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Antioxidant properties of semi-Synthesized and isolated 3-(3, 4-dimethoxyphenyl)-4-oxochroman-7-yl-5-methoxy-2- 4- Citrus Aurantium benzoate variants substituted

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Abstract

In this work, the antioxidant activity of a methanolic extract, isolated hesperitin and hesperidin, and a semi-synthetic hesperitin derivative from Citrus aurantium were assessed. Citrus aurantium orange peels are extracted using a Soxhlet apparatus to get a methanolic extract. This is followed by the separation of two essential flavonoids glycosides, hesperitin and hesperidin, and the synthesis of a novel hesperitin derivative. The 1,1-diphenyl-2-picryl hydroxyls (DPPH) scavenging of free radicals techniques were used to assess the antioxidant activity. The IC50 Value was used to assess the antioxidant potential. When compared to other derivatives, the isolated hesperitin Aa shown greater biological activity, while the semi-synthesised derivative (Aa-03) had strong antioxidant activity.

Keywords: Hesperidin, hesperitin, citrus aurantium, and antioxidant activity

1.Introduction

The outer layer of the Rutaceae family, whether fresh or dried, is orange peel. Citrus aurantium pericarp, one of the world's most significant citrus fruits [1,2]. Because of their eye-catching hues and delectable flavours, citrus fruits are grown all over the world and are mostly grown in tropical and subtropical climates. Globally, citrus fruits are produced to a total of 102 million tonnes. Citrus fruits are consumed in large quantities as nutritious food and juice, but the peel is most usually thrown away as trash since it contains a large number of secondary components with significant antioxidant activity in comparison to other fruit parts[3, 5].Although the bitter orange, or Citrus aurantium, has different characteristics, it is in line with the orange. Fruits are a rich source of substances known as phytonutrients, which have antioxidant qualities. Examples of these substances include ascorbic acid, flavonoids, carotenoids, phenolic acid, tocopherol, and compounds containing sulphur. Rutaceae, the family that includes citrus fruits, is one of the principal tree species that are planted worldwide. Citrus fruits are more abundant in bioactive substances including vitamin C, carotenoids, flavonoids, limonoids, essential oils, alkaloids, and minerals that are good for human health.[8,9] Non-nutritious plant compounds known as phytochemicals have anti- or preventive qualities against some illnesses. The majority of

phytochemicals contain antioxidant properties that guard against oxidative damage to our cells. lower the chance of getting certain cancers. About 90% of the volatile oil in bitter orange peel is limonene. Other ingredients include flavonoids, triterpenes, monoterpene, vitamin C, carotene, and citric acid. In traditional Chinese medicine, the peel of the immature fruit is used to treat diarrhoea, constipation, dysenteric vomiting, and indigestion [12,15].A polyphenolic bioflavonoid type of flavanone, hesperidin is widely distributed in the peel and membraneous sections of oranges and other citrus fruits. The natural ingredients included in citrus peels—sugars, flavonoids, volatile oils—are useful for both human health and the food industry. Orange peels are also a significant source of useful substances originating from plants and can be utilised as an antioxidant. Historically, leaves, fruit, bark, flowers, and roots have been utilised to treat illnesses. Citrus aurantium, commonly known as sour orange, Bigarade orange, Seville orange, or Narangam Tamil in Khatta, is a plant growing in India whose fruits are utilised for a variety of medical uses.[10–11] Even though bitter oranges are an excellent source of edible fruits, their unique medical properties have been recognized[18].

(A) Citrus Aurantium plant

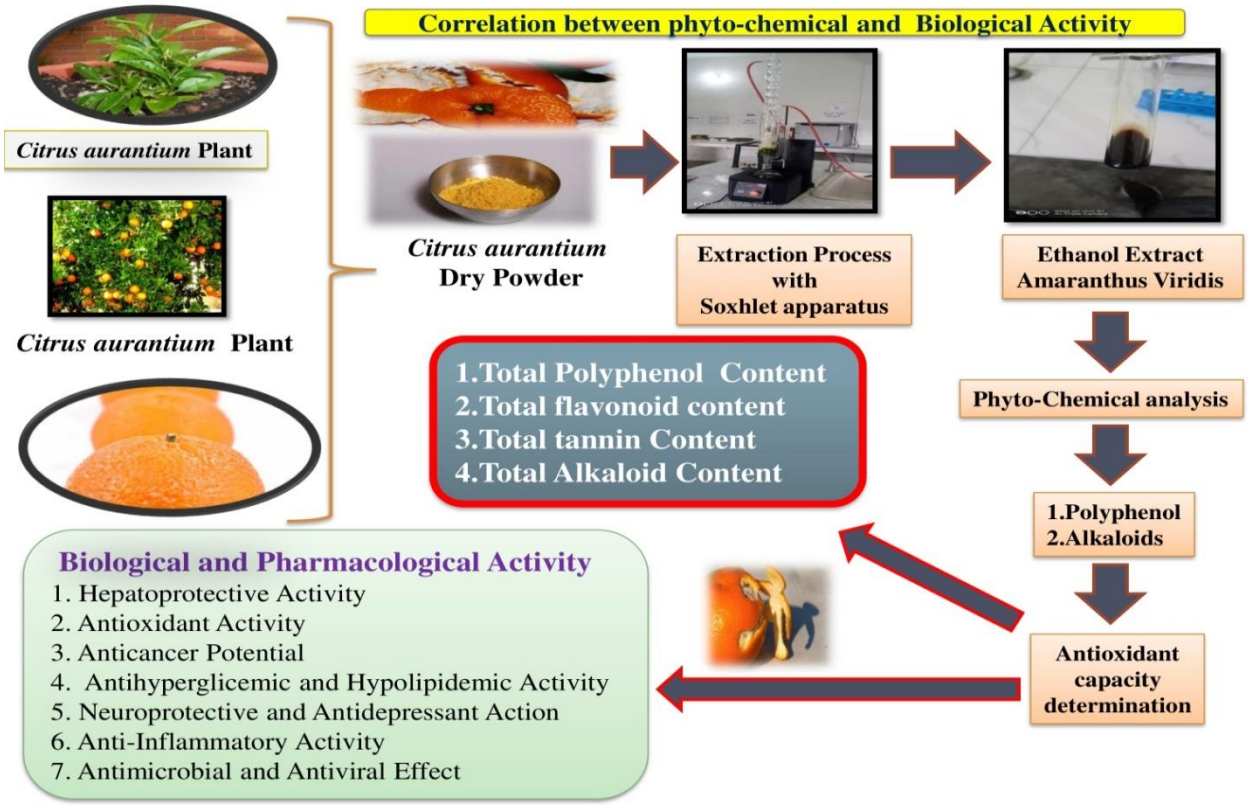
(B) Citrus Aurantium plant

(C) Citrus Aurantium plant

(D) Citrus Aurantium fruit

(E) Citrus Aurantium Dry peel

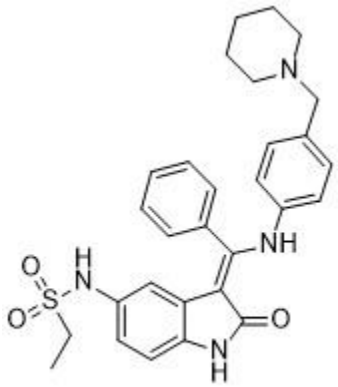
(F) Citrus Aurantium Dry peel Powder



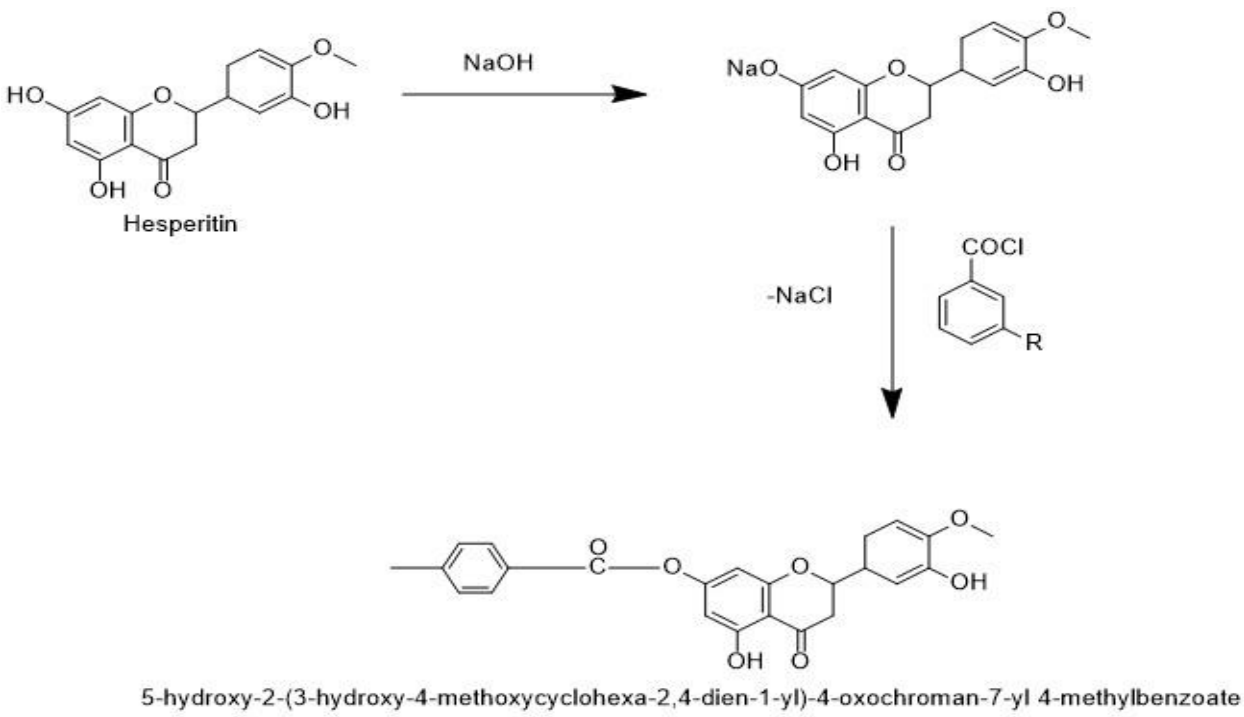
Hesperidin: Importance and use

Hesperitin-7-beta-rutinoside is the chemical compound known as hesperidin. Its aglycone is 4'-methoxy-3', 5,7-trihydroxy flavanones, and the C-7 position of the aglycone is connected to the sugar moiety rutinose. It is the main flavonoid found in sweet orange and lemon. Hesperetin, a disaccharide coupled to rutinose, aglycone, and flavanone form the glycoside hesperidin. One molecule each of glucose and rhamnose makes up the disaccharide unit [20]. The primary flavonoid of citrus species, it is mostly found in the peel and membranes of or Furthermore, it possesses a wide range of biological actions, such as reducing capillary fragility and having anti-inflammatory, antibacterial, antioxidant, and anti-carcinogenic properties[22].

Hesperitin



2.Scheme



5-hydroxy-2-(3-hydroxy-4-methoxycyclohexa-2,4-dien-1-yl)-4-oxochroman-7-yl 4-methylbenzoate

Types of substituted benzoate

Compound	**R**
C1	-OCH_3
C2	-Cl
C3	-OH

3.Material and method

3.1Collection of plant material

Citrus aurantium peels used in the current investigations were gathered from the local Raebareli, Uttar Pradesh, India market. NBRI, Lucknow, 226015, India, identified, verified, and authenticated the plant. (Reference No.: 2024-25/15 Authentication/LWG/PDSH)

3.2 Extraction of Crude Hesperidin

Orange peel that had been air-dried was extracted using a Soxhlet assembly and a variety of solvents, including n-hexane, water, chloroform, petroleum ether (40–60), and ethanol at a concentration of 95%.Different extracts' average extractive values were computed [21].

3.3 Procedure: Extraction of hesperidin from *Citrus aurantium*

400 ml of petroleum ether (40–60´C) mixed with 100 gm of powdered citrus aurantium peel, both on water and refluxed for one hour. Dry the marc at room temperature after filtering the contents through a Buchner funnel while it's still hot.The powder extract was refluxed for three hours with 400 millilitres of methanol. 50 cc of hot methanol is used to wash the marc and filter it while it's still hot. Include in the filtrate's washing process. After concentrating the mixed filtrate according to low pressure to produce a bulk extract that was syrupy; the additional moisture was removed and the extract was stored in desiccators for further isolation.

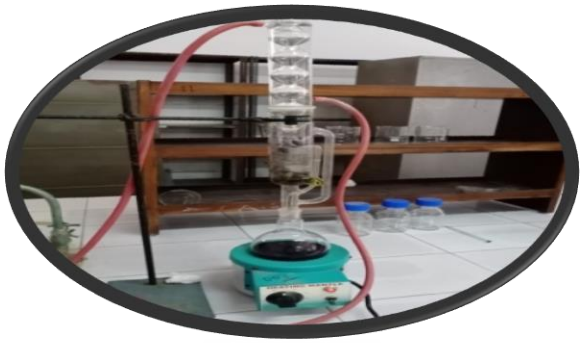
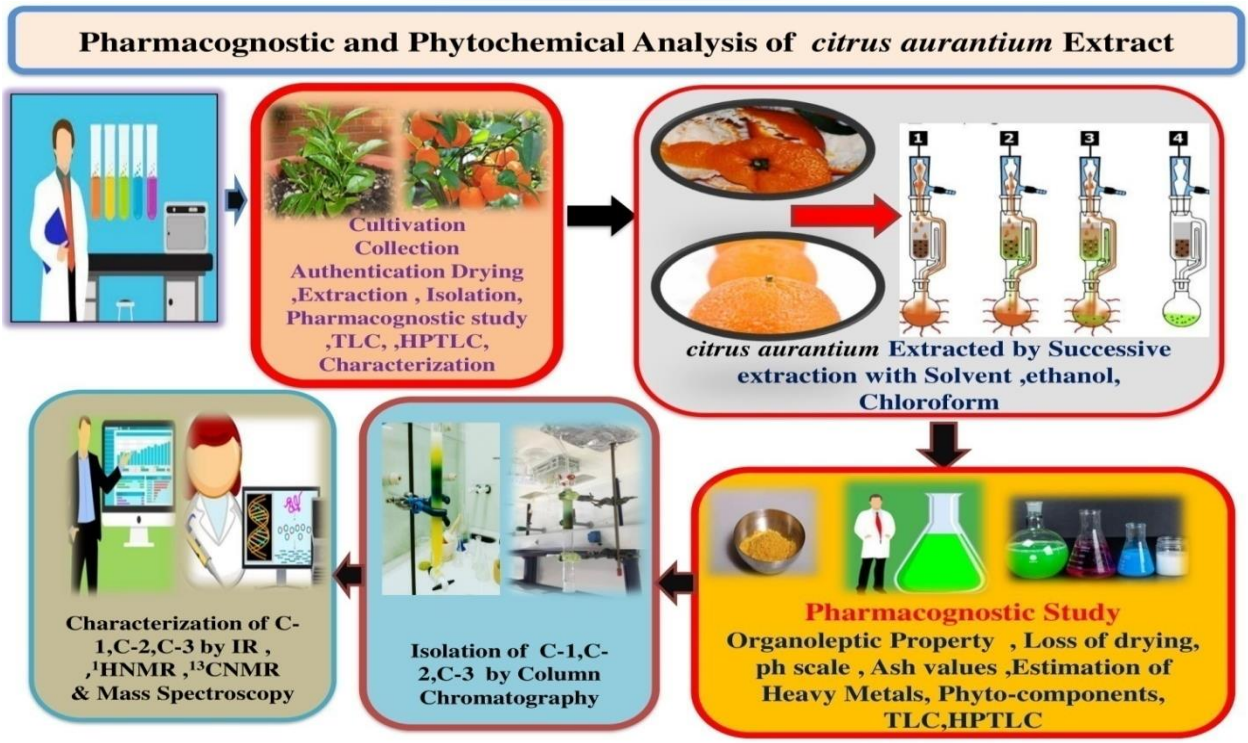


Fig 2: Extraction by Soxhlet Apparatus



3.4 Isolation of Phytoconsituents from the *Citrus aurantium*

Orange peel is dried & 50g of the fined powder is subjected to reflux with 200 ml of alcohol for 1.5 to 3 hrs. After the crude hesperidin was added to 7 ml of dimethylformamide then added acetic acid solution at 60 ^{0}C. The solution was filtered through a Buchner funnel and dilute with water allow to stand for 4hrs the Deabsorbed & extracted in to chloroform. White crystals of Hesperidin are separated out.

3.5 Conversion of hesperidin into hesperitin

Hesperidin, methanol, and concentrated sulfuric acid were stirred together and heated for 8 hours at reflux. After cooling and concentration, the homogeneous solution is diluted with ethyl acetate. The organic solution is washed and dried with magnesium suphate after being washed with water. Purification of hesperitin was achieved by dissolving the crude product in a small amount of acetone and then adding the standard solution to a vigorously stirred solution of water and acetic acid. Water is used to wash and cool precipitated hesperitin in an ice bath. Hesperitin is purified into a pure yellow powder.[7]

3.6 Recrystallization

Rarely are the solid organic components pure when separated after extraction. They are frequently tainted by other substances, or impurities, produced during the same procedure as the intended result. Typically, a suitable solvent or solvent combination in crystalline form is used to purify impure crystalline substances. Solids are purified via crystallisation, which takes advantage of differences in solubility in a particular solvent or combinations of solvents.

1. Dissolving the impure material in a solvent of appropriate boiling point.
2. Filtration to separate the solution from dust particles and insoluble contaminants.
3. Letting a reaction mixture cool down until the substance that has dissolved crystallises.
4. Extracting the crystals from the mixture and raising them to the surface in order to isolate them. Utilise technology or mother's wine. Following drying, the resultant solid is purified by TLC, spectroscopy, or melting point measurement. If impure, it is recrystallised using a new solvent. Until the chemical was entirely pure, the process was repeated.

3.7 Procedure for synthesis hesperitin derivatives

Hesperidin, methanol, and reflux-heated concentrated sulphuric acid were combined and heated for seven hours. Ethyl acetate is added to the homogenous solution after it has cooled and been

concentrated.After being cleaned with water, the organic solution is dried and cleaned with magnesium sulphate. Hesperitin was purified by first combining the raw material with a little amount of acetone, which and thenvigorously stirring a solution of water and acetic acid with the standard solution added. Hesperitin that has precipitated is washed and cooled with water in an ice bath. A pure yellow powder is obtained by purifying hesperitin.after the hesperitin response. Additionally, hesperitin reacted with sodium hydroxide and refluxed for two hours until the reaction was finished and formed intermediate-level substance. Subsequent intermediates create substituted derivatives when they react with benzyl chloride.
To create ester derivatives of (C-1) (S), hesperitin (0.01 mol) is dissolved in diethyl ether and refluxed with different phenolic (0.01 mol) acids.Methoxy -2-(3,4-dimethoxyphenyl) - 5-4-hydroxybenzoate (C-2) oxochroman-7-yl 4-hydroxybenzoate5-(3,4-dimethoxyphenyl) methoxy-2-(C-3) (S) 4-oxochroman-7-yl 4-methoxybenzoateNewly synthesised hesperitin derivatives, 2-(3,4-dimethoxyphenyl)-5-methoxy-4-oxochroman-7-yl 4-chlorobenzoate.

3.8 Identification of an isolated compound

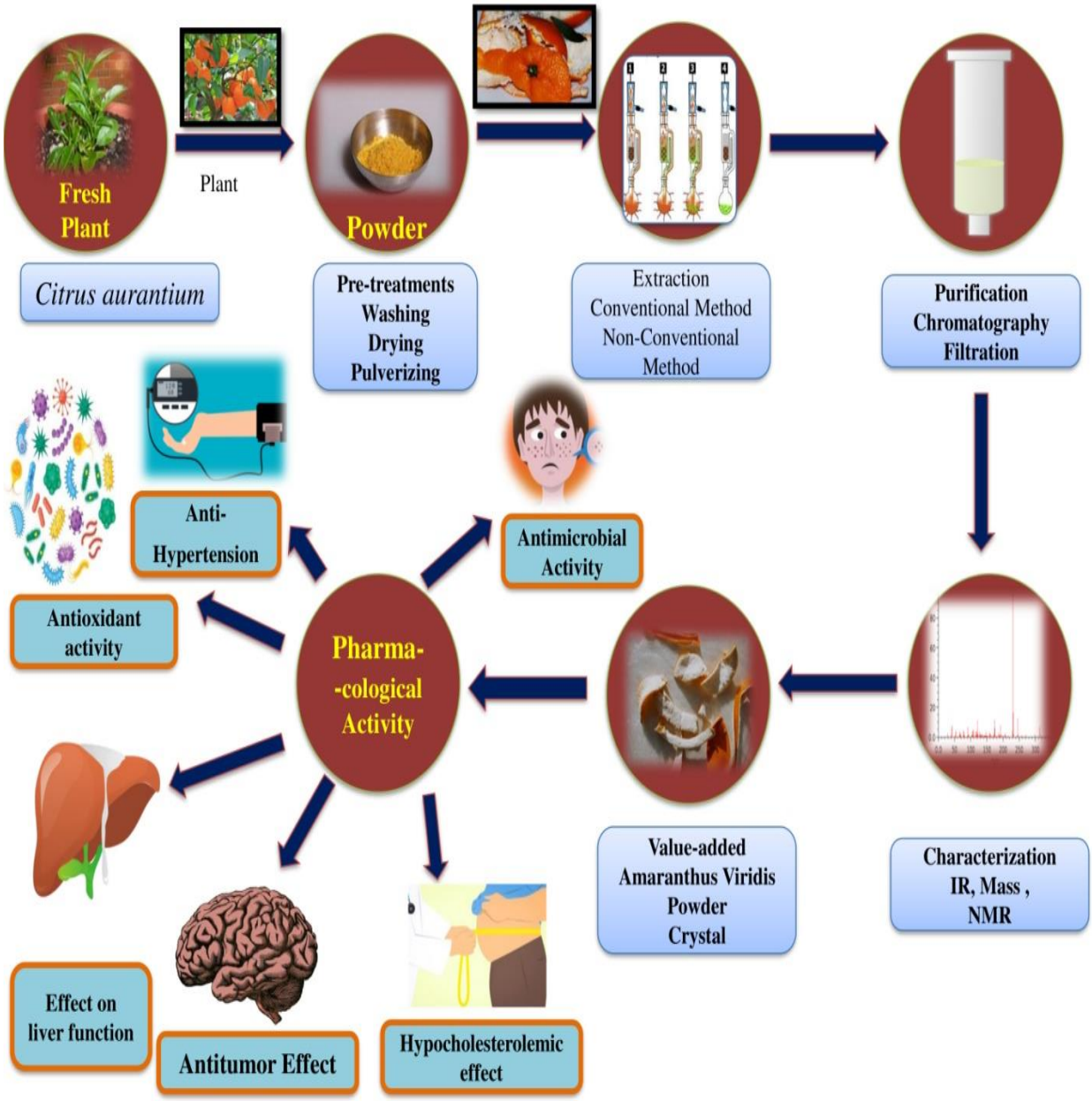
The following method was used to identify the isolated components from the citrus aurantium herbal extracts.

3.9 Determination of melting point range

Using an open capillary method and a melting point device, the compounds' melting points were determined. On one end, compounds were placed in a sealed capillary, and on the other, capillary-specific caves were constructed. There was a thermometer placed up in the caverns. The temperature range that separates a compound's initial melting point and its final melting point is known as its melting point range.

3.10 The creation of thin-layer chromatography (TLC) methods

The glass dishes with a silica gel G coating weresubjected to TLC. The adsorbent silica gel G was applied on 20 x 5 cm TLC plates that had been cleaned beforehand, with a conventional spreader, to a 0.3 mm thickness, roughly. After thirty minutes of baking at 110 degrees Fahrenheit in a hot air oven, the silicon plates were energised.. The compounds were put in a spot about 2 cm above the powered plate's lowest point. The mobile phases were chosen based on the items' polarity. -Butanol One kind of alcohol applied to the mobile stage was butanol. water, acetic acid, and butanol (3:1:1). Sports were photographed using a UV chamber (340 nm) after the mobile process had finished processing 3/4 of the plate. The Rf value was calculated.



3.11 Solubility studies

To dissolve the 10 mg extracted products in a 50 ml beaker, several solvents including water, toluene, methanol, ethanol, chloroform, acetone, n-hexane, and petroleum ether were utilised. Measurements for the isolated chemical were made.

Description of solubility

S.No.	Descriptive terms	Pergram of solvent, the approximate amount of the solvent in milliliters
1	Very soluble	Less than 1
2	Freely soluble	From 1 to 10
3	Soluble	From 10 to 30
4	Sparingly soluble	From 30 to 100
5	Slightly soluble	From 100 to 1000
6	Very slightly soluble	From 1000 to 10000
7	Insoluble or practically insoluble	More than 10000

3.12 Spectral analysis

3.13 UV spectral analysis

Compounds weighing 10 mg are dissolved in suitable solvents and then diluted to a 20 ml capacity using the same solvent. The resolution mentioned above was diluted from 2.0 mL to 50% capacity. The UV-Visible Spectrophotometer is used to collect UV spectra was employed.

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3.14 IR spectral analyses

IR of chemicals utilising the Perkina Elmer Spectrum RXI FTIR instrument using potassium bromide molecules. There were records of spectrums.

3.15 Preparation of KBr pellet of compounds

Precise measurements were made for After adding 1.0 mg of the chemical and 100 mg of anhydrous KBr (AR grade), the mixture was thoroughly triturated. The mixture was squeezed for five minutes at a pressure of five to six tonnes in an evacuated die. A lucid disc was used to record the IR spectra, and it was subsequently placed within a pellet container.

3.16 1H NMR spectral analysis

Using a Bruker Avance-300 NMR, 1H NMR spectra of several substances were recorded using TMS as a corporate benchmark. (parts per million of the chemical shift δ)

3.17 Biological activity of the isolated compound

3.18 Antioxidant Activity

The related extracts' potential forThe purple DPPH methanol solution was used to determine an atom of hydrogen or electron donation. The stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH, or) is the reagent used in this spectrophotometric assay.. One millilitre of a 0.1 mM methanol solution of DPPH in methanol was mixed with an effective result that ranged in concentration from 20, 40, 100, 200, and 400 μg/mL. The solution mixture's absorbance is calculated to be 517 nm after 30 minutes in the dark. The UV-VIS spectrophotometer (UV-1800, Shimadzu Corp., Japan) was used with a blank, 1 mL of DPPH solution.

3.19 Pharmacological studies

Antioxidant activities assay

On 1, 1-diphenyl-2-picryl Free hydrazyl (DPPH), radical scavenging action

Preparation of 0.004% w/w solution of DPPH

After After increasing quantity to a volumetric flask with a volume of 100 m and dissolved 4 mg of DPPH in 40 ml of methanol, the collection underwent incubation for 30 minutes in a dark environment. made a 0.004% DPPH solution.

Preparation of stand. ascorbic acid solution

S.No.	Compound Code	Molecular Weight	Molecular Formula	TLC Rf value	Melting point (°C)	Percentage Yield	Colour
1.	C-01	450.13	$C_{25}H_{22}O_8$	0.57	219	65	Yellowish
2.	C-02	468.88	$C_{25}H_{21}ClO_7$	0.61	225	67	Yellowish
3.	C-03	464.15	$C_{26}H_{24}O_8$	0.72	227	70	Yellowish

(S)- 3-(3,4-dimethoxyphenyl)-5-methoxy-2-oxochroman-7-yl C-1,4-hydroxybenzoate

Solubility data: Acetone, methanol, ethanol, ethylacetate,acetonitril, water

Spectral data:

FTIR (KBr, cm-1): 3509 cm-1 (O-H stre), 3055cm-1 (C-H stre (arene), 3600 (Are-OH Stre), 2947cm-1 (C-H stre, alkane), 2839 CH stre.(alkane-OCH_3), 1642 (C=O stre), 1612 (Ar-C=C stre), 1492 (C-C.(Aro), 1198 (-O- ether), 950(C-C stre. Ali), 750 C-Cl stre).

1H NMR (DMSO-d6) δ (ppm): -5.51-6.59 (m 4H, Ar-CH), 3.13- 3.38 (s, 2H CH_2),6.30 (s, 2H), 3.73 (s, 3H,CH_3), 6.88-7.97 (m, 4H, Ar-CH),7.97 (s, 1H, CH), 5.0 (Ar-OH), 3.73(6H, CH_3).

10 mg Ascorbic acid in 5 ml methanol and volume make up to the 10 ml volumetric flask.

Prepared 1 mg/ml solution of ascorbic acid.

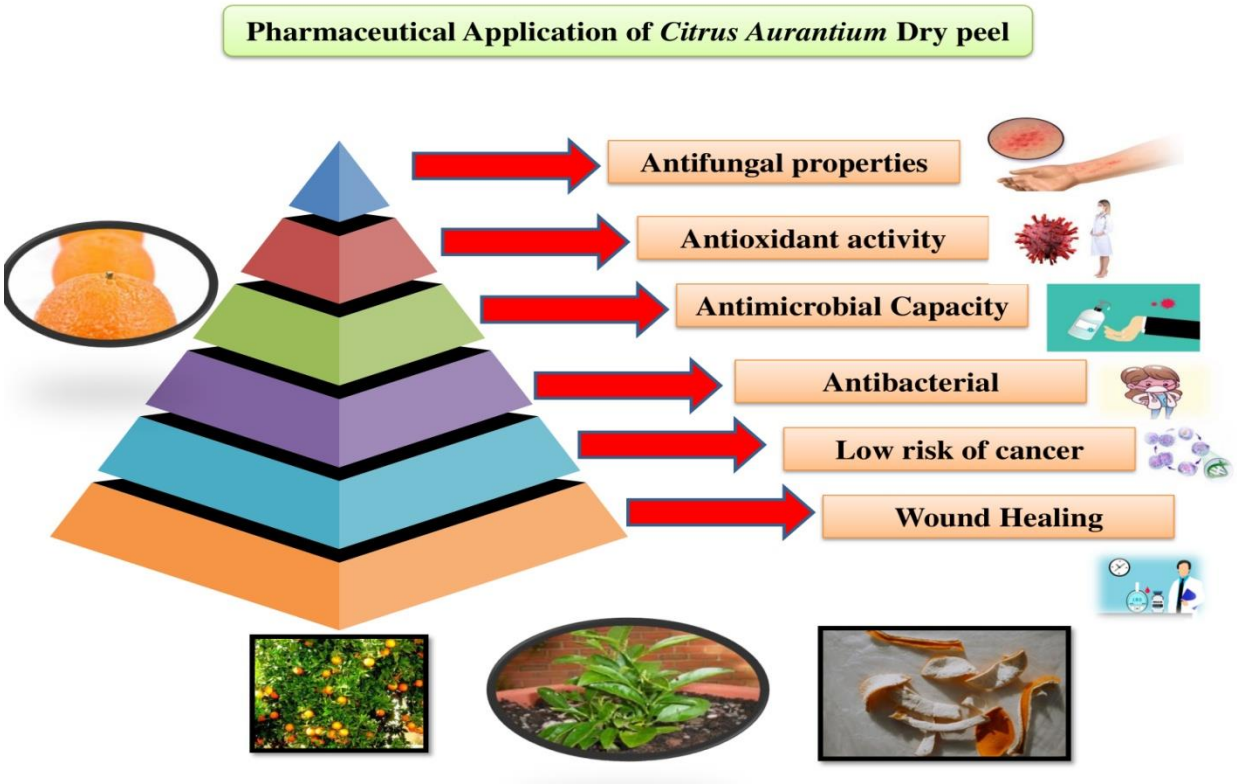
To begin, take five test tubes and divide the ascorbic acid concentrate into five aliquots (20, 40, 100, 200, and 400 µl/ml). Then, put the ascorbic acid concentrate into three millilitres of DPPH solution in a test tube.Repeat the process to isolate the chemical in the test tube, add 3 ml of DPPH, and incubate in a dark area for half an hour. Next, measure the absorbance at 517 nm using a UV-visible spectrophotometer. noted the observed value after observing the absorbance of the testing and standard solutions.

DPPH scavenge activity (%) Inhibition = Abs – Abc /Abs×100

Where,

Abs: Absorbance of blank sample

Abc: Absorbance of the sample



4.Result and discussion

Table 3: Physical Character of hesperidin and hesperitin

S. No	Parameter	Observation Hesperidin	Observation Hesperitin (A1)
1.	**Color**	Yellowish brown	Yellowish
2.	Odor	Characteristic and Aromatic	Characteristic and Aromatic
3.	Melting point range	242-244 ^{0}C	226 ^{0}C
5.	TLC:-n-butanol: Acetic acid: Water (4:1:5**)**	0.61	0.72

S.No	Compound Code	Colour	Odour	Taste
1.	C-1	Yellowish Colour	Aromatic	Bitter or pungent
2.	C-2	Yellowish Colour	Aromatic	Bitter or pungent
3.	C-3	Yellowish Colour	Aromatic	Bitter or pungent

2-(3,4-dimethoxyphenyl)-5-methoxy-2-oxochroman-7-yl Chlorobenzoate (4-chloro)
Solubility data: Acetone, methanol, ethanol, ethylacetate,acetonitril, water

Spectral data:

FTIR (KBr, cm-1): 35091cm-1 (O-H stre), 3055cm-1 (C-H stre (arene), 2947cm-1 (C-H stre, alkane), 2839 CH stre.(alkane-OCH_3), 1642 (C=O stre), 1612 (Ar-C=C stre), 1492 (C-C.(Aro), 1198 (-O- ether), 950 (C-C stre. Ali), 750C-Cl stre).

1H NMR (DMSO-d6) δ (ppm): 5.51-6.59 (m 4H, Ar-CH),3.13- 3.38 (s, 2H CH_2), 6.30 (s, 2H), 3.73 (s, 3H,CH_3), 7.42-8.08 (m, 4H, Ar-CH), 3.73 (s, 6H, CH_3). 3.37(s, 3H,CH3), 3.36 (s, 3H, CH3), 3.30 (s, 3H, CH3(S)- 3-(3,4-dimethoxyphenyl)-5-methoxy-2-oxochroman-7-yl C-3, 4-methoxybenzoate

Solubility data

Freely soluble**:** Water, acetic acid, ethanol, methanol, ethylacetate

Spectral data

FTIR (KBr, cm-1): 35091cm-1 (O-H stre), 3055cm-1 (C-

H stre (arene), 2947cm-1 (C-H stre, alkane), 2839 CH stre.(alkane-OCH_3), 1642 (C=O stre), 1612 (Ar-C=C stre), 1492 (C-C.(Aro), 1231(-OCH_3 stre), 1198 (-O- ether), 950 (C-C stre. Ali).

1H NMR : 1H NMR (DMSO-d6) δ (ppm): -5.51-6.59 (m4H, Ar-CH), 3.13- 3.38 (s, 2H CH_2), 6.30 (s, 2H), 3.73 (s,3H,CH_3), 3.73-8.03 (m, 5H, Ar-CH), 3.73 (s, 9H, CH_3)

4.1 Antioxidant activity

demonstrated the isolated chemical (A1-01) (S)-5-methoxy -2-(3,4-dimethoxyphenyl)'s inhibitory concentration.To scavenge 50% of DPPH free radicals, -4- oxochroman-7-yl 4-hydroxybenzoate is needed (IC50 in μg/mL). Even so, the isolated chemical as a whole demonstrated the potential to scavenge free radicals. The chemical known as (C-1) (S)- 3-(3,4-dimethoxyphenyl)-5-methoxy-4-oxochroman-7-yl 4-hydroxybenzoate was discovered to have an IC50 value of 2.15 to 35.34 μg/ml, indicating a high level of DPPH free radical scavenging capacity. The least effective compound of hesperitin for scavenging free radicals was isolated. The typical ascorbic acid compound exhibited a significant level of DPPH scavenging activity as well. acid ascorbic.and the isolated chemical had high IC50 values but a relatively limited capacity to scavenge DPPH radicals. The capacity to scavenge DPPH radicals varied significantly between species and cultivars.

4.2 Evaluation of antioxidant activity

Results for antioxidant activity of the hesperitin test from the ascorbic acid standard by DPPH method.

DPPH.Scavenging activity (%) Inhibition = [(Abs – Abc)/Abs]×100

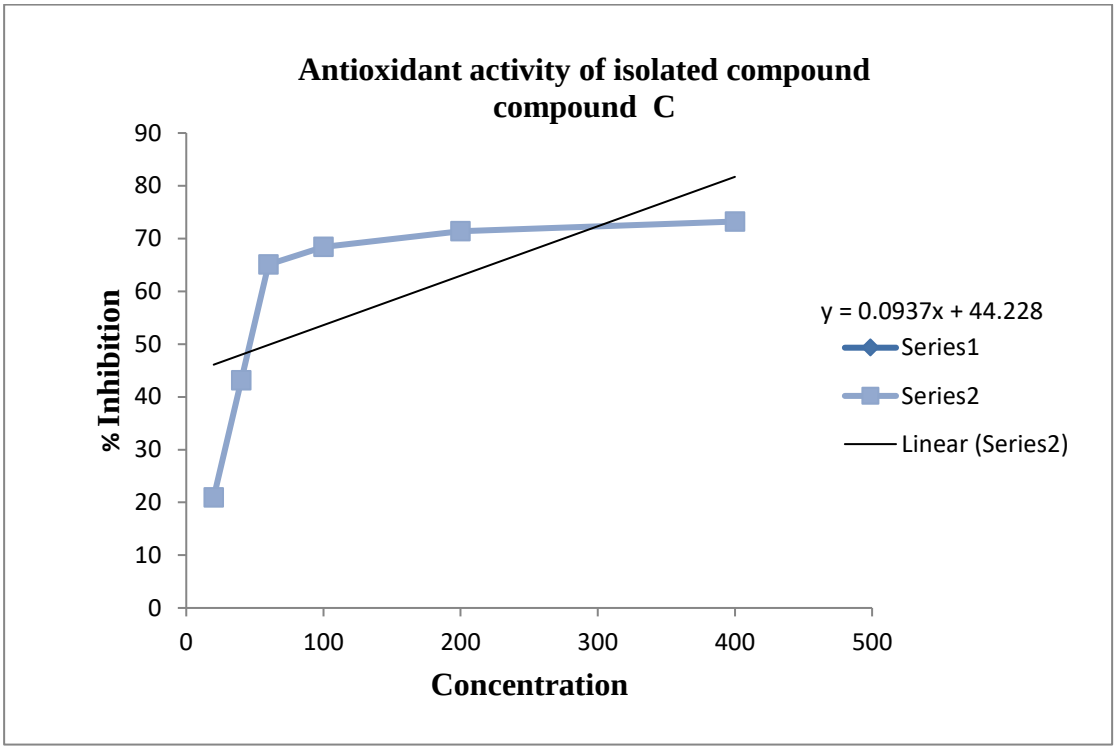
Where, Abs = absorbance of blank sample Abc = absorbance of sample

Table 6: Compound-C

S.No.	Concentration µg /ml	Absorbance (std)	% Inhibition
1	20	0.278	72.52
2	40	0.210	80.4
3	60	0.115	88.63
4	100	0.017	98.32
5	200	0.010	99.0
6	400	0.008	99.02

Table 7: percentage Inhibition antioxidant activity of Ascorbic acid (Standard

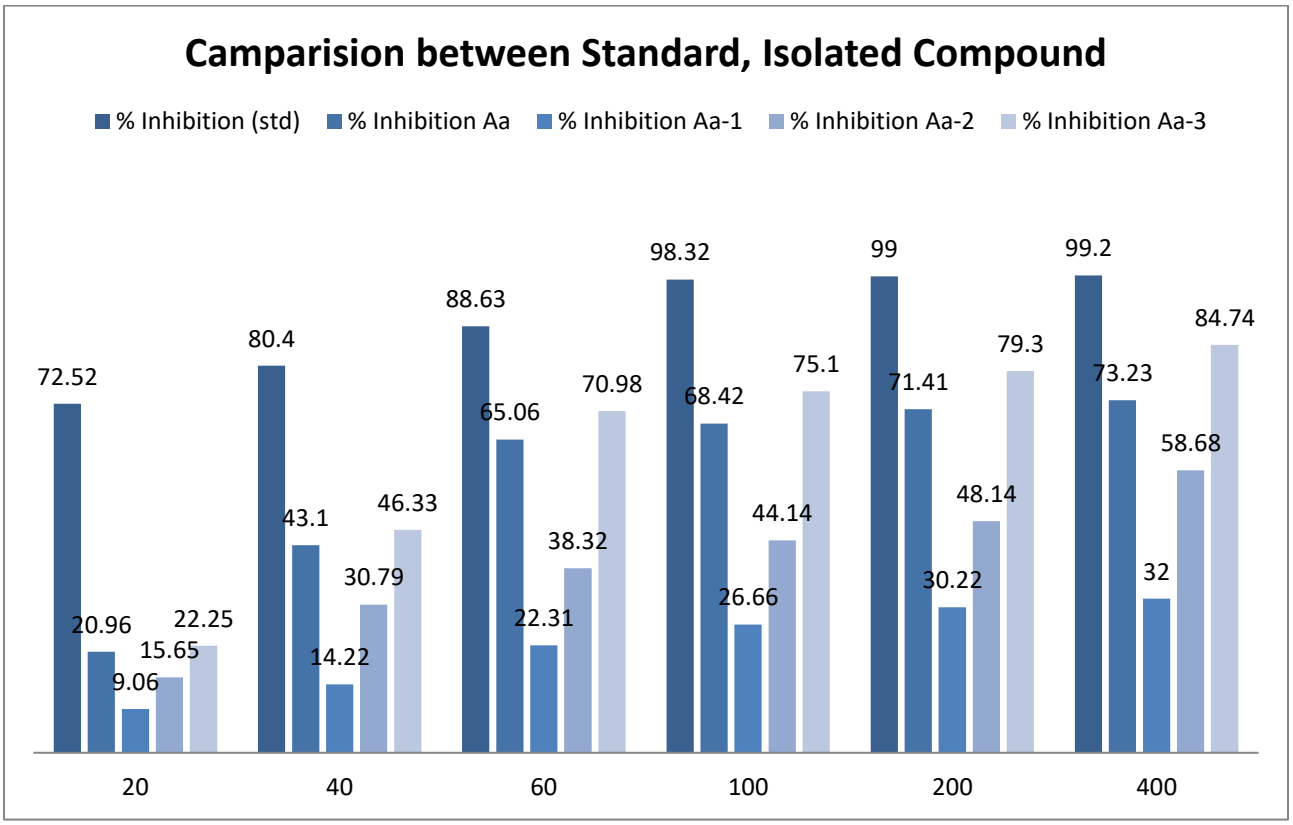
S. No.	Concentration µg /ml	Absorbance(std)	% Inhibition	IC50 (µg/ml)
1	20	0.871	20.96	
2	40	0.627	43.10	
3	60	0.385	65.06	65.15
4	100	0.348	68.42	
5	200	0.315	71.41	
6	400	0.295	73.23	



Graph 1: The graph shows the derivative (C) compound increased antioxidant activity with increasing conc.

Table 3: Evaluation of the norm, separated compound's and its analogues' antioxidant properties

Concentration (µg/ml)	20	40	60	100	200	400
% Inhibition (std)	72.52	80.4	88.63	98.32	99	99.2
% Inhibition C	20.96	43.1	65.06	68.42	71.41	73.23
% Inhibition C-1	9.06	14.22	22.31	26.66	30.22	32
% Inhibition C-2	15.65	30.79	38.32	44.14	48.14	58.68
% Inhibition C-3	22.25	46.33	70.98	75.1	79.3	84.74



Graph 2: The graph shows that high activity in ascorbic acid (Std) then isolated compound (C), and its derivatives (C-1, C-2, C-3)

Conclusion

In this work, the antioxidant activity of a methanolic extract, isolated hesperitin and hesperidin, and a semi-synthetic hesperitin derivative from Citrus aurantium were assessed. Citrus aurantium orange peel was extracted using a soxhlet apparatus to yield a methanolic extract. This was followed by the isolation of two essential flavonoids glycosides (hesperitin and hesperidin), which were extracted and isolated from the orange peel. The results were described by chemical tests such as melting point and thin layer chromatography (TLC). The IC50 Value was used to assess the antioxidant potential. When compared to derivatives, the isolated hesperitin (C) exhibited stronger antioxidant activity.demonstrated the isolated compounds C-1, C-2, and C-3's inhibitory concentrations needed to scavenge 50% of DPPH free radicals (IC50 in µg/mL). Even so, the isolated chemical as a whole demonstrated the potential to scavenge free radicals. The isolated compounds C-1, C-2, and C-3 were found to have an IC50 value of 15.65 to 58.68 µg/ml, or 22.25 to 84.74, indicating a strong capacity to scavenge DPPH free radicals. The least effective compound of hesperitin for scavenging free radicals was isolated. The typical ascorbic

acid compound exhibited a significant level of DPPH scavenging activity as well. With high IC50 values, ascorbic acid and the isolated chemical demonstrated comparably limited DPPH ability to scavenge radicals. The ability to scavenge radicals DPPH levels varied significantly between types and hesperitin species.the separation and synthesis of a novel derivative of hesperitin. The free radical 1,1-diphenyl-2-picryl hydroxyls (DPPH) was used to measure the antioxidant activity.strategies for scavenging activities. The IC50 Value was used to assess the antioxidant potential. Hesperitin (C) that has been isolated and its semi-synthesised derivatives, C-1 and C-2, have less powerful antioxidant action than derivative (C-3). When compared to other derivatives, the isolated hesperitin derivative (C-3) demonstrated strong antioxidant activity.

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