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# Identifying And Quantifying Flavonoids In Three Medicinal Plants By Hplc

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***Abstract:***

*Natural antioxidants such as flavonoids are natural disease preventing, health-promoting and has therapeutic applications as anticancer, antidiabetic, diuretic, anti-inflammatory. The project aims at identification of flavonoids and quantitative screening for finding the total flavonoid content in three different medicinal plants gymnema sylvestre, andrographis paniculata, cassia angustifolia. This involves taking these plant extracts , detecting the flavonoids by using high performance liquid chromatography and estimation of total flavonoid content by using uv-spectrophotometer by taking quercetin as a standard. The results revealed the presence of the following flavonoids: epicatechin, apgeninin, catechin, luteolin, myricitin, quercetin, kaempferol, rutin, tangeretin, hesperetin, taxifolin, and eriodictyol.*

***Keywords:*** *antioxidants, flavonoids, flavonoid content, gymnema sylvestre, andrographis paniculata, cassia angustifolia, hplc, uv-spectrophotometer, quercetin.*

**1. Introduction**

Flavonoids or bioflavonoid (from the Latin word flavus meaning yellow), also collectively known as Vitamin P and citrin are a class of plant secondary metabolites or yellow pigments having a structure similar to that of Flavones. Flavonoids are widely distributed in plants fulfilling many functions. Flavonoids are the most important plant pigments for flower coloration producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. Flavanoids such as the catechins are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. Flavonols, the original bioflavonoids such as Quercetin, are also found ubiquitously, but in lesser quantities. The wide spread distribution of flavonoids, their variety and their relatively low toxicity compared to other active plant compounds for instance alkaloids mean that many animals, including humans, ingest significant quantities in their diet. Preliminary research indicates that flavonoids may modify allergens, viruses, and so may be biological response modifiers. Invitro studies show that flavonoids also have anti-allergic, anti-inflammatory, anti-microbial, anti-cancer and anti-diabetic properties. Flavonoids might induce mechanisms that affect cancer cells and inhibit tumor invasion. In preliminary studies, Cancer researchers proposed that smokers who ate foods containing certain flavonoids, such as catechins found in strawberries and green and black teas, Kaempferol from brusselsprouts and apples, and quercetin from beans, onions and apples, may have reduced risk of obtaining lung cancer.

***2. Gymnema Sylvestre***

*2.1. Plant Classification*

Kingdom : Plantae

Family : Asclepediacea

Genus : Gymnema

Species : sylvestre

Binomial name : *Gymnema sylvestre*.

Common name : Podapatri.


*Figure 1:Gymnema Sylvestre*

The leaves contain hentriacontane, pentatriacontane, α-and β-chlorophylls, phytin, resins, tartaric acid, formic acid, butyric acid, anthraquinone derivatives, inositol, d -quercitol and gymnemic acid. The plant is stomachic, stimulant, laxative and diuretic. It is good for cough, biliousness and sore eyes. If the leaves of the plant are chewed, the sense of taste for sweet and bitter substances is suppressed. The leaf powder is tasteless with a faint pleasant aromatic odour. It stimulates the heart and the circulatory system. Traditional healers from diverse parts of India use this plant in various ailments.

*2.2. Uses*

Preliminary human evidence suggests that Gymnema may be effective in the management of blood sugar levels in type 1 and type 2 diabetes, as an adjunct to conventional drug therapy, for up to 20 months.

Gymnema appears to lower serum glucose and glycosylated hemoglobin (HbA1c) levels following chronic use, but may not have significant acute effects.

It is used to reduce the absorption of glucose into the body, and also reduce the sweetness of foods, both of which are desirable for those wishing to lose weight and to reduce the level of sugar in their blood.

It was used for this purpose in Ayurvedic medicine, subjects being given the leaves to chew. As with many other ancient Ayurvedic remedies, this use of Gymnema sylvestre has passed into modern times, and has sound scientific basis.

Preliminary human research reports that gymnema may be beneficial in patients with type 1 or type 2 diabetes when it is added to diabetes drugs being taken by mouth or to insulin.

Gymnema sylvestre extract has been shown to be effective for weight loss when used in combination with other products.

*2.3.Mostly Used For*

Allergy, Cancer, Cardio vascular diseases, Constipation, Cough, Dental problems, Diuresis, High blood pressure, Laxative, Liver diseases, Rheumatoid arthritis, Metabolic disorders, Snake venom antidote, Stomach ailments, Uterine stimulant.

**3. Andrographis Paniculata**

*3.1. Plant Classification*

Kingdom : Plantae
Family : Acanthaceae
Genus : Andrographis
Species : paniculata
Binomial name : Andrographis paniculata
common name : Nelavemu, Nelavema.

*Figure 2: Andrographis Paniculata*

Andrographis paniculata contains bitter andrographolide, a bicyclic diterpenoid lactone and Kalmeghin (upto 2.5%). The leaves contain the maximum active content while in the stem it is in lesser amount. Andrographis and its various components have demonstrated a variety of effects in the body. Aspects stimulate the general immune activities, others inhibit the body's inflammatory mechanism and still others demonstrate not only anti-microbial abilities, but also are instrumental in killing certain tumor cells. Studies have also indicated that the active chemical, Andrographolide, helps to stop the clumping of blood platelets which is the clotting process that can lead to heart attacks.The plant is bitter, acrid, cooling, laxative, vulnerary, antipyretic, antiperiodic, anti-inflammatory, expectorant, depurative, soporific, anthelmintic, digestive and useful in curing respiratory disorders, buring sensation, wounds, ulcers, chronic fever, malarial and intermittent fevers, inflammations, cough, bronchitis, skin diseases, leprosy, colic, flatulence, diarrhoea, dysentery, haemorrhoids etc. Kalmegh is also a reputed Homoeopathic drug. It has been proved to be hepatopratective drug. Andrographis paniculata contains many flavonoids, which have anti-inflammatory properties. Andrographolide, an active component of andrographis paniculata, inhibits inflammatory responses. The Methanolic extract of Andrographis possesses anti diabetic property. The extract also produced a prolongation of the pentobarbitone-induced sleeping time and lowered the body temperature in different experimental animal models. Andrographis paniculata plant extract is known to possess a variety of pharmacological activities. Andrographolide also enhanced the tumor necrosis factor α production and CD marker expression, resulting in increased cytotoxic activity of lymphocytes against cancer cells, which may contribute for its indirect anticancer activity. These results suggest that Andrographolide is an interesting pharmacophore with anticancer and immuno modulatory activities and hence has the potential for being developed as a Cancer therapeutic agent.

**4. Cassia Angustifolia**

*4.1. Plant Classification*

Kingdom : Plantae

Family : Caesalpinaceae

Genus : Cassia

Species : angustifolia

Binomial name : Cassia angustifolia

Common name : Senna.

*Figure 4: Cassia Angustifolia*

Cassia angustifolia consists of Flavanol (Isorhamnetin, kaempferol), Anrathquinone (Rhein, Emodin), Sennoside A, Sennoside B, Menitol, Sodium potassium tartarte, Salicilic acid, Crisophenic acid, Volatile oils, Resins, Calcium oxalate.In the leaf Sennosides A and B based on the aglycones Sennidin A & B, Senosides C & D which are glycosides of hetero dianthrones of aloe-emodin and rhein are present. Others includes palmidin A, rhein anthrone and aloe-emodin glycosides, some free anthraquinones and some potent, novel compounds of as yet undetermined structure. Cassia angustifolia usually contains more of the sennosides. In the fruit, sennosides A and B and closely related glycoside sennoside A. Naphthalene glycosides, tinnevellin glycoside and 6-hydroxymusizin glycoside Miscellaneous, mucilage, flavonoids, volatile oil, sugars, resins etc. This is mostly used for treating constipation, jaundice, dyspepsia, helminthiasis, cough, and bronchitis. Useful parts are Leaves.

*4.1. Uses*

Cassia angustifolia purifies blood and restores the metabolic imbalance lost due to indigestion.

The herb stimulates liver for proper secretion of enzymes in the body.

It helps in lowering bowels and increasing the peristaltic movement of the intestines.

The powder made from crushing leaves is helpful in treating constipation and indigestion.

It is useful in relieving people from the condition of Osteoarthritis, Gout and Rheumatoid arthritis.

The herb is used as an expectorant, wound dresser, antidysentric, carminative and laxative.

*4.2. Cassia Angustifolia Is Handy In Treating*

Loss of appetite, hepatomegaly, spleenomegaly, malaria, skin diseases, jaundice and anemia. The herb has purgative, antihelminthic, antipyretic, cathartic, laxative, vermifuge and diuretic properties.

It act as purgatives and are similar to aloe and rhubarb in having active ingredients anthraquinone derivatives and their glycosides. The latter are called sennosides or glycosides.

Cassia angustifolia is used in modern medicine as a laxative, acting on the lower bowel, it is especially useful in alleviating constipation.

It increases the peristaltic movements of the colon by irritating the colonic mucosa.

Cassia angustifolia is powerful cathartic used in the treatment of Constipation.

**5. Materials And Methods**

*5.1. Chemicals Required*

| | |
|---|---|
| Methanol (HPLC GRADE) | - MERCK, Mumbai. |
| Water (HPLC GRADE) | - MERCK, Mumbai. |
| Aluminum nitrate | - SD Fine Chem Limited, Maharashtra. |
| Potassium acetate | - SD Fine Chem Limited, Maharashtra. |
| Quercetin | - SD Fine Chem Limited, Maharashtra. |

*5.2. Instruments Used*

| | |
|---|---|
| Electronic weighing balance – ELB-234 | - Denver, Mumbai. |
| Digital pH meter | - Agilent, Delhi. |
| Sonicator | - Orion, Himachal Pradesh. |
| Hot air oven | - Ancillary, Mumbai. |

| | |
|---|---|
| Incubator | - Orion, Himachal Pradesh. |
| Plant crushing machine | - Zenith, Mumbai. |
| Vacumm filter | - Agilent, Delhi. |
| U.V Spectrophotometer- UV2301 | - Orion, Himachal Pradesh. |
| RP HPLC | - Waters Corporation, U.S.A. |

*5.3. Peak 7000 Isocratic Hplc With Following Configurations*

PEAK 7000 delivery system.

Rheodyne manual sample injector with switch (77251)

Analytical column Kromasil 100-5 (C18-COLUMN) 250 X 4.6mms.

*5.4. Initial Chromatographic Conditions*

5.4.1.Column

Kromasil C18, 250 X 4.6mm.

5.4.2.Mobile Phase

The mobile phase was prepared by using methanol and water (50:50) and was filtered through 0.45 µ liter nylon membrane, pH was maintained at 5.4

5.4.3.Mobile Phase Ratio

Methanol and Water (50:50).

5.4.4.Detector Wave Length

298 nm.

5.4.5.Operating Pressure

9.5 MPa.

5.4.6.Temperature

Ambient.

5.4.7.Injection Volume

20 µl.

5.4.8.Run Time

30 mins.

5.4.9.Flow Rate

1.0 ml per min.

**6. Methodology**

*6.1. Preparation Of Plant Extracts*

Healthy fresh Plants were taken and was completely shade dried, crushed in to fine dry powder. Plant extract was prepared by using HPLC gradient methanol solution and was incubated in a incubator for about 24 hrs. Sample was filtered by using nylon6, 6 ultipore membrane filter papers which has pore size of 0.45 µm and diameter of 13 mm .Vacuum filter was used for the filtration of the plant extract.

*6.2. Preparation Of Mobile Phase*

Mobile phase was prepared by taking methanol and water (HPLC GRADE) in 50:50 ratio. This solution was mixed and pH was adjusted to 5.4.Mobile phase was filtered by using Nylon 6,6 ultipore filter membranes having pore size of 13 µm and 47 mm diameter.

*6.3. Sample Injection*

Sample injections was done by means of a syringe pump in to HPLC sample injector port by maintaining constant steady flow rate at 1.0 ml/min and by maintaining wavelength at 298nm, flavonoids gets separated and was identified. Peaks obtained in HPLC represents flavonoids and by comparing with the standard chromatogram flavonoids are detected.

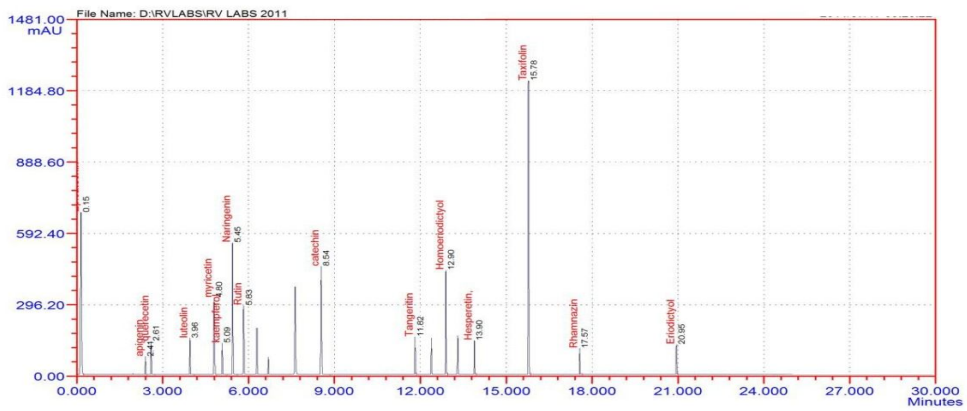
*6.4. Spectrophotometer Methods*

UV spectrophotometer was used for estimating the total flavonoid content in three leaf extracts. Aluminium nitrate method was followed for the detection of total flavonoid content in plant extracts of Gymnema sylvestre, Andrographis paniculata, and Cassia angustifolia.

6.4.1.Total Flavonoid Content Was Determined By Using Aluminium Nitrate Method

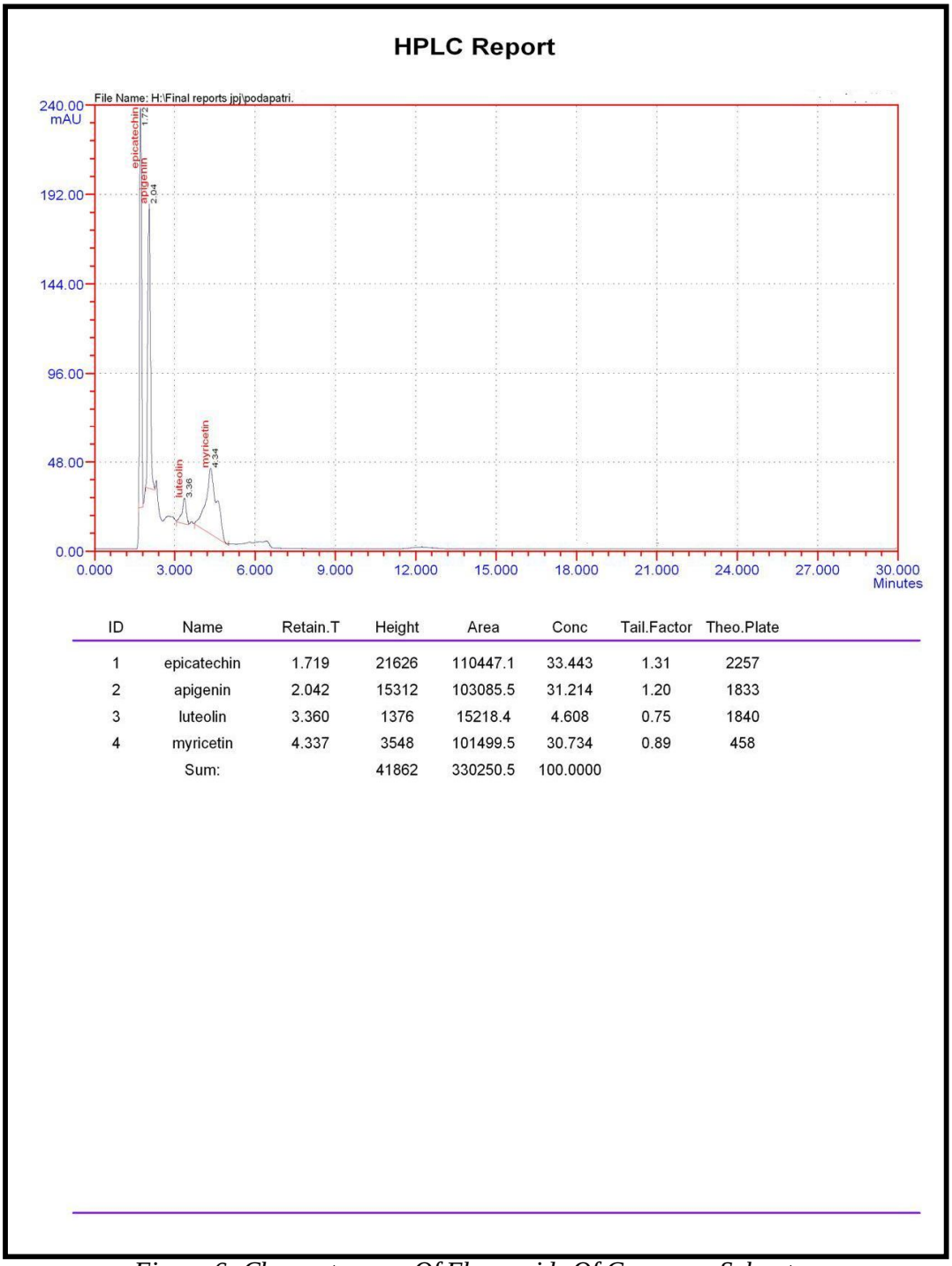
To 5ml of different crude extracts of Gymnema sylvestre, Andrographis paniculata, Cassia angustifolia, 0.5ml of 10% aluminium nitrate and 0.5ml of 1M aqueous potassium acetate was added. Solution was make up to 10ml with distilled water. The mixture was incubated for 40 minutes in incubator at 40°C temperature, and sample was absorbed spectrophotometrically by using UV-Spectrophotometer at 415nm, against reagent blank prepared similarly with out extract. Quercetin was used as a standard for the calibration curve and expressed as µg/ml.

## 7. Results And Discussion




| ID | Name | Retain.T | Height | Area | Conc | Tail.Factor | Theo.Plate |
|---|---|---|---|---|---|---|---|
| 1 | Epicatechin | 0.151 | 68650 | 173438.1 | 18.802 | 1.27 | 71 |
| 2 | apigenin | 2.410 | 8807 | 13131.6 | 1.424 | 1.21 | 52054 |
| 3 | querecetin | 2.606 | 15241 | 23172.2 | 2.512 | 1.46 | 58567 |
| 4 | luteolin | 3.963 | 16560 | 32680.1 | 3.543 | 1.40 | 80377 |
| 5 | myricetin | 4.805 | 33230 | 63724.9 | 6.908 | 1.15 | 125111 |
| 6 | kaempferol | 5.091 | 14451 | 24162.6 | 2.619 | 1.78 | 184783 |
| 7 | Naringenin | 5.451 | 56293 | 87320.3 | 9.466 | 2.37 | 246152 |
| 8 | Rutin | 5.835 | 30574 | 59640.5 | 6.465 | 0.87 | 178309 |
| 9 | catechin | 8.541 | 46349 | 121179.2 | 13.137 | 2.05 | 212706 |
| 10 | Tangeritin | 11.824 | 16294 | 22406.9 | 2.429 | 1.27 | 1473602 |
| 11 | Homoeriodictyol | 12.901 | 44018 | 65035.1 | 7.050 | 1.32 | 1519694 |
| 12 | Hesperetin, | 13.899 | 14763 | 20761.8 | 2.251 | 1.24 | 1946875 |
| 13 | Taxifolin | 15.785 | 122548 | 182630.9 | 19.798 | 1.39 | 2235926 |
| 14 | Rhamnazin | 17.573 | 11496 | 15506.4 | 1.681 | 1.21 | 3382880 |
| 15 | Eriodictyol | 20.953 | 13076 | 17666.8 | 1.915 | 1.24 | 4793401 |
| | Sum: | | 512350 | 922457.4 | 100.0000 | | |

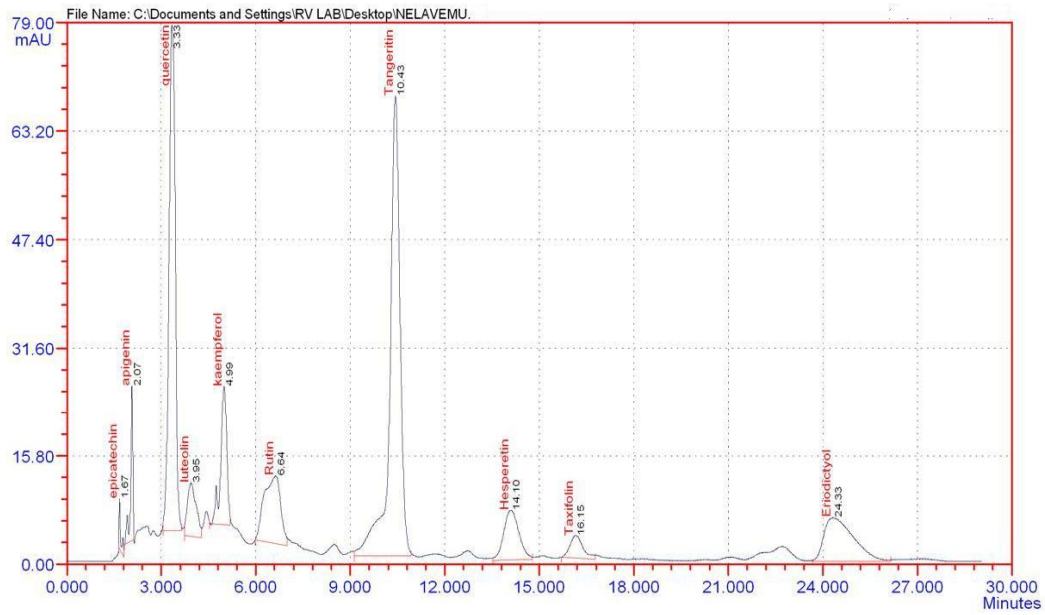
*Figure 5: Chromatogram Of Standard Flavonoids*




| ID | Name | Retain.T | Height | Area | Conc | Tail.Factor | Theo.Plate |
|---|---|---|---|---|---|---|---|
| 1 | epicatechin | 1.719 | 21626 | 110447.1 | 33.443 | 1.31 | 2257 |
| 2 | apigenin | 2.042 | 15312 | 103085.5 | 31.214 | 1.20 | 1833 |
| 3 | luteolin | 3.360 | 1376 | 15218.4 | 4.608 | 0.75 | 1840 |
| 4 | myricetin | 4.337 | 3548 | 101499.5 | 30.734 | 0.89 | 458 |
| | Sum: | | 41862 | 330250.5 | 100.0000 | | |

*Figure 6: Chromatogram Of Flavonoids Of Gymnema Sylvestre*

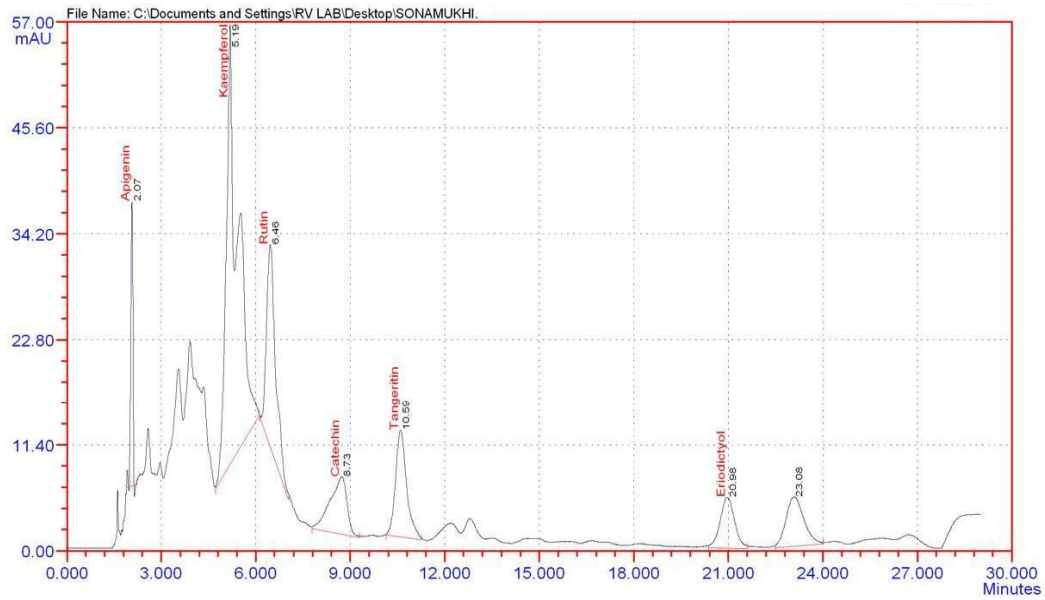
**HPLC Report**




| ID | Name | Retain.T | Height | Area | Conc | Tail.Factor | Theo.Plate |
|---|---|---|---|---|---|---|---|
| 1 | epicatechin | 1.673 | 776 | 2576.6 | 0.609 | 3.99 | 5063 |
| 2 | apigenin | 2.070 | 2271 | 10764.1 | 2.546 | 0.63 | 3802 |
| 3 | quercetin | 3.335 | 7391 | 102188.9 | 24.172 | 1.08 | 1160 |
| 4 | luteolin | 3.948 | 781 | 15144.8 | 3.582 | 1.27 | 826 |
| 5 | kaempferol | 4.993 | 2028 | 27549.1 | 6.516 | 0.04 | 2693 |
| 6 | Rutin | 6.637 | 989 | 35592.0 | 8.419 | 0.78 | 678 |
| 7 | Tangeritin | 10.432 | 6711 | 152612.6 | 36.099 | 0.69 | 4194 |
| 8 | Hesperetin | 14.102 | 722 | 24440.8 | 5.781 | 1.11 | 3459 |
| 9 | Taxifolin | 16.148 | 331 | 9680.2 | 2.290 | 1.19 | 6077 |
| 10 | Eriodictyol | 24.333 | 641 | 42210.6 | 9.985 | 1.87 | 2721 |
| | Sum: | | 22641 | 422759.6 | 100.0000 | | |

*Figure 7: Chromatogram Of Flavonoids Of Andrographis Paniculata*

**HPLC Report**




| ID | Name | Retain.T | Height | Area | Conc | Tail.Factor | Theo.Plate |
|---|---|---|---|---|---|---|---|
| 1 | Apigenin | 2.072 | 3072 | 14552.9 | 5.419 | 0.14 | 3811 |
| 2 | Kaempferol | 5.187 | 4772 | 120781.5 | 44.974 | 1.87 | 837 |
| 3 | Rutin | 6.462 | 2186 | 43686.4 | 16.267 | 1.04 | 2084 |
| 4 | Catechin | 8.730 | 619 | 22882.3 | 8.520 | 0.71 | 1112 |
| 5 | Tangeritin | 10.592 | 1148 | 28217.3 | 10.507 | 1.18 | 3701 |
| 6 | Eriodictyol | 20.975 | 547 | 17391.3 | 6.476 | 1.04 | 8674 |
| 7 | | 23.078 | 531 | 21044.7 | 7.836 | 1.28 | 6758 |
| | Sum: | | 12875 | 268556.4 | 100.0000 | | |

*Figure 8: Chromatogram Of Flavonoids Of Cassia Angustifolia*

**8.Calibration Curve Of Quercetin Standard**

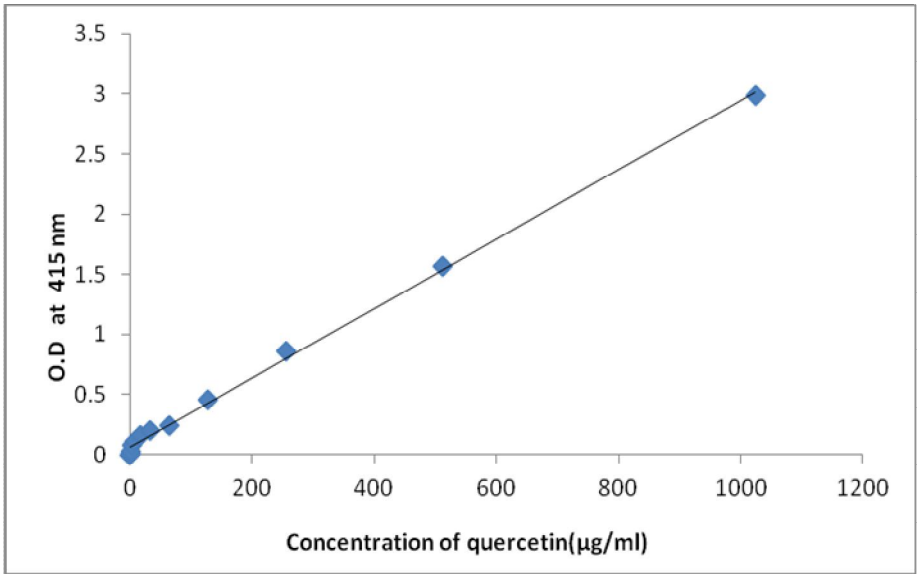



*Figure 9: Calibration Curve Of Quercetin Standard*

**9.Obtained Values**

Gymnema sylvestre : 0.403

Andrographis paniculata : 0.364

Cassia angustifolia : 1.113

Calculation of Absorbance was done by using the formula Y= mx+c.

Absorbance = slope x concentration + intercept.

Intercept=0.06637.

Slope: 0.00288.

Correlation coefficient: 0.9989.

Gymnema sylvestre (Podapatri)=0.403-0.06637/0.00288=116 ppm.

Andrographis paniculata (Nelavemu)=0.364-0.06637/0.00288=103 ppm.

Cassia angustifolia (Senna)=1.113-0.06637/0.00288=363 ppm.

**10. Discussion**

In this study the presence of Flavonoids and total content of Flavonoids in Gymnema sylvestre, Andrographis paniculata, Cassia angustifolia plants were determined . All these plants are medicinally important in Ayurveda. These are used to treat diseases such as Diabetes, Cancer. Antioxidants in plants like flavonoids and phenolic compounds involves in curing the diseases. So the presence of flavonoids was studied in this work,

An isocratic system was chosen to minimize the price and for high resolution, good accurate results in HPLC.The mobile phase, as well as the other chromatographic conditions, showed high performance in the separation of the flavonoids.

The methanol extract from the leaves of Gymnema sylvestre , Andrographis paniculata, Cassia angustifolia were subjected to preliminary phytochemical screening and further the methanolic extract were subjected to liquid–liquid fractionation using methanol and water solvents. The chromatographic conditions applied for flavonoids include an isocratic elution with methanol: water (50:50 v/v) for 30 minutes and flow rate at 1.0 ml/min, at 298 nm, Detector Wavelength and Kromasil C18 Column was used. 20 µl of methanol extract is injected.

These were analyzed by HPLC method to show the number of compounds present in these extracts. Flavonoids are natural Antioxidants which are having Anticancer, Diuretic, Anti diabetic, Anti-inflammatory properties. The Flavonoids found in Gymnema sylvestre was Epicatechin (R.T-1.72) Apigenin (R.T-2.04) Luteolin (R.T-3.36) Myricitin (R.T-4.34). Due to the presence of these flavonoids in this plant it exhibits medicinal properties. These are useful for controlling the loss of body weight in diabetic patients.

Andrographis paniculata commonly called as Nelavemu, consists of Flavonoids which are useful for treating several diseases, as this plant contains flavonoids such asQuercetin(R.T3.33),Luteolin(R.T3.95),Kaempferol(R.T4.99),Rutin(R.T6.64),Tangeretin(R.T-10.43),Taxifolin(R.T-16.15),Eriodictyol(R.T-24.33).

Cassiaangustifolia,hasfollowingflavonoidssuchasApigenin(R.T2.07)Kaempferol(R.T5.1) Rutin(R.T6.46)Catechin(R.T8.73)Tangeritin(R.T10.59)Eriodictyol(R.T-20.98). Due to the presence of these flavonoids in this plant it exhibits medicinal properties, used in Ayurvedic medicines in treating Diabetes and Cancer, Viral fevers.

Aluminium nitrate reacts with flavonoids present in the plant extracts and leads to formation of pale yellow color after incubation. The absorbance values of the solutions are proportional to the concentrations of flavonoids present in the plant extracts. By using the Aluminium nitrate method the total flavonoid content present in the Gymnema sylvestre was found to be 116 ppm, and Andrographis paniculata was 103 ppm and Cassia angustifolia was 363 ppm. Therefore Estimation of total flavonoid content in Gymnema sylvestre, Andrographis paniculata, and Cassia angustifolia was done by using Spectrophotometer.

**11.Conclusion**

This study revealed the presence of Flavonoids, such as Epicatechin, Apigenin, Quercetin, Luteolin, Myricitin, Kaempferol, Naringenin, Rutin and Catechin in plant extracts. Apigenin were detected in all the three plant extracts. Epicatechin was eluted first and it is dominant. Luteolin and Eriodictyol are in low quantities. Flavonoids are water soluble polyphenolic compounds, which are extremely common and wide spread in the plant kingdom as their glycosides. Flavonoids are natural Antioxidants which are having disease controlling properties. Recent advances and research on flavonoids and phenolic compounds have been proved that these flavonoids are widely useful in treating several diseases .As flavonoids are having this type of properties these can be used extensively in manufacturing of Medicinal drugs which can cure diseases like Diabetes, Cancer, Rheumatoid arthritis, Viral fevers etc.

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