of ageing. The human skin is the tissue that is most noticeably impacted. Age-dependent/chronological ageing and premature aging/photoaging are the two types of skin ageing. The earlier study found that indicators like deep furrows, dark/light pigmentation, and a leathery look are induced by external factors. Skin wrinkles are an obvious sign of natural ageing. The epidermis, dermis, and subcutaneous tissue are the three layers that make up the skin [1]. The outermost layer of the skin is called the extracellular matrix (ECM), and it is made up of proteins like collagen and elastin as well as fibroblasts. The extracellular matrix (ECM) offers a structural foundation that is critical for skin flexibility and growth, and it is crucial for the preservation of physiological processes.

Degradation of the extracellular matrix (ECM), which has been directly connected to skin ageing, is correlated with increased activity of various skin-aging-related enzymes, including hyaluronidase, elastase and collagenase. One of the fundamental components of the skin, collagen is an essential part of hair, nails, and connective tissue. It controls the suppleness and strength of the skin while maintaining its flexibility [2]. Hyaluronic acid helps the skin retain moisture, maintain its structure, and be flexible. Additionally, it contributes to the rapid proliferation, regeneration, and repair of tissue. It also facilitates the exchange of waste materials and nutrients. It is possible to analyse the chemical components of plant-based medicine directly by combining mass spectrometry and gas chromatography. For non-polar components, fatty acids, volatile essential oils, and lipids, GC-MS analysis is strongly

recommended. When identifying different chemicals from plant extracts, such as alkaloids, flavonoids, organic acids, amino acids and GC-MS is a dependable method [3].

Additionally, computer-based instruments have developed into sophisticated methods for drug discovery that can be applied to the screening of medications derived from bioactive components found in medicinal plants. Furthermore, computer-aided drug discovery techniques have evolved into sophisticated methodologies that can be used to evaluate drugs made from bioactive components present in medicinal plants [4].

The root vegetable crop *Raphanus sativus* L. is a member of the *Brassicaceae* family. Radishes come in a variety of skin colours, including red, purple, black, yellow, and pink, while their meat is usually white. Furthermore, there are regional variations in the flavour, size, and length of the edible radish root worldwide. Radishes' roots and leaves are rich in essential nutrients and a variety of secondary metabolites that have antioxidant qualities. A dietary source rich in nutrients is carrots. A significant root vegetable, they are well-known for their health benefits and nutraceutical properties due to their abundance of naturally occurring bioactive components. The chemical substances found in carrots (*Daucus carota* L.) include ascorbic acid, polyacetylene, carotenoids, and phenolics [5].

Root vegetables like carrots (*Daucus carota* subsp. sativus) are typically orange in hue, though there are cultivars that are purple, black, red, white, and yellow as well. Carotenoids, flavonoids, polyacetylenes, vitamins, and minerals are all present in carrots, a root vegetable that offers a host of health and nutritional advantages [6]. In addition to supporting the traditional belief that carrots are healthy for eyes, carrots' carotenoids, polyphenols, and vitamins function as immune boosters, antioxidants, and anticarcinogens.

The aim of this study to evaluate the anti-aging activity and GC-MS analysis of aqueous peel extracts of *Raphanus sativus* and *Daucus carota* was carried out.

2. Materials and Methods

2.1. Collection of Vegetables

The fresh vegetables of *Raphanus sativus* (RS) and *Daucus carota* (DC), will be purchased from a local market in Tirupattur used for the study. These vegetables are washed thoroughly with distilled water to devoid of any impurities. "The skin of cleaned RS, DC was peeled off carefully with a skin peeler. "The peeled skin is shade dried for dehydration for about a week. The completely dried skin is made in to a fine powder using electric mixing grinder". The ground powder is sieved stored in an air tight container and used whenever it was needed". The Fig1 shows the *Daucus carota* and *Raphanus sativus* are follows,

Fig1: Raphanus sativus

Daucus Carota

3.2. Preparation of Peel Extracts

The preparation of peel extracts was taken separate test tube as 10g of RS + 100 ml of DDW [Double Distilled water] and 10g of DC + 100 ml of DDW. "This will be kept in boiling water bath at room temperature for 10 minutes. The test tube is removed after that period and allowed to cool to room temperature". Then the contents will be filtered through whatman No:1 filter paper. "The filtrate can be used for further studies by storing in refrigerator at $4^{O}C$ not more than a week .

3.3 Antityrosinase Inhibition Activity

The tyrosinase inhibition activity was determined using the dopachrome method described by Masuda *et al.* with slight modifications . The extracts were diluted with 50% (w/w) dimethyl sulfoxide. tyrosinase reactions were performed in a 96-well plate, with each well containing 40 µL of extracts at different dilutions, and 140 µL of 0.1 M phosphate buffer (pH 6.6) followed by 40 µL of tyrosinase solution (31 U/mL) After incubation for 10 min, 40 µL of 2.5 mM L-DOPA was added to the mixture .

The 96-well plate was allowed to stand for 10 min at room temperature, and the absorbance of the solution was measured at 475 nm using a microplate reader . Each sample was accompanied by a blank. All the components, except L-DOPA, used kojic acid as the reference substance [7] .

3.4. Anticollagenase Inhibition Activity

The collagenase activity was investigated using fluorescein-conjugated gelation as the substrate following Zinger *et al.* with some modifications. The enzyme solution was added to the substrate solution followed by a reaction buffer (0.5 M Tris-HCl, 1.5 M NaCl, 50 mM $CaCl_2$, 2 mM NaN_3, pH 7.6). The fluorescence intensity of each sample was measured every 2 min for 20 min using a multimode reader with excitation set at 485 nm and emission at 530 nm . Collagenase concentration was calculated using a calibration curve generated by collagenase samples of known concentrations [8] .

3.5. GCMS analysis

An auto sampler, mass spectrometer, and gas chromatograph were employed. An Agilent HP-5MS (5% Phenyl methyl siloxane) capillary column of 30 m 0.25 mm ID 0.25 m in thickness was utilised with the gas chromatograph (GC). As the carrier gas, high purity 99.999% helium was used at a steady flow rate of 1 mL/min. The injector and mass selective detector transfer line had respective temperatures of 250 C and 280 C. The initial temperature of the column was set at 50℃, and it was held there for two minutes. The temperature was then

raised to 200 ∨C with a hold on time of two minutes using multiple ramp rates of 25 ¯C/min, and it was then elevated to 280 ¯C at a rate of 10 ¯C/min with a hold on time of seven minutes [9].

The electron ionisation (EI) mode of the mass spectrometer was operated at 230 ´C for the ion source and 150 ℃ for the quadruple temperature. The complete mass range of 30 to 500 mass to charge ratio (m/z) was selected for the MS scan range. Seventy eV was the electron ionisation voltage. After being processed, the DC peel extract was dried into a powder. To prepare the sample for GCMS analysis, 1% dry extract was dissolved in methanol. A split-less mode injection of 1.0µL of methanolic sample was performed. The entire GC run time was thirty-five minutes [10]. A comparison was made between the DC peel extract spectrum acquired using GCMS and the mass spectral library spectrum kept at the National Institute of Standards and Technology (NIST).

4. Results and Discussion

4.1. The Antityrosinase Inhibition Activity

The tyrosinase enzyme is responsible for catalysing the synthesis of melanin in animal eyes, skin, and hair bulbs. Tyrosinase inhibitor activity may be exploited for hyperpigmentation and cosmetic purposes including skin lightening, according to extracts from natural substances. To assess the extracts' potential as skin-whitening agents, their inhibitory effects on tyrosinase enzyme activity were ascertained [11]. The results of antityrosinase activity of aqueous peel extract of *Daucus carota* showed maximum inhibition of antityrosinase activity when compared to standard and least percentage inhibition tyrosinase activity was exhibited by *Raphanus sativus* were respectively. The Fig 2 shows the antityrosinase activity of aqueous peel extract of *Daucus carota* are follows,

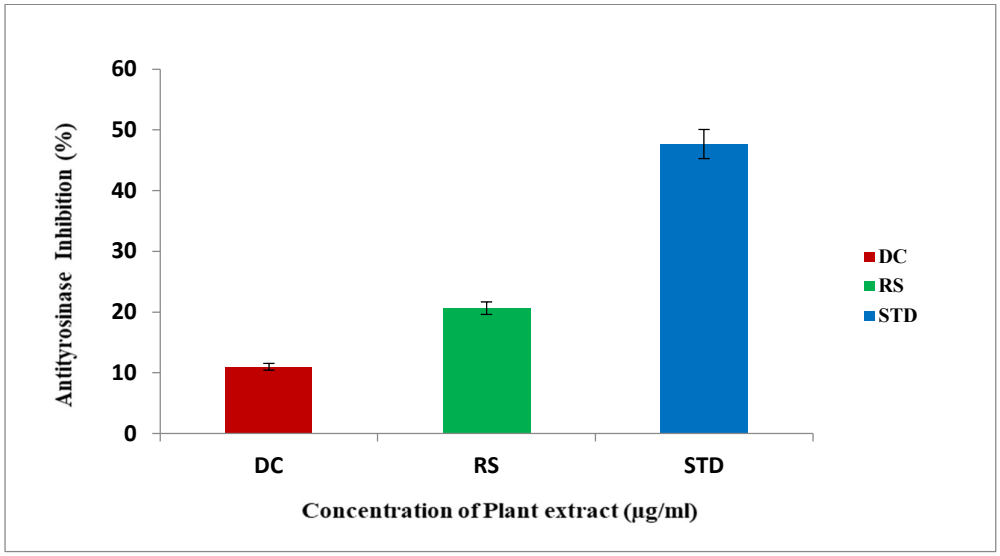

Fig 2 : Antityrosinase activity of aqueous peel extract of *Daucus carota*

4.2. Anticollagenase Inhibition Activity

The results of anti-collagenase activity of aqueous peel extract of *Daucus carota* showed maximum inhibition of anti-collagenase activity when compared to standard and least

percentage inhibition of anti-collagenase activity was exhibited by *Raphanus sativus* were respectively. The Fig 3 shows the anticollagenase activity of aqueous peel extract of *Daucus carota* are follows, The bioactive compounds are flavonols, as opposed to flavones, isoflavones, and flavanones, have a greater inhibitory effect on collagenase and tyrosinase activity.

Consequently, it is believed that the significant anti-collagenase activity of *A. occidentale* stems from the flavonol group of compounds, which includes quercetin and kaempferol [12].

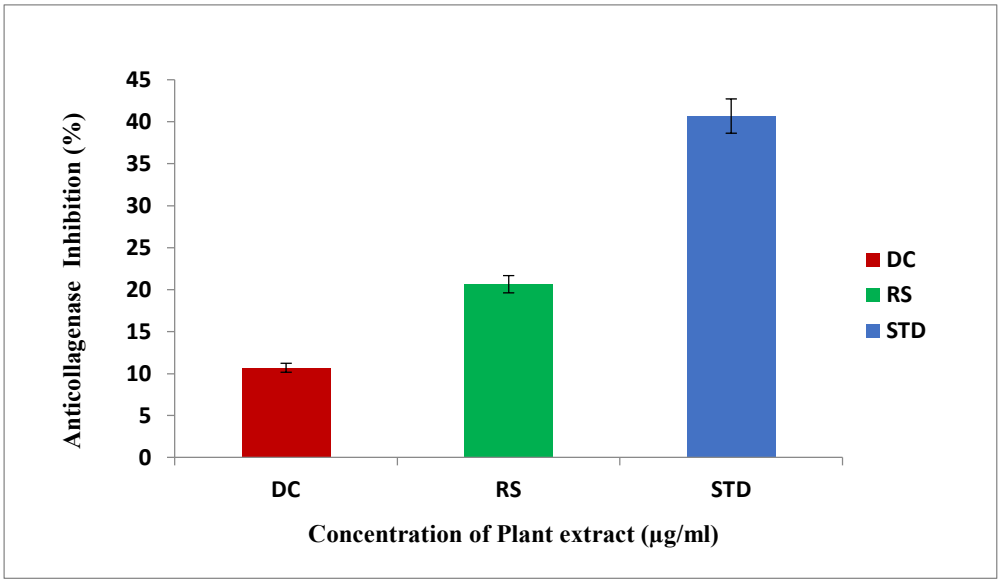

Fig 3 : Anticollagenase activity of aqueous peel extract of *Daucus carota*

4.3. GC MS analysis of bioactive compounds of aqueous peel extract of of *Daucus carota*

A variety of chemical compounds from the GC fractions of the *Daucus carota* aqueous peel extract are identified as a consequence of the GC-MS analytical results. Nineteen bioactive compounds were identified and their retention time (RT), % of peak area, molecular formula, molecular weight and biological activities are presented in Table 1 and Fig. 3 as follows,

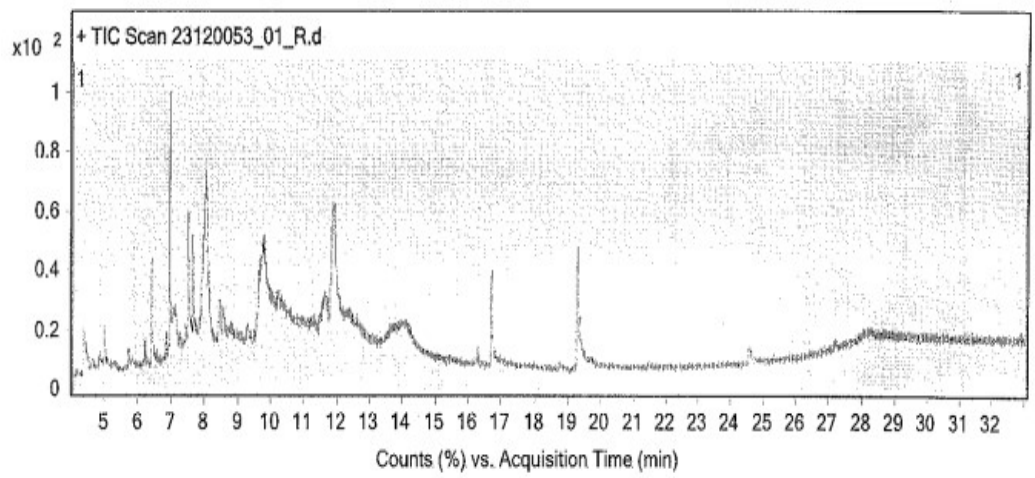

Fig: 3 GC-MS Chromatogram of *Daucus carota*

Table 1: Bioactive compounds from aqueous peel extract of *Daucus carota*

Bioactive compounds	Molecular Weight	Molecular Formula	Retention Time (%)
9, 12-octadecadienoic acid (z z)	280.4455	$C_{18}H_{32}O$	19.289
n hexadecanoic acid	*256.4241*	$C_{16}H_{32}O$	16.712
dodecanoic acid 3-hydroxy	216.32	$C_{12}H_{24}O3$	11.908
melezitose,	522.45	$C_{18}H_{32}O_{16}$	9.778
L- Glucose	*180.16*	$C_6H_{12}O_6$	8.029
5 hydroxymethylfurfural	*126.11.*	$C_6H_6O_3$	7.524
2,3-dihydro-3,5-dihydroxy-6-methyl	144.1253	$C_6H_8O_4$	6.958

The GC- MS results of *Daucus carota* peel extract showed that seven bioactive compounds were identified. The Prevailing compounds are namely 9, 12-octadecadienoic acid (z z) (19.289%), n hexadecanoic acid (16.712%), dodecanoic acid 3-hydroxy- (11.908%), melezitose (9.778%) , L- Glucose (8.029%), 5 hydroxymethylfurfural (7.524%) and 2,3-dihydro-3,5-dihydroxy-6-methyl (6.958%) were respectively.

The study of the bioactive substances found in medicinal plants and their effects has grown in the modern era. GC-MS is one of the best techniques for quantitative analysis of volatile and semi-volatile substances because it combines the best separation technique, GC, with the best identification approach [13]. Bioactive Compounds that were detected with higher percentages, such as 5-hydroxymethylfurfural (7.524%), hexadecanoic acid, and palmitic acid ester ethyl ester (16.712%), demonstrated a broad range of robust bioactivity [14].

These phytochemicals have a variety of pharmacological effects, including antibacterial and antioxidant properties. Food's creaminess and bitterness are both increased by the sweetness enhancer maltol [15]. Due to varying gene expression regulation and macrophage activation, different types of dietary fat alter the risk of numerous acute and chronic inflammatory disorders. Unsaturated fatty acids, such as omega-6 fatty acids like 9, 12-Octadecadienoic acid, methyl ester, are essential for healthy cell growth, blood cholesterol reduction, and skin lubrication [16].

5. Conclusion

It can be concluded that the aqueous peel extract of *Daucus carota* showed significantly increased antityrosinase and anticollagenase activities of when compared standard and *Raphanus sativus*. The GC- MS results of *Daucus carota* peel extract showed that seven bioactive compounds such as 9, 12-octadecadienoic acid (z z), n hexadecanoic acid, dodecanoic acid 3-hydroxy-, melezitose, L- Glucose, 5 hydroxymethylfurfural and 2,3-dihydro-3,5-dihydroxy-6-methyl were respectively. Ageing is now a day one of the biggest issues faced by specially women all over the world and its onset is around when they reach the age over 40. The need for creating substances with anti-ageing effects is because the life style, now a days

people are maintaining is not good for them which can lead to earlier health issues like denaturation of collagen which helps in maintaining the elasticity property of the skin and the need for going to create with natural substances is because they have got least side effects and almost no toxicological effects. Hence, *Daucus carota* peel extract the presence of phytochemicals is responsible for their therapeutic effects. Further investigation is required for possible development of novel drugs.

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Conflict of Interest: Nil

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