Design and development of Walnut kernel septum solution for thyroid function

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Abstract

In this study, we investigated the test of the septum in walnut kernel (Juglans regia L). Walnut septum has been widely used in Iranian traditionally used to treat diabetic patients and other different diseases like skin or colon cancer and acts as an antidiabetic, anti-inflammatory, antioxidant, anti-allergic, antimicrobial, antiviral, cardio protective, and neuroprotective agent etc.

Crushing walnuts in the laboratory using a wooden hammer, by braking of walnut fruit separating the Walnut kernel septum membranes. carefully removing the walnut septa. Each walnut septum sample originating from the same tree. The walnut membrane septum was dried by natural method approximately 7 days, in a dark place, in order to remove the moisture initially present. The walnut septum membrane was divided into two parts. In one method the directly walnut septum membrane soak into ethanol and distilled water directly.

Another part of Walnut kernel septum membranes was powdered by electric grinder and then The powdered material was soak into ethanol and distilled water. The solvents used were Ethanol and distilled water with different ration, at room temperature with occasionally shaking for 21 days. The solution was holding on in an exceedingly airtight dry, clean and away from light. Afterwards, the mixture was filtered using whatman filter paper and collected the concentrated extract (Walnut Septum Solution) WSS for further evaluation. Obtained extract was stored in a refrigerator for further tests.

The evaluation parameter like, Moisture content, Total Ash values, boiling point, Specific gravity, Density, Viscosity, PH, Appearance, HPTLC analysis/HPLC Analysis, *in vitro* drug release profile of extracted solution and stability studies shows all solutions are under the range. The solution F1 give excellent drug release.

With the help of HPLC method, we find the walnut septum membranes solution are having the flavonoids: catechin, rutin, myricetin, luteolin, quercetin, apigenin, and kaempferol were Present.

Keywords., Walnut, Juglans regia L. Kernel, Septum, solution

INTRODUCTION

In Turkish folk drug, the fruits and leaves of Juglans regiaL. have been extensively used as an herbal remedy for the treatment of endocrine conditions similar as diabetes mellitus, anorexia, thyroid dysfunctions, etc. The effect of fruits of J. regia on the thyroid hormone situations of mice was delved using two excerpts prepared from the fruits by different styles. The acute venom of these two excerpts in mice were assessed as well. On the base of our findings attained, the excerpts prepared from the fruits of J. regia enhanced thyroid hormone situations, while they wielded minimum acute toxin in mice.

Figure No. 1. Walnuts Kernel with Septum

walnuts are helpful in managing thyroid is because they've omega 3 which reduces inflammation. The rate of inflammation is high in thyroid and to combat that, walnuts can help. Healthy fats especially from nuts and factory- grounded sources are the key for restoring thyroid function and so, consuming walnuts in a regulated quantum can attack thyroid inflammation. still, it should not be considered the sole food to boost thyroid function Walnuts can be an important part of any healthy diet, being a good source of healthy fat, protein and antioxidants. However, you should see a croaker to know If you have a thyroid condition. about the part your diet plays in your overall health.

Thyroid disorders and natural treatments. Worldwide, incidence of endocrine diseases including thyroid disorders is increasing. Thyroid disorders are generally classified into hyper and hypothyroidism. Women are more likely to have alteration in thyroid function as compared to men. In general, patients with thyroid disorders may have decreased circulating thyroid hormones (hypothyroidism) or increased levels of thyroid hormones (hyperthyroidism). Hypothyroidism is one of the most prevalent endocrine disorders characterized by low levels of thyroid hormones (T3 & T4) in the serum and high thyroid stimulating hormone (TSH).

Maceration is one of the oldest and simplest extraction methods in which coarse and powdered plant material is soaked in solvents such as methanol, ethanol, ethyl acetate, acetone, hexane etc.

Materials and Methods

Preparation of Walnut Kernel Septum Membranes:

The Walnut fruit was purchased from traditional medicine shopkeeper in local market of Hyderabad, Telangana, INDIA and confirmed by an expert botanist. By braking of walnut fruit separating the Walnut kernel septum membranes. The walnut membrane septum was dried by natural method approximately 7 days, in a dark place, in order to remove the moisture initially present. The walnut septum membrane was divided into two parts. In one method the directly walnut septum membrane soak into methanol and distilled water directly.

Figure. No. 2. Walnut kernel septum membranes

Figure. No. 3. Walnut kernel septum membranes powdered

Preparation of Walnut Kernel Septum Membranes solution:

Another part of Walnut kernel septum membranes was powdered by electric grinder and then The powdered material was soak into ethanol and distilled water. The solvents used were ethanol and distilled water with different ration, at room temperature with occasionally shaking for 21 days. The solution was holding on in an exceedingly airtight dry, clean and away from light. Afterwards, the mixture was filtered using Whatman filter paper and collected the concentrated extract for further evaluation. Obtained extract was stored in a cool dark place for further tests.

Figure No. 4. Walnut kernel septum membranes solution F1 to F5.

Figure No. 5. Walnut kernel septum membranes solution F6 to F10.

Method	Ratio Alcohol : Water	Alcohol	Water	Formulation No.
walnut septum membrane	100:0	250ml	0	F1
	0:100	0	250ml	F2
	75:25	187.5ml	62.5ml	F3
	50:50	125ml	125ml	F4
	25:75	62.5ml	187.5ml	F5
walnut septum membrane in powder form	100:0	250ml	0	F6
	0:100	0	250ml	F7
	75:25	187.5ml	62.5ml	F8
	50:50	125ml	125ml	F9
	25:75	62.5ml	187.5ml	F10

Table. No. 1. Different Walnut Kernel Septum Membranes solution

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

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

30, 35 and 40 µg/ml at 360 nm. The drug obeys Beer - Lambert's law in the range of 0 - 40 µg/ml.

EVALUATION PARAMETER

1. **To determine the boiling point of** Walnut Septum Solution**:** Take a capillary tube and close its one end by holding the end in the flame and rotate it for 2-3 minutes, transfer a few mL of septum membranes solution to the fusion tube. fusion tube keeping the sealed, start heating the Note down the temperature soon as the regular streams of bubbles are seen out of liquid in the fusion tube.
2. **Determination of Moisture content of Walnut septum:** Moisture content is determined via a thermos gravimetric method i.e. by loss on drying. In which, the sample is heated & the weight loss due to evaporation of moisture is recorded. Taking Walnut kernel septum membranes powder (0.5gm). Weigh the empty porcelain dish and note the reading. (W1**).** Weigh the porcelain dish with Walnut kernel septum membranes in it and note the reading. (W2). Keep the porcelain dish in hot air oven for 15 minutes at 100°C-105'C. After heating, keep the porcelain dish in desiccator for 15 minutes. Take out the dish from desiccator, and weigh the porcelain dish with dried sample. (W3). After weighing all calculate the moisture content.
3. **Determination of total ash of walnut septum:** Find out the weight of a clean dry crucible. Place about 2 g of Walnut kernel septum membranes powder sample and weigh this to find out accurate weight of the sample taken. weighed crucible over electric burner. The crucible should be partially opened. The sample will get charred with initial expulsion of smoke. Place the crucible in a muffle furnace and heat to 600°C. Keep it for 2 hours. At this temperature all organic matter will be burnt leaving behind minerals. Remove the crucible from the furnace carefully and cool it in a desiccator to room temperature and weight again.
4. **Determine of density of Walnut Septum Solution:** Take the weight of empty dry bottle with capillary tube stopper (W1). Calculate weight in grams of distilled water (W3). Weight bottle with Walnut kernel septum membranes solution on analytical balance (W2). Calculate the density.
5. **Determination of Specific gravity of Walnut Septum Solution:** Take weight of empty, Clean and dry bottle with capillary tube stopper (W1). Fill the bottle with distilled water and weight (W2). Weight bottle with Walnut kernel septum membranes solution (W3).

6. **Determination of Viscosity of the Walnut Septum Solution**; Take clean the Ostwald viscometer, Fill water in dry viscometer up to mark G. Count time required, in second for water to flow from mark A to mark B. Rinse viscometer and take Walnut kernel septum membranes solution and then fill it up to mark A, find out the time required for liquid to flow to mark B. Determination of densities.
7. **pH Determination of pH of the Walnut Septum Solution**: The pH determination of Walnut kernel septum membranes solution by using pH meter.
8. **Determination of Organoleptic Characters of the Walnut Septum Solution:** Organoleptic evaluation resources the study of drugs using organs of senses. It refers to the approaches of analysis like colour, odour, taste.
9. **To study *in vitro* drug release profile of prepared Walnut Septum Solution**

Osmosis is the phenomena in which solvent particles permit through a semi-permeable membrane (parchment paper) from an area of higher concentration to an area of lesser concentration.

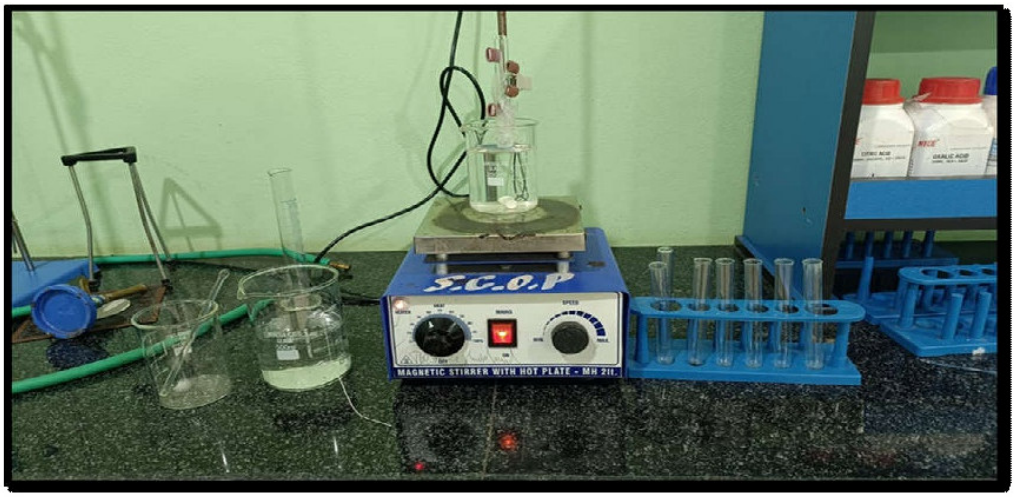

Figure. No. 6. Assembly of instruments for Walnut Septum Solution release.

In-vitro studies of Walnut kernel septum membranes solution were studied by osmosis method by test tube attached with parchment paper as semipermeable membrane and using magnetic stirrer apparatus employing a magnetic bead as stirrer. 250 ml of phosphate buffer of pH 7.4 was used as dissolution medium at 50 rpm. The temperature of 37 ± 0.5°C was maintained throughout the experiment. Walnut kernel septum membranes solution was used in each test. 1 ml of sample of dissolution medium were withdrawn by

means of syringe fitted with pre-filter at known intervals of time and analyzed for drug release by measuring the absorbance at 360 nm after suitable dilution with phosphate buffer. The volume withdrawn at each time interval was replaced with fresh quantity of dissolution medium. The amount of Walnut kernel septum membranes solution released was calculated and plotted against time.

10. **HPLC method**

1. Chemicals and Reagents

Methanol (MeOH) and acetonitrile (ACN), HPLC grade, were acquired from Merck. Acetic acid 99% and trifluoroacetic acid (TFA) 99% were obtained from Merck. The LiChrolut RP-18 (C18, 3 mL, 500 mg) SPE containers used were supplied by Merck (Darmstadt, Germany). Ultrapure water was provided by a Milli-Q decontamination system (Millipore, Bedford, MA, USA). The flavonoids catechin 98%, rutin 98%, myricetin 98%, luteolin 98%, quercetin 98%, kaempferol 98%, and apigenin 98% were supplied by Sigma-Aldrich (Steinheim, Germany). Stock standard solutions at 1000 mg/L concentration level were prepared and stored in dark brown glass bottles at -20 °C. Working standard solutions were ready in MeOH after suitable dilution of the stock solutions each laboratory day, before analysis.

2. Instrumentation

A quaternary low-pressure incline HPLC–DAD system by Shimadzu (Kyoto, Japan) was used for examination. The HPLC system consisted of: (a) an FCV-10ALVP mixing system, (b) a Rheodyne 7725i injection valve, and a 20 µL loop for sample injection, (c) an LC- 10ADVP pump equipped with a Shimadzu SCL-10ALVP System Controller, (d) an SPD-M10AVP photodiode array detector. Real time examination monitoring and post run dispensation were carried out using the software Lab Solutions-LC solutions, supplied by Shimadzu. A glass space filtration apparatus, acquired by All tech Associates, and nylon 0.2 µm membrane Filters were utilized for the filtration of the mobile phase, and a DGU-10B de-gassing unit with helium was used for degassing. A vortexer purchased from FALC Instruments was used for sample agitation. Centrifugation was carried out using a HermLe centrifuge, model Z-230. An ultrasonic bath (MRC: DC-150H) by MRC was used for specimen preparation. For disappearance, after SPE extraction, a ReactiVap 9-port evaporator model 18,780 by Pierce was utilized. For sample filtration, prior to the injection in the chromatographic system, Q-Max RR syringe filters (0.45 µm nylon membrane) were purchased from Frisenette ApS.

3. Chromatographic Separation and Analysis

The chromatographic parting of the flavonoids was attained on a C18 Universe column (250 mm 4.6 mm, 5 μm), supplied by Fortis Technologies Ltd. on, A reverse-phase HPLC test was carried out using a gradient scheme with 1 mL/min flow rate, thermos stated at 30 ◦C. The mobile phase consisted of (A) 1% acetic acid in water, and (B) ACN. The gradient elution program begun with 80:20, v/v (A: B), gradually increasing to 50:50, v/v (A: B), in the following 25 min, and then outstanding constant for the next 5 min. The initial circumstances were restored for 10 min, prior to the next injection. The injection volume was 20 μL of solution and the total run time was less than 25 min for each injection. For peak identification, the Rts of the peaks of the real samples were compared with the Rts of the standard mixtures, along with the spectral information providing by the DAD sensor that operated over the variety 280–400 nm. Peak nursing and quantitation were achieved at the maximum wavelength of each analyte.

4. Sample Collection

walnut septa models were created after crushing walnuts in the workroom using a wooden hammer, and carefully removing the walnut septa. Each walnut septum taster was a bulk sample that contained of ten walnut septa originating from the same tree. In this way, bulk walnut septum samples were created in the laboratory. All the walnut samples were collected during the harvesting period of November 2022.

Stability Testing:

Stability Testing of the prepared Walnut kernel septum membranes solution was performed on keeping the sample at accelerated temperature conditions. Ten portions of the final Walnut kernel septum membranes solution A, B and C were taken kept at accelerated temperature at Room temperature and 40 respectively. The solution was tested for all the physicochemical parameters, turbidity and homogeneity at the interval of one month, two months and Three months to observe any change.

Results and Discussion

The Moisture content of walnut kernel septum membrane is 15.9 %. The total ash of walnut septum 1.78 g. The other evaluation of Walnut kernel septum membranes solution parameters are as follows

Sl. 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.2. Result of evaluation parameters of F1 to F5

Sl. No.	Parameter	F6	F7	F8	F9	F10
1	Boiling Point	75	86	78	84	80
2	Density	0.9215	0.9217	0.92218	0.9216	0.9217
3	Specific gravity	0.928	0.919	0.924	0.921	0.925
4	Viscosity	75.4	73.5	73.6	72.8	73.7
5	pH Determination					
	b) pH meter	7.0	6.1	6.8	6.8	6.2
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. 3. Result of evaluation parameters of F6 to F10

Construction of Calibration Curve walnut septum membrane solution:

SL. No.	Concentration of Drug (µg/ml)	Absorbance at 360 nm
1	0	0
2	5	0.109
3	10	0.223
4	15	0.339
5	20	0.448
6	25	0.561
7	30	0.671
8	35	0.790
9	40	0.900

Table. No. 4: Standard Graph of walnut septum membrane solution

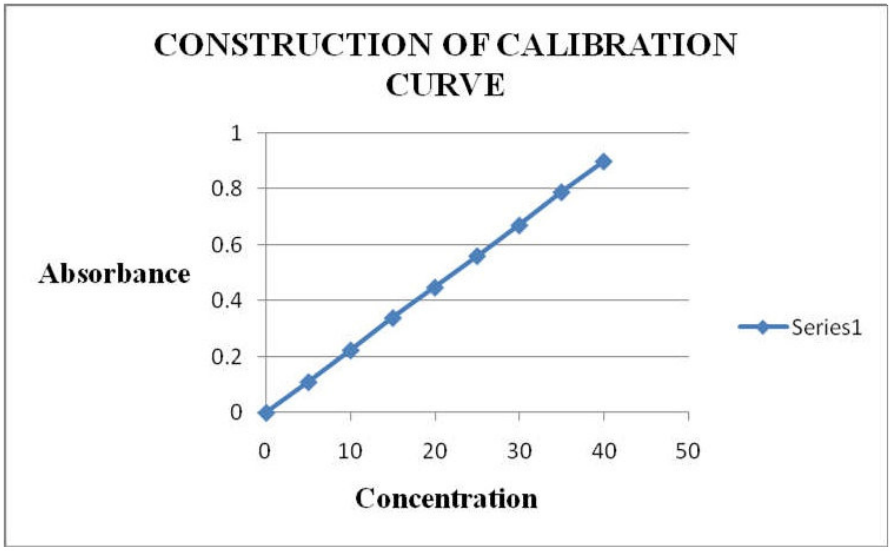

Fig. No. 7: Standard Calibration Curve for walnut septum membrane solution

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	15.11	17.01	19.11	21.22	17.12	17.50	14.05	16.75	2.01
10	33.51	19.79	21.37	27.31	32.32	27.41	24.20	20.14	32.50	11.07
15	44.23	30.95	29.72	39.15	40.91	34.54	28.70	31.72	41.60	16.72

20	55.47	42.15	45.77	51.57	53.21	49.14	34.50	45.13	52.80	25.65
25	69.21	50.67	57.41	61.72	62.25	56.27	47.25	52.59	60.75	36.04
30	77.69	62.95	65.92	70.97	74.61	67.11	55.80	64.17	72.80	44.34
35	86.61	72.11	72.96	83.01	85.29	77.91	67.30	74.77	80.25	58.34
40	97.41	84.43	83.79	91.01	92.11	85.01	75.95	81.15	87.10	70.39

Table No. 5: In- vitro Drug Release Profile of different formulation in Phosphate Buffer pH 7.4

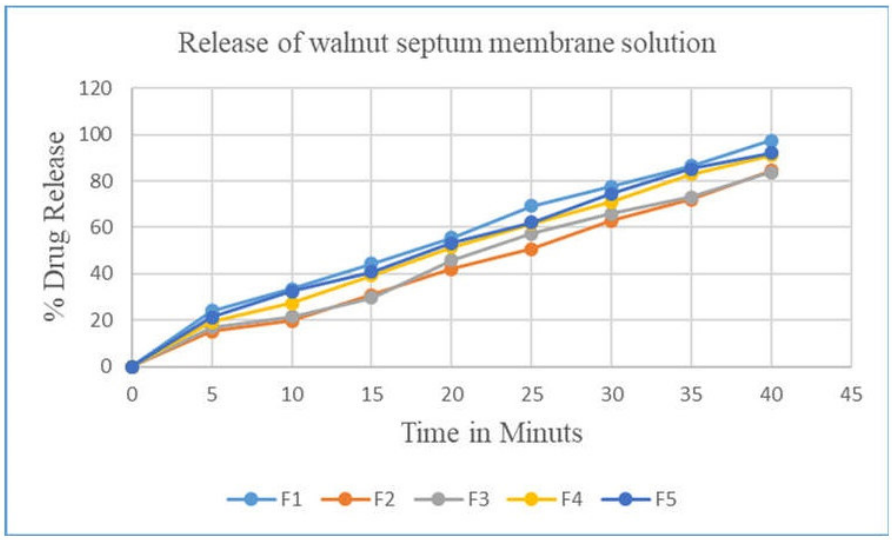

Figure No. 8. Percent release of walnut septum membrane solution F1 to F5

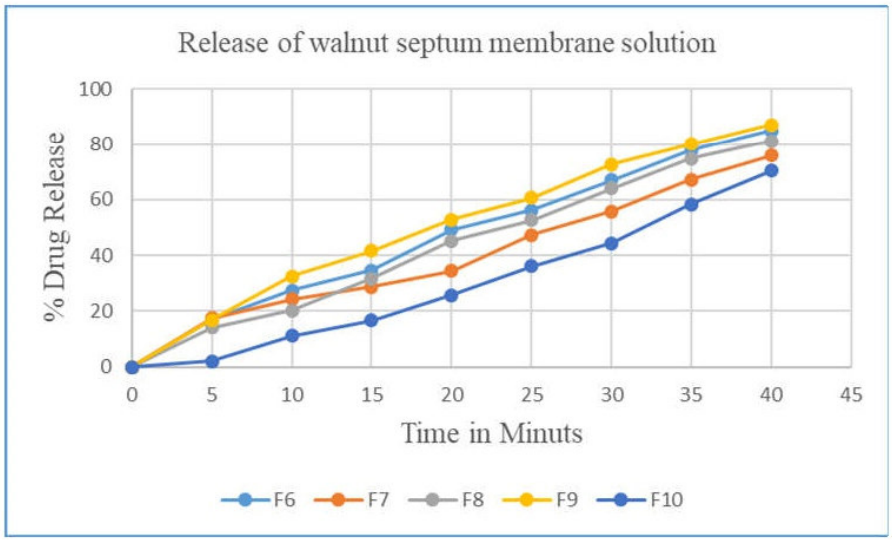

Figure No. 9. Percent release of walnut septum membrane solution F6 to F10

***In-Vitro* Drug release:**

Drug release data of the various walnut septum membrane solution is indicated in table. The walnut septum membrane solution released the drug for 40 min (formulations: F1 to F10. All the formulations F1 to F10 showed marked variation in the drug release at the end of 40 miniuts. Formulation F1 showed the highest release rate (97.41%) and formulation F10 least release rate (70.39%) at the end of 40 minutes. Among all 10 walnut septum membrane solution (F1 to F10), the 3 formulations (F4, F5 and F6) gives good release as compared to other walnut septum membrane solution. As per the drug content and osmosis studies are concerned, it indicated that F1 formulation gives best drug content and shows best walnut septum membrane solution release.

Method Development and Validation

Method Progress and Validation A HPLC-DAD organization was developed and validated to measure the flavonoid profile of walnut septum and all the analytical parameters, including the calibration curves, linear range, the determined coefficients (r2), accuracy and precision, restrictions of detection (LODs), and limits of quantification (LOQs) are presented in Table. The analytical curves presented an adequate fit when acquiesced to the lack-of-fit test (F calculated was less than F tabulated in all cases), with r2 above 0.99, showing that they can be used for the quantification of the flavonoids. The LOQs were found to range between 0.30 μg/g and 0.90 μg/g, while the LODs were planned over the range 0.10 μg/g to 0.30 μg/g. The RSD% of the within day (n = 6) and between-day assays (n = 3 × 3) were lower than 6.2, and 8.5, respectively, showing adequate accuracy. The accuracy was assessed by means of relative proportion of recovery (%R) at low, medium, and maximum concentration levels of 1, 5, and 10 μg/g, and the results were acceptable, ranging from 90.8 (apigenin, at 10 μg/g concentration level) to 97.5% (catechin, at 10 μg/g concentration level) for within-day assay (n = 6), and from 88.5 (myricetin, at 1 μg/g meditation level) to 96.2% (catechin, at 5 μg/g meditation level) for between-day assay (n = 3 × 3).

Compound	Calibration Equation $y = (a \pm Sa) + (b \pm Sb)x$ (Linear Range: 1–10 μg/g)	r^2	F_{calc}	F_{tab}	LOD (μg/g)	LOQ (μg/g)
Apigenin	$y = (1732 \pm 152) + (1745 \pm 665)x$	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

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

Table No. 6. HPLC-DAD method analytical parameters.

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.

Table No. 7. %Recoveries (%R, $n = 6$) for the evaluation of repeatability.

Walnut Septum Analysis

walnut septum membranes solution was analyzed in triplicate and the flavonoids: catechin, rutin, myricetin, luteolin, quercetin, apigenin, and kaempferol were resolute. The chromatographic documentation results, counting the retention times (Rts) of the analytes, and their respective maximum absorption wavelengths (λ, nm) are presented in Table. Figure

illustrates the chromatographic parting of the flavonoids in a walnut septum extract that was experiential at 280 nm.

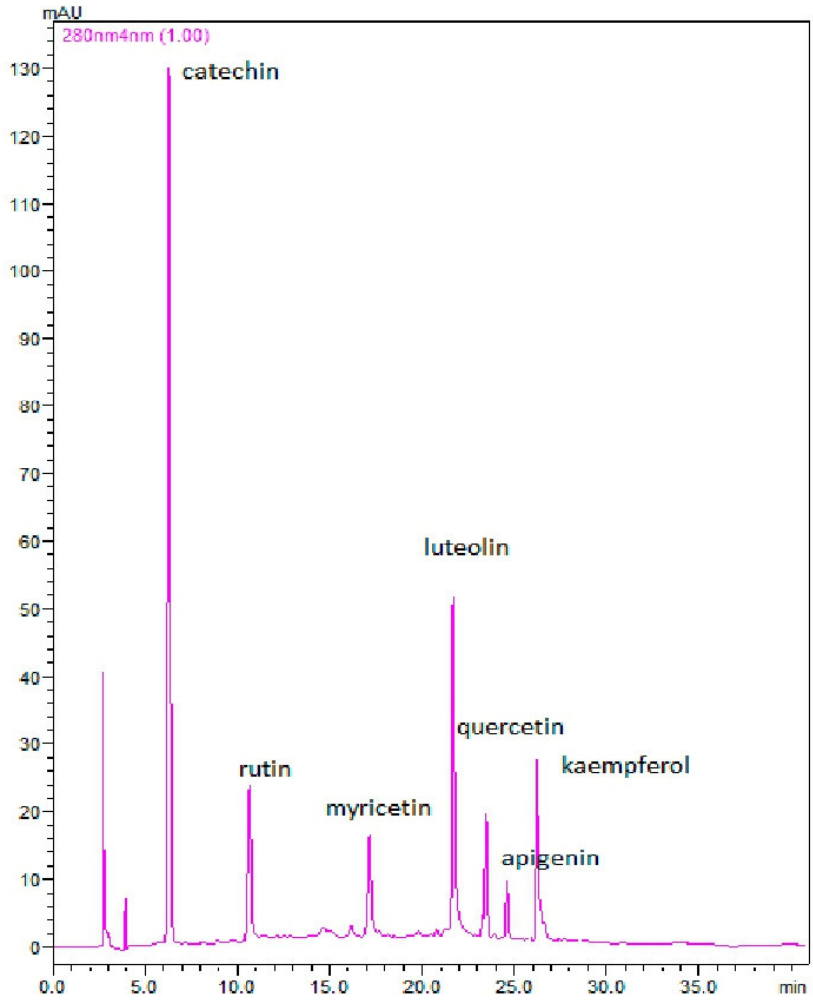

Figure No. 10. Characteristic chromatogram of a walnut septum extract; monitored at 280 nm.

Stability Testing:

Stability Testing of the prepared Walnut kernel septum membranes solution was performed on keeping the sample at accelerated temperature conditions. The solution was tested for all the physicochemical parameters, turbidity and homogeneity at the interval of one month, two months and Three months observed no changes in the solution. There is no major changes at all.

Conclusion

Walnut membrane septum are appreciated plants which contain an inspiring amount of biologically active substances that have a wide range of uses. During this study it was investigated the influence of water/ethanol solvent mixtures at different concentrations on the level of total polyphenol content of extracts from walnut membrane septum. Obtained experimental data are demonstrated in figure. During HPLC DAD analysis walnut septum membranes solution was analyzed in triplicate and the flavonoids: catechin, rutin, myricetin, luteolin, quercetin, apigenin, and kaempferol were resolute.

In this study water and ethanol, two environmentally and food safe solvents were used to optimize solid-liquid withdrawal of phenolic mixtures from walnut membrane septum. Total polyphenol content of walnut septum extracts was evaluated. It was established that optimal solvent for antioxidant extraction from walnut membrane septum is 100% mixture of ethanol. From prepared 10 solutions of walnut membrane septum with different concentrations of ethanol and water, the F1 gives excellent in-vitro drug lease. The walnut kernels septum need farther studies to make formulations for thyroid treatment.

We consider walnut septum to be a significant biological medium, a rich accepted source of bioactive compounds that deserves to be investigated in the upcoming in order to be fully exploited in the food, cosmetic, or pharmaceutical industry.

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