RESULT AND DISCUSSION

5.1 Moisture content

Five grams of walnut kernel septum membranes was weighed into a dish. For two hours, the plate was dried at 102 C. Until a steady weight was achieved, this drying process was repeated. The mass loss compensated for a walnut kernel septal membrane yielded a moisture content of 15.9%. The moisture level of the walnut kernels was low. Since low moisture satisfied lowers the likelihood of microbial growth and numerous unwanted biochemical variations typically related with these processes, it is essential for maintaining the quality and shelf life of kernels.

Table No.5.1 Result of evaluation parameters of F1 to F5

Sr. No.	Parameter	F1	F2	F3	F4	F5
1	Boiling Point	87	82	90	78	72
2	Density	0.9216	0.9217	0.9215	0.9217	0.92218
3	Specific gravity	0.919	0.927	0.920	0.929	0.923
4	Viscosity	72.8	73.7	75.4	73.5	73.6
5	pH Determination					
	pH meter	6.2	6.8	7.0	6.1	6.8
6	Organoleptic Characters					
	1) Color	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown
	2) Odor	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic
	3) Taste	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
	4) Appearance	Good	Good	Good	Good	Good

Table No. 5.2 Result of evaluation parameters of F6 to F10

Sr. No.	Parameter	F6	F7	F8	F9	F10
1	Density	0.9215	0.9217	0.92218	0.9216	0.9217
2	Specific gravity	0.928	0.919	0.924	0.921	0.925
3	Viscosity	75.4	73.5	73.6	72.8	73.7
4	pH Determination					
	b) pH meter	7.0	6.1	6.8	6.8	6.2
5	Organoleptic Characters					
	1) Color	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown
	2) Odor	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic
	3) Taste	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
	4) Appearance	Good	Good	Good	Good	Good

5.2 DETERMINATION OF λ MAX OF DRUG:

Determination of λmax of walnut septum membrane solution was done in Phosphate Buffer pH 7.4 solutions. Spectrums obtained for individually showed constant peak i.e. wavelength of maximum at 360 nm.

5.3 CONSTRUCTION OF CALIBRATION CURVE:

The absorption spectrum of UV showed λmax to 360 nm. The standard curves of walnut septum membrane solution in pH 7.4 Phosphate Buffer gotten are shown in Figure. The graph of absorbance v/s concentration for walnut septum membrane solution was create to be linear in the concentration range of 0, 5, 10,15, 20, 25, 30,

35 and 40 µg/ml at 360 nm. The walnut septum membrane solution obeys Beer - Lambert's law in the range of 0 – 40 µg/ml.

Table. No. 5.3: Standard Graph of walnut septum membrane solution

Sl. no.	µg/ml Concentration of Drug.	360 nm Absorbance
1.	0.	0.
2.	5.	0.109.
3.	10.	0.221.
4.	15.	0.338.
5.	20.	0.448.
6.	25.	0.562.
7.	30.	0.672.
8.	35.	0.791.
9.	40.	0.901.

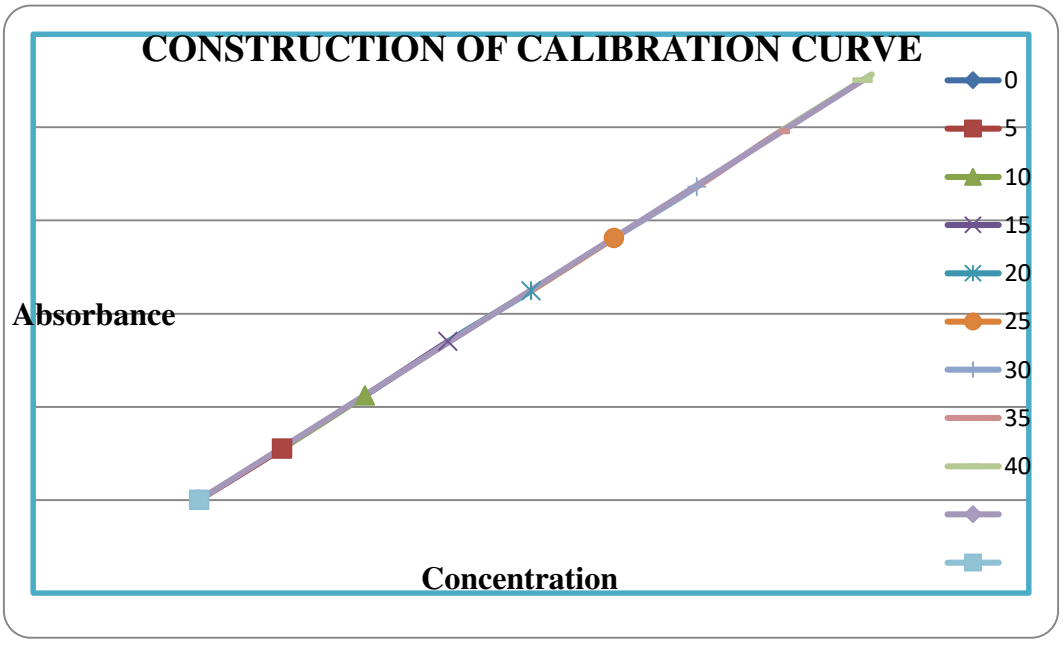


Fig. No. 5.1 : Standard Calibration Curve for walnut septum membrane solution

Table No. 5.4: Different formulation in Phosphate Buffer pH 7.4 (In- vitro Drug Release Profile)

Time (Min) .	Percent release of walnut septum membrane solution									
	F1.	F2.	F3.	F4.	F5.	F6.	F7.	F8.	F9.	F10.
0.	0.	0	0.	0.	0	0	0.	0.	0.	0.
5	23.9	02.23	21.22	09.11	05.22	14.05	05.91	05.72	04.20	3.00
10	33.51	3.00	32.32	11.31	08.32	20.14	09.21	10.20	09.34	03.10
15	44.23	05.03	40.91	17.12	12.91	31.72	12.34	16.50	12.45	05.03
20	55.47	08.76	53.21	27.41	18.21	45.13	17.50	32.50	16.75	07.76
25	69.21	11.07	62.25	34.54	21.22	52.59	24.20	41.60	32.50	12.07
30	77.69	16.72	74.61	49.14	32.32	64.17	28.70	52.80	41.60	17.72
35	86.61	25.65	85.29	56.27	40.91	74.77	34.50	60.75	52.80	24.65
40	97.41	36.04	92.11	67.11	53.21	81.15	47.25	72.80	60.75	37.04

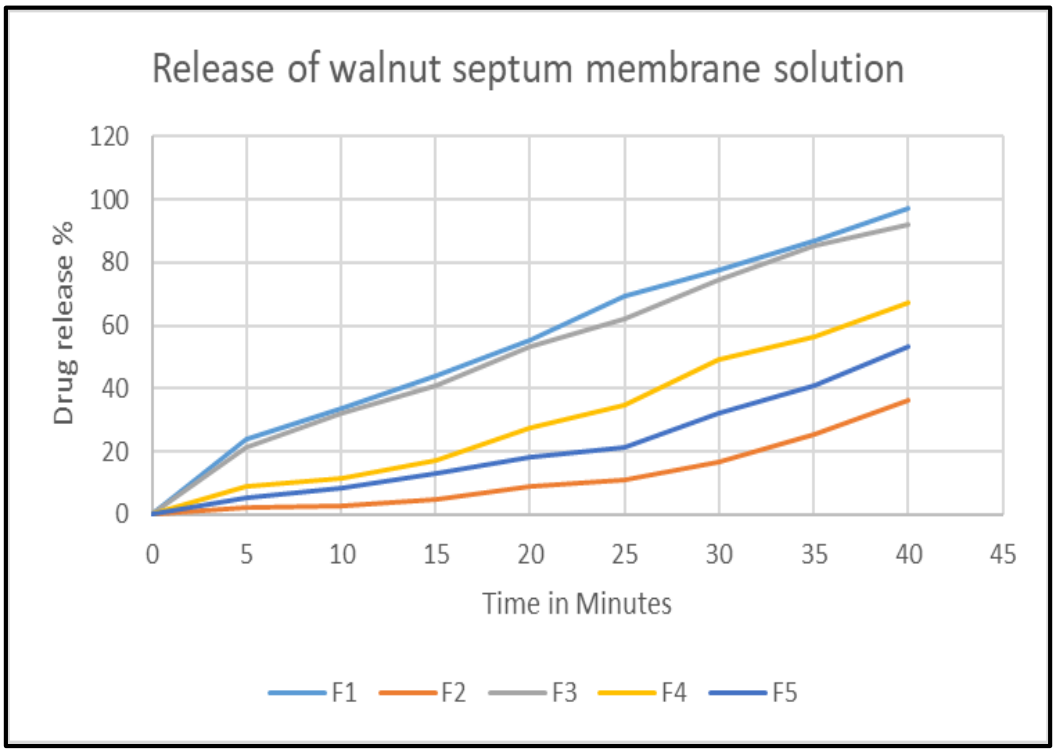


Figure No. 5.2 Percent release of walnut septum membrane solution F1 to F5

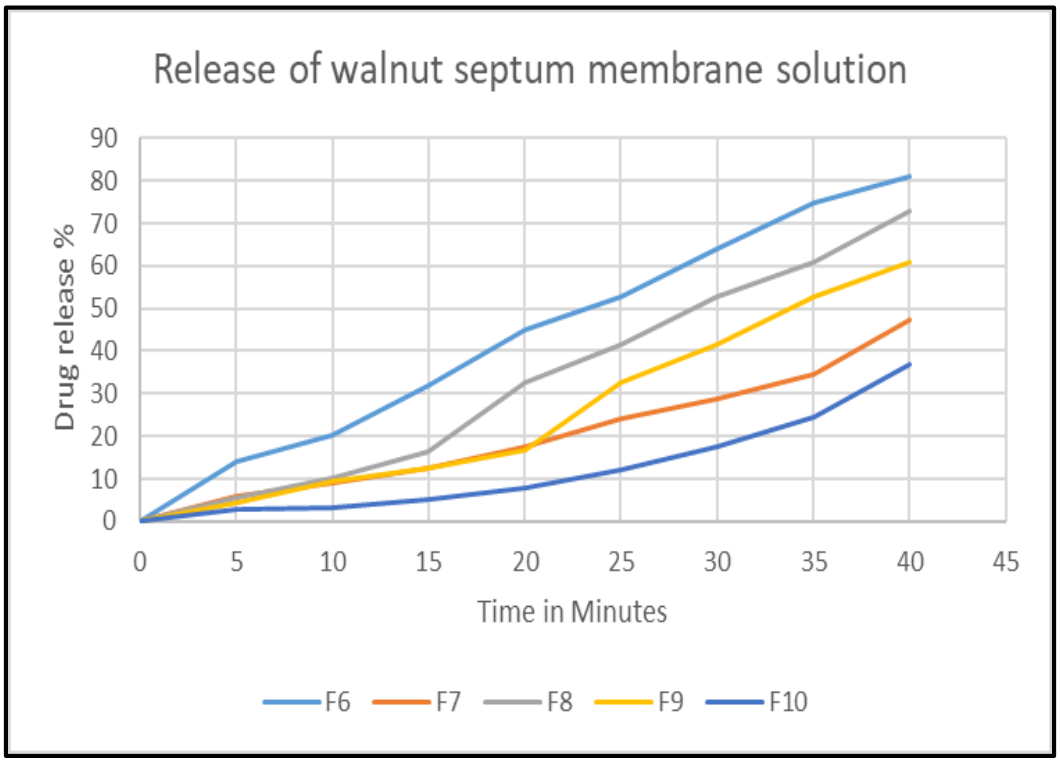


Figure No. 5.2 Percent release of walnut septum membrane solution F6 to F10

5.4 In-Vitro Drug release:

The table displays the drug release information for each walnut septum membrane solution. For forty minutes, the medication was released via the walnut septum membrane solution (formulations F1 through F10). At the end of 40 minutes, there was a noticeable difference in the drug release for all formulations F1 through F10. After 40 minutes, formulation F1 had the maximum release rate (97.41%) while formulation F10 had the lowest release rate (36.04%). When compared to other walnut septum membrane solutions, the three formulations (F4, F5, and F6) provide superior release out of the ten walnut septum membrane solutions (F1 to F10). According to research on drug content and osmosis, the F1 formulation walnut septum membrane solution release exhibits the optimum drug content and performance.

1) HPTLC analysis

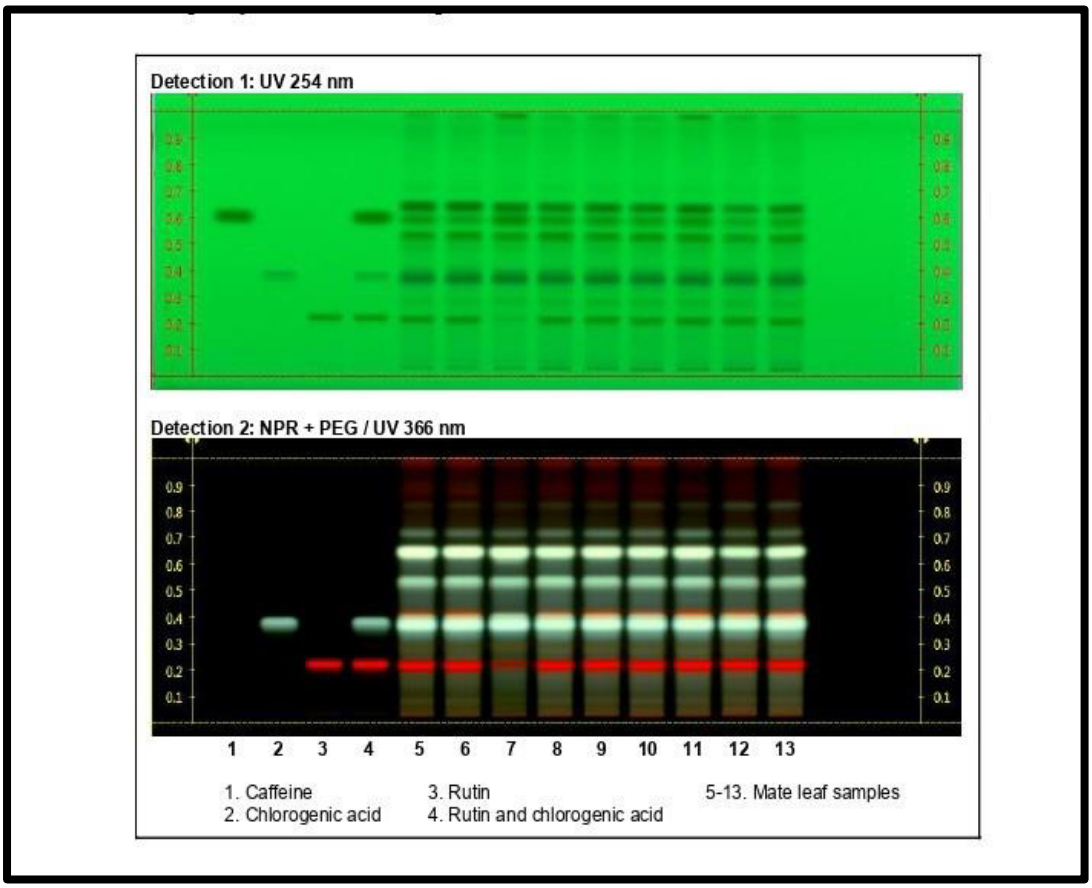


Fig. No. 5.3. Chromatography spots with HPTLC

The walnut kernel septum membranes solution, At 1740C, it had a sharp melting nature. The compound's mass spectrum revealed a molecular ion peak at m/z 173 [m-1] +, indicating that C10H6O3 is the molecular formula. Absorption bands were seen at 3444 cm-1 (-O-H stretching), 1717, 1653 cm-1 (α-β unsaturated ketone), 1644 cm-1 (double bond), and 1575, 1487 cm-1 (aromatic stretching) in the infrared radiation spectrum. At 423 nm, the UVVIS spectrum has λ max assigned. NMR spectra of the 1H and 13C were likened to those of the real material.

Method Development and Validation

Method Development and Validation The flavonoid profile of walnut septum was measured using an HPLC-pater association that was created and validated. All logical parameters, such as estimation angles, direct range, determined portions (r2), delicacy and perfection, limits of discovery (LODs), and restrictions of quantification (LOQs), are shown in Table. When subjected to the lack-of-fit examination, the logical angles showed a good fit (F computed was lower than F tabulated in every case), with r2 exceeding 0.99, indicating their suitability for flavonoid measurement. The LODs were planned across the range of 0.10 µg/g to 0.30 µg/g, whereas the LOQs were set up to range between 0.30 µg/g and 0.90 µg/g. Acceptable elicacy was demonstrated by the RSD of the between-day (n = x 3) and within-day (n = 6) assays, which were independently less than 6.2 and 8.5. The consequences were respectable, ranging from 90.8 (apigenin, at 10 µg/ g attention position) to 97.5 (catechin, at 10 µg/ g attention position) for within-day assay (n = 6), and from 88.5 (myricetin, at 1 µg/ g contemplation position) to 96.2 (catechin, at 5 µg/ g contemplation position) for between-day assay (n = 3 × 3). The delicacy was measured using relative proportion of recovery (R) at low, medium, and maximum attention ituations of 1, 5, and 10 µg/g.

Ftab: Ftabulated., Fcalc.: Fcalculated., LOD: limit of detection., LOQ: limit of quantitation.

Table No. 5.5 HPLC-DAD method analytical parameters.

Compound.	Calibration Equation y = (a±Sa) . + (b±Sb)x. (Linear Range: 1–10 µg/g)	r^2	F_{calc}	F_{tab}	LOD µg/g	LOQ µg/g
Apigenin.	Y. = (1732± 152) + (1745± 665)x	0.994	4.6×10^{-7}	0.2334	0.29	0.87
Catechin.	Y. = (1095 ±1115) + (11808 ± 305)x	0.997	7.9×10^{-9}	0.2334	0.31	0.94
Kaempferol.	Y. = (1710 ± 54.3) + (19045 ± 685)x	0.996	1.7×10^{-9}	0.2334	0.29	0.90
Luteolin.	Y. = (1017 ± 1608) + (17008 ± 440)x	0.995	2.9×10^{-9}	0.2334	0.20	0.60
Myricetin.	Y. = (989 ± 1450) + (20005 ± 424)x	0.993	5.6×10^{-9}	0.2334	0.24	0.72
Quercetin.	Y. = (−1032 ± 1128) + (18404 ± 153)x	0.993	6.5×10^{-9}	0.2334	0.20	0.60
Rutin.	Y. = (389 ± 1200) + (19857± 204)x	0.995	1.9×10^{-9}	0.2334	0.20	0.60

Table No. 5.6 %Recoveries (%R, *n* = 6) for the evaluation of repeatability.

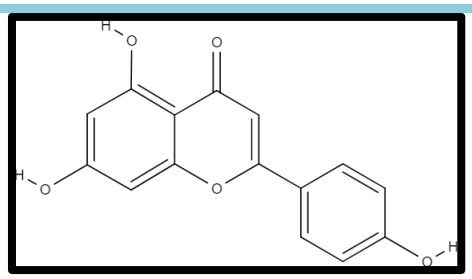
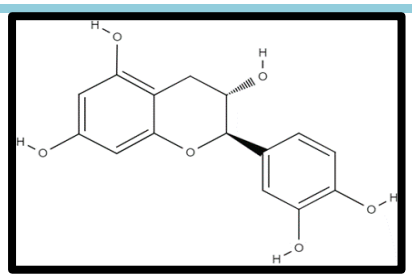
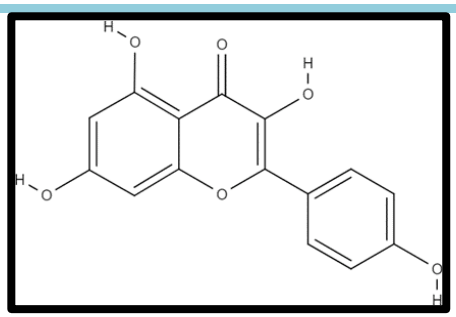
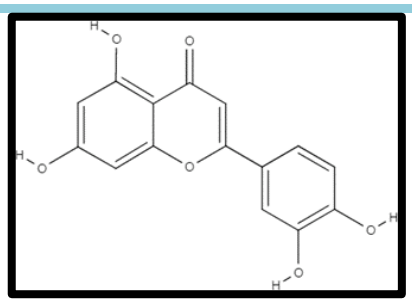
Compound	%R Medium. Conc. Level. (5. µg/g)	%RSD.
Apigenin	91.7	6.1
Catechin	96.4	6.2
Kaempferol	93.5	3.2
Luteolin	95.6	4.6
Myricetin	94.4	5.2
Quercetin	98.8	4.2
Rutin	92.5	4.5

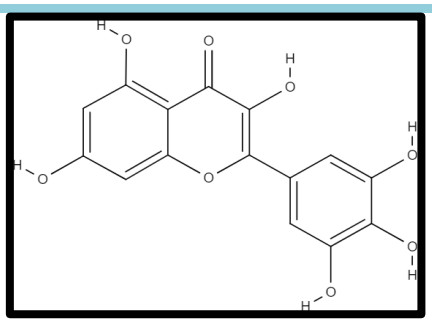
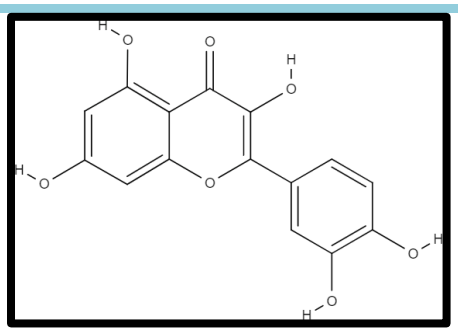
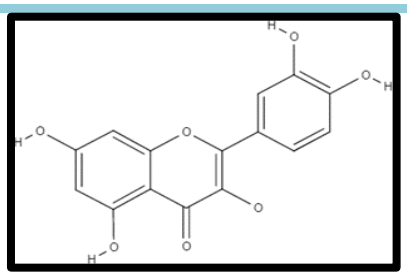
Conc.: Concentration.

Analysis of walnut Septum

walnut septum membranes solution was examined three times, and the flavonoids that were found were rutin., myricetin., luteolin., quercetin., apigenin., and kaempferol. Table displays the findings of the chromatographic documentation, counting the maximum absorption wavelengths (λ, nm) and retention durations (Rts) of the analytes. The flavonoid chromatographic separation in a walnut septum extract that was experienced at 280 nm is shown in Figure.

Table No. 5.7 Maximum absorption wavelength of the determined flavonoids

Compound	Chemical Structure	Rt	λ (nm)
Apigenin		24.5	360
Catechin		5.8	278
Kaempferol		26.1	360
Luteolin		21.1	356

Myricetin		16.5	370
Quercetin		22.7	378
Rutin		10.1	353

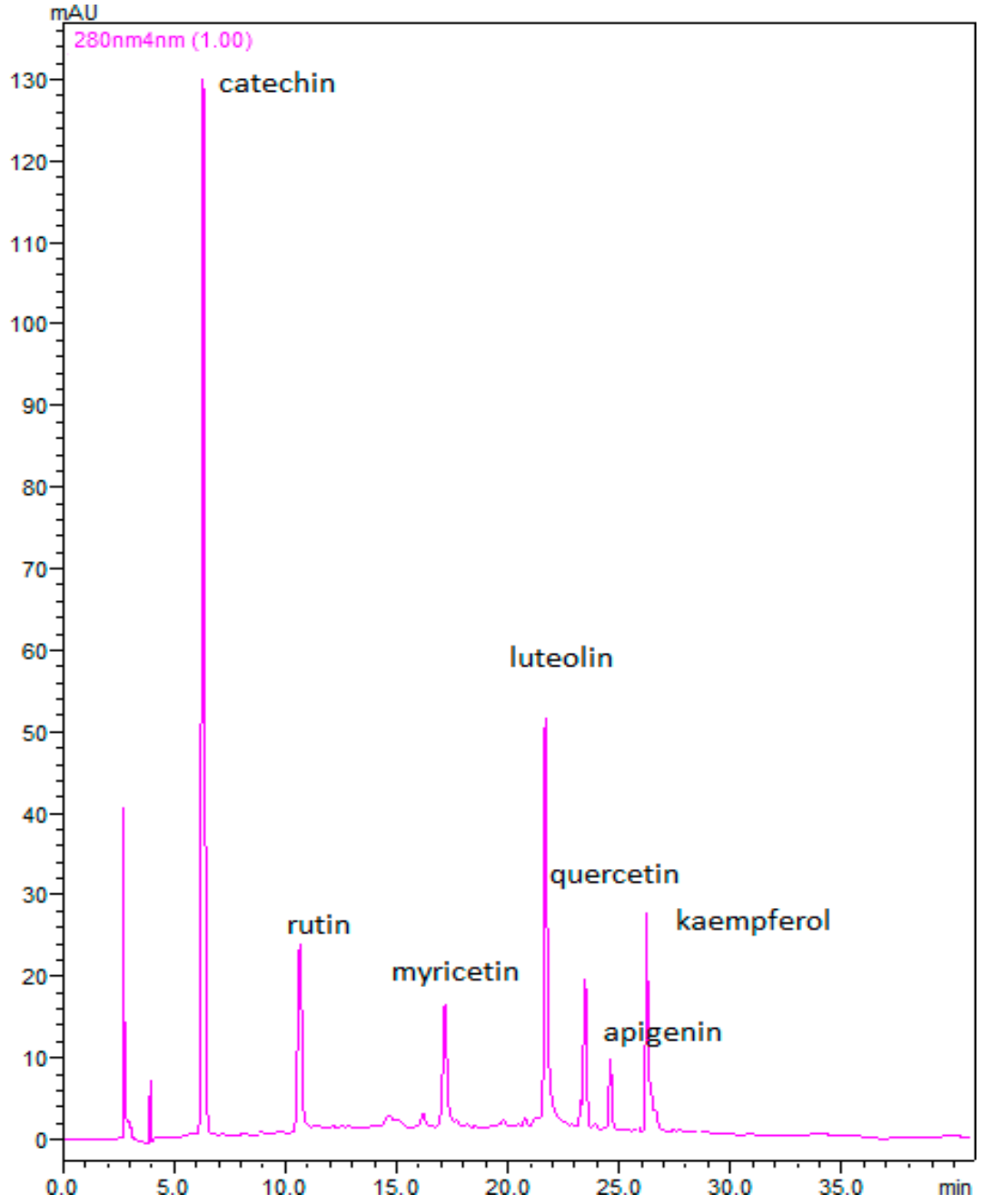


Figure No. 5.4 A walnut septum extract's characteristic chromatogram, measured at 280 nm.

1. Apigenin

The mean attention of walnut septa was found to contain 6 µg/g of apigenin. According to the ANOVA analysis, there was no statistically significant difference ($p = 0.147$) between the apigenin attention in the various forms of septa that were analyzed. In the past, apigenin-rich extracts, oils, and teas were utilized for their sedative properties as a mild painkiller, anesthetic, and sleep aid, supporting the

notion that walnut septa might be utilized as an infusion wood. Moreover, apigenin plays a role in food sedulity as a seasoning or adjuvant ingredient, strengthening the body's reaction to antigens. It may produce anti-metastatic and anti-proliferative products, reducing conformation and transforming undesirable excrescence cells. Likewise, it functions as an antioxidant, anti-inflammatory, and may even help prevent skin or colon cancer, it could help skin or colon cancer and acts as an anti-inflammatory., antioxidant., anti-allergic., antimicrobial., antiviral., cardio defensive, and neuroprotective agent.

2. Catechin

The flavonoid found in advance in all walnut septa was called catechin, and its concentration was 32 µg/g. Altogether of the walnut septa of the various varieties showed a statistically significant variation in catechin attention, as indicated by the ANOVA analysis ($p = 0.04$). The walnut septum is rich in catechin, as indicated by the attention ranges. It can be used as a food accretive to boost the Anti-Oxidant potential of food products, enhance foods, or serve as a functional component of newly developed practical food products, such as teas, infusions, soft drinks, etc. Catechins have been linked to a number of health benefits because they have shown promise as preventative measures against diabetes., obesity., metabolic disorders., arterial hypertension., and ischemic stroke. Additionally, catechins show a strong natural defense against bone cancer, Alzheimer's disease, Parkinson's disease, and mouth cancer. Similarly, catechin has special properties that account for a number of pharmaceutical and natural products since it can prevent chain reactions brought on by ROS, which makes it a potential antioxidant. Additionally, it exhibits strong anti-diabetic properties through hepato-defensive, anti-neurodegenerative, insulin-epigonic, and amyloid conformation-hindering effects.

3. kaempferol

The highest recorded mean concentration of kaempferol was 6 µg/g. Regarding the attention of kaempferol among the septa of the various sorts, no statistically important difference was found ($p = 0.06$). Regarding its inherent properties, kaempferol is a naturally occurring beneficial flavonoid that may function as a chemopreventive agent, protecting in contradiction of oxidative stress and inflammatory chronic diseases.

4. Luteolin

The walnut septa's mean attention levels for luteolin were 3 µg/g. The luteolin attention measured in the septa of the various kinds showed statistically significant differences ($p = 0.039$). Regarding its inherent properties, luteolin is a obviously occurring flavonoid with a pusillanimous liquid look. Research has shown that it may exhibit several cellular benefits, perhaps having a positive impact on overall health. In addition to acting as an antitumor, Anti-Inflammatory, antibacterial, or Estrogenic nonsupervisory conflation and aiding hepatic problems, it may showcase antioxidant packets, shielding cells from ROS-induced damage.

5. Myricetin

The walnut septa's mean attention levels for myricetin were 8 µg/g. According to the ANOVA analysis, there is a statistically significant difference ($p = 0.002$) between the septa of the various varieties. Myricetin, which has been demonstrated to have antioxidant, anti-inflammatory, antiviral, and anti-carcinogen effects, has sparked a great deal of scientific interest. It functions as an antineoplastic agent in situations of death since it has shown to have potent suppressive effects on the activity of many cancer cell types (such as cancer cell invasion or metastasis), controlling apoptosis and acting as an inhibitory factor on the growth of these cells.

6. Quercetin

The level of quercetin in walnut septa was found to be 9 µg/g. According to the ANOVA study, there is a statistically significant difference between the examined sorts and quercetin attention ($p = 0.0008$). The works claims that quercetin gives food its bitter flavor. Lately, it has been utilized as a food supplement to protect meat products from the growth of bacteria when combined with myricetin and other phenolic mixtures. It has a number of noteworthy health promoting properties, such as Antioxidant, antidiabetic, Anti-ulcer, cardio-defensive, and chemo-preventative properties. By inhibiting the lipoxygenase and cyclooxygenase pathways, it may also result in the production of Anti-Inflammatory and Anti-Allergy products by lowering the amount of pro-inflammatory or pro-oxidant compounds.

7. Rutin

Three to six µg/g of rutin was found in walnut septa. For the attention of rutin, there was a statistically significant difference between the septa of the various sorts (p = 0.002). Rutin is a vital component of many physiological processes in the mortal body and has substantial natural benefits. It also offers promising antioxidant potential. The literature suggests that rutin has a wide range of therapeutic benefits, including anti-inflammatory, anti-protozoal, antiviral, anti-hypertensive, vasoactive, cyto-defensive, anti-allergic, antispasmodic, anti-carcinogenic, antibacterial, and antiplatelet effects. Similarly, because of its strong ability to scavenge radicals, rutin helps to strengthen the capillaries that make up the blood vessels, protecting people from hemorrhagic disorders linked to fragility.

Analysis of Flavonoids by Quantitative methods

Triplet (n = 3) walnut septa were anatomized. Based on their maximum immersion wavelengths, the related flavonoids (myricetin., luteolin., quercetin., apigenin., and kaempferol) were assessed. A number of beneficial health properties and other bioactive meanings that have previously been documented in the nonfiction were associated with the presence of bent flavonoids, highlighting the potential functional exertion of the anatomized derivative. displays the mean values (± SD) and quantification ranges for the flavonoids originate in the walnut septa. To display the distributional properties and visually represent the flavonoid concentration, box and whisker plots were created. The quantification outcomes were further anatomized with ANOVA to examine if there are statistically important differences between the attention of the strongminded flavonoids and the variations of the walnut septa. FT-IR studies

2) To study *in vitro* drug release profile of prepared solution

3) To ascertain release mechanics and kinetics of drug release.

4) Comparative study of marketed product if available solution with Allopathy, Ayurveda, Homeopathy etc.

5) To perform stability studies as per ICH guidelines.

Stability Testing:

Consistency The sample was reserved at an accelerated temperature for the purpose of testing the set walnut kernel septum membranes. Ten sections of the final walnut kernel septum membrane results, A, B, and C, were held at room temperature and 40 degrees Celsius, respectively, at an accelerated temperature. At one-, two-, and three-month intervals, the result was checked for all physicochemical characteristics, including turbidity and unity, and no changes were found.