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Phytochemical screening,FT-IR,GC-MS analysisof Isolated Bioactive compound (Fisetin)fromEthanolic fruit Extract of *Fragaria xananassa* and their Antioxidant activity

Mythily Panneerselvam[1], Dr.P.T.Devika[2*]
PG & Research Department of Biochemistry, Mohamed sathakcollege of arts & science, Sholinganallur, Chennai, India[1, 2].*
Corresponding Author:Dr.P.T.Devika

Abstract:*This study was designed for isolation of bioactive flavonoid molecule Fisetin from Ethanolic fruit Extract of Fragaria xananassa. Crude extract of strawberry was prepared using ethanol. The ethanolicfruit extract wassubjected for photochemical analysis and theisolated compound was subjected to FT-IR, GC-MS and their free radical scavenging activity was studied.Then the characterization techniques confirmed that the isolated compound was found to be Fisetin. The free radical scavenging activity proposes that the isolated compound fisetin could act as aeffective source of antioxidant.The bioactive flavonoid compound fisetinwas isolated effectively from the Ethanolic fruit Extract of Fragaria xananassa.and their antioxidant activity was studied.*

Keywords:*Antioxidant activity, FT-IR ,fisetin , GC-MS.*

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I. Introduction

A diet rich in fruits and vegetables is associatedwith a lesser incidence of several progressive pathologies,including obesity, cardiovascular and neurological diseases,and cancer[1,2]therefore, increasing intake of fruitmay be a practical approach for prevention. Strawberries (*Fragaria xananassa*)are one of the richest natural sources, which represents an essential factor in disease prevention and health promotion[3]. Indeed, strawberries are best known fortheir antioxidant and anti-inflammatory action. The major class ofstrawberry polyphenols are flavonoids, mainly anthocyanins, followed by flavonols and phenolic acids[4,5,6]. Flavonoids are primarily known as the pigments responsible for the colours of leaves, fruits of plants. Fisetin is a plant polyphenol from the flavonoid group. It is found in various fruits and vegetables, mostly present in strawberries, apples, onions, and cucumbers[7]. Berries contain more amount of fisetin as compared to other fruits. It is known to show anti-cancerous, anti-diabetic, antioxidant, anti-inflammatory and memory stimulator effects[8] .The search for naturally occurring fisetin has a moreattention in industries as well as in scientific research.In this study, we focused at investigating the phytochemical screening, antioxidant activity and FT-IR,GC-MS analysis of bioactive compound(fisetin) present in the ethanol extract of *Fragaria xananassa*.

II. Materials And Methods

Sample Preparation and extraction:

500 g of strawberries were chopped into fine slices and were kept for oven drying for 12 hrs at 100°c, then grind in mixture and 37 g fine powder was prepared. This powder was used as sample.37 gof strawberry powder was taken in a petri dish followed by adding (ethanol / water 80/20: v/v) for 48 hours, in the dark at room temperature, with renewal of solvent every 12 hours. The Solution was filtered by using cheese cloth and evaporated at 50°C. Refrigeration for 24hr after adding 100ml distilled water followed filtration[9].

Preliminary phytochemical screening:

Preliminary phytochemical screening of*Fragaria xananassa* extract was performed using standard chemical methods[10].

GC-MS analysis:

The selected isolated extract compound was subjected to GC-MS analysis using CP3800 Saturn 2200 Gas Chromatography-Mass Spectrometer system. The temperature was setto 80°-350°C at the rate of 3°C/min and held at 350°C at 55 min. The ion source temperature was 200°C with 20-500 amu scan range. The spectrum of isolated component was compared with Wiley and NIST libraries.

FTIR Spectroscopic Analysis:
Fourier transform infrared spectrophotometer (FTIR) is the most powerful tool for identifying the functional groups present in compounds. IR spectra were recorded on Bruker Alpha TKBR and ATR spectrophotometer.

Determination of Antioxidant activity:
The scavenging effect of DPPH•, hydroxyl radical and superoxide anion radicals were determined by the methods of Mensoretal.[11], Halliwellet al.[12]and Nishimiki et al.[13], respectively. The total antioxidant activity (ABTS+ assay) of isolated flavonoid fisetinwas also determined by the method of Miller et al.[14].

III. Results And Discussion

World over, the scientists are discovering the potential of utilizing pharmacologically active compounds from medicinal plants and fruits. Herbal medicines are used by 70% of the people worldwide due to its cheap cost, high efficiency and fewer side effects[15]. In the present study, the assessment of phytochemical screening with Ethanol extract of*Fragaria xananassa* revealed the presence of carbohydrate, flavonoid, phenol, alkaloid, tannin and terpenoid, which are known to have remedial activityagainst pathogenic diseases.(Table no 1). Previous reports suggest that the presence of these bioactive constituents in plant preparations could contributeto the antioxidant, antibacterial, antifungal and antiviral properties[16,17].Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and improvement.

Table no 1: Qualitative analysis of phytochemical screening of ethanol extract of*Fragaria xananassa*

S.NO	Phytochemical constituents	Ethanol extract
1.	Carbohydrates	+
2.	Protein	-
3.	Saponins	-
4.	Tannins	+
5.	Polyphenols	+
6.	Flavonoids	+
7.	Steroids	-
8.	Terpenoids	+
9.	Alkaloids	+

The FT-IR spectrum of isolated compound was shown in figure no 1 and their corresponding characteristic peak positions were listed in table no 2. The absorption peaks positioned confirms that the isolated compound is flavonoid fisetin. This result is in good agreement with the previous literature for molecular structure of fisetin[18].

Figure no 1: FT-IR spectrum of isolated compound

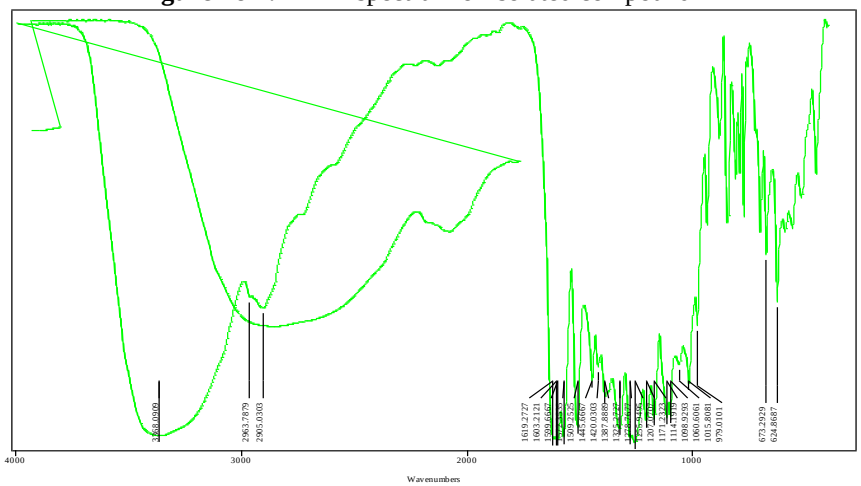


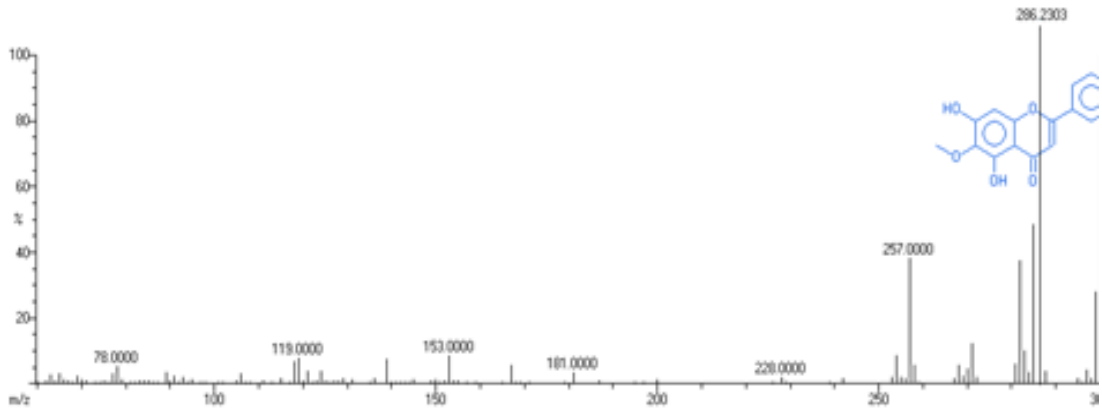
Table no 2: *FTIR* Peak Values of isolated compound.

Peak At	Functional groups
3368.0909	O-H stretching vibration presence of alcohols, phenols
2963.7879	C-H stretching vibration presence of alkenes
2905.0303	O-H stretching vibration presence of carboxylic acids
1619.2727	C=O Aryl ketonic stretch

1603.2121	-C=C- stretching vibration presence of alkenes
1572.3535	C=O aromatic stretch
1445.6667	C=C aromatic stretch
1402.0303	C-C stretching vibration presence of aromatics
1387.8889	O-H bending of phenols
1325.2727	C-H bond in Aromatic hydrocarbon
1278.7677	C-O s stretching vibration presence of alcohols, carboxylic acids, esters, ethers
1255.9495	C-O stretch of Aryl ether
1171.2323	C-CO-C stretch and bending in ketone
1114.1919	C-N s stretching vibration presence of aliphatic amines
979.0101, 673.2929	C-H bending of aromatic hydrocarbons

The isolated fruit compound was analyzed by GC-MS. It has been applied for a quick separation and identification of the isolated compounds from strawberry extract. The chromatogram of the isolated compound was shown in figure no 2. The fragment pattern m/z 286.23 was found and it is speculated that they may correspond to the fragment patterns offisetin. Comparison to the reference substance and a mass spectral library system confirmed that the isolated compound is found to be fisetin. The previous literature showed a similar peak, which confirms that the isolated compound is proved to be fisetin[18].The GC-MS analysis of the identified compound from ethanol extract of the *Fragaria xananassa* specific activities such as antimicrobial, anti-inflammatory, neuroprotective, and potent antioxidant activity have also been reported .

Figure no 2:GC-MS spectrum of isolated compound fisetin from ethanol extract



Radical scavenging activities are most important due to the deleterious effect of free radicals in biological systems.Figure no 3 shows the percentage *in vitro* scavenging effects of ethanol extract isolated fisetin on DPPH radical and ABTS radical (total antioxidant activity) .As seen in Fig no 3, fisetin scavenges the above mentioned radicals *in vitro* in a concentration-dependent manner (10, 20, 30, 40 and 50µM). The percentage scavenging activity offisetin increases with increasing concentration. At the concentration of 50µMfisetin, the percentage scavenging of ABTS+ (75%) was higher than that of DPPH radical (56%).

In this study, free radical scavenging activity of ethanol extract of Fisetin was determined using DPPH•method. Reports have shown that DPPH• is widely used to investigate the free radical scavenging effects of various antioxidant substances, and polyhydroxy aromatic compounds[19]. ABTS ,that have been applied to the total antioxidant activities of solutions of pure substances, aqueous mixtures and beverages[20]. ABTS activity have effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group's substitution[21]. In this study, ethanol extract of fisetin*in vitro* scavenges DPPH• and ABTS+ dose dependently. The highest percentage scavenging effect of isolated compound fisetin on DPPH• and ABTS+ at the concentration of 50µM were 56% and 75%, respectively.

Figure no 3. *In vitro* scavenging effects of fisetin on DPPH and ABTS radicals.

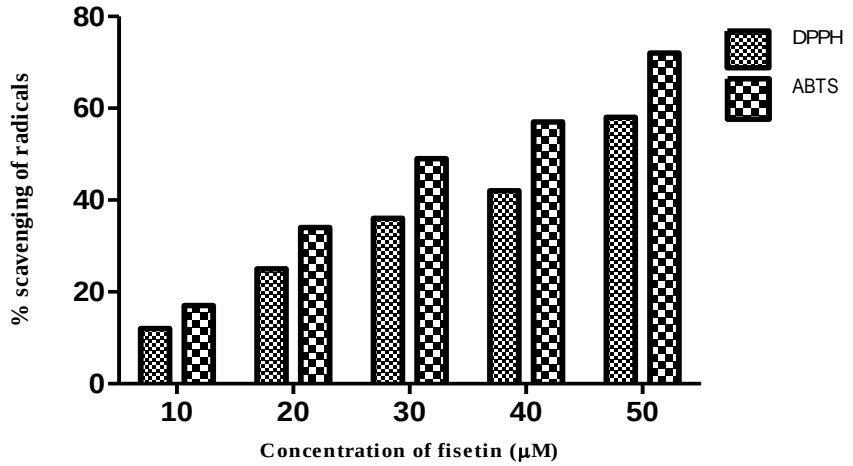
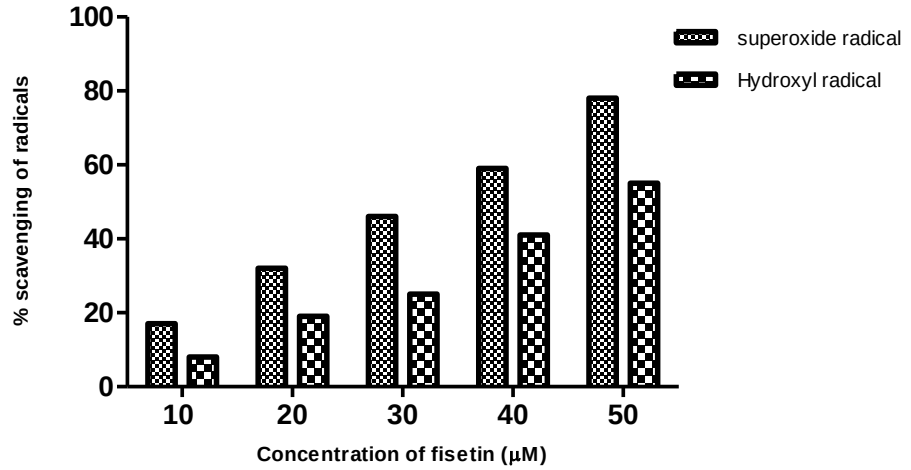


Figure no 4 shown the percentage *in vitro* scavenging effects of fisetin on superoxide radical and hydroxyl radical. As seen in Figure no 4, isolated compound fisetin scavenges the above mentioned radicals *in vitro* in a concentration-dependent manner (10, 20, 30, 40 and 50µM). The percentage scavenging activity offisetin increases with increasing concentration. Moreover, fisetin (50µM) showed the highest percentage superoxide radical scavenging activity (79%) than hydroxyl radical scavenging activity (57%). Wickens[22] reported that superoxide radicals directly initiate peroxidation process. Superoxide anion is a precursor to active free radicals that have potential of reacting with biological macromolecules and thereby inducing damage of tissue. Yen and Duh[23] have reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical. Hydroxyl radical (OH•) is chiefly responsible for lipid peroxidation, which impairs the normal function of cell membranes,motility and permeability. In this study, fisetin*in vitro* exhibits 79% of superoxide radical and 57% of hydroxyl radical scavenging activity at the concentration of 50µM fisetin.

Figure no 4. *In vitro* scavenging effects of fisetin on superoxide and hydroxyl radicals.



IV. Conclusion

From the present work, the compound Fisetin has been extracted successfully from *Fragaria xananassa.* Ethanol plays the main role in the extraction of plant constituents,it showed the presence of more flavonoid content, when compared to other solvents. The identification of flavonoid fisetin was attempted by direct comparison with its retardation factor. The isolated constituent of fisetin was identified through FT-IR and mass spectroscopy. The isolated compound fisetin shows increased antioxidant activity with an increase in the treated concentrations.

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