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PHENOLIC ACIDS AND FLAVONOIDS QUANTIFICATION OF FRACTIONS ISOLATED FROM *VIGNA MUNGO* AND *VIGNA RADIATA*

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ABSTRACT

Two plant species of the genus Vigna were selected for the study. These are *Vigna mungo* L Hepper and *Vigna radiata* L. R. Wilczek. Both were authenticated and identified from the BSI, Pune. Seeds of both plants were selected for the analysis. Plant material was extracted by using ethanol and water as a solvent. A column chromatographic technique was used for the separation of the compounds from the extract by using n-hexane, chloroform and methanol in different concentrations. Total four fractions were selected for the study. Those are VMF1, VMF2, VRF3 and VRF4. Preliminary phytochemical tests were performed. EE of *Vigna mungo* was showing presence of carbohydrates, saponins, flavonoids, alkaloids and tannins in preliminary analysis. Carbohydrates, saponins, flavonoids, phenolics and tannins were identified in the EE of *Vigna radiata.* VMF1 and VMF2 was indicating presence of the flavonoids and phenolic compounds in significant concentrations. VRF3 and VRF4 of *Vigna radiata* were indicating the flavonoid and phenolic compounds. Phenolic acids and flavonoids quantification of fractions isolated from

Vigna mungo and *Vigna radiata* was performed by using 10 standards by using HPLC-DAD-MS/MS system. Extracted ion chromatogram of *Vigna mungo* VMF1 shows presence of rutin and quercetin.VM fraction 2 shows rutin and quercetin. Gallic acid, syringic acid, quercetin and kaempferol are present in *Vigna radiata* fraction 3. Syringic acid was found to be present in higher concentration. Fraction 4 of *Vigna radiata* shows presence of syringic acid, cinnamic acid and quercetin.

Keywords: Phenolic Acids and Flavonoids Quantification, *Vigna Mungo*, *Vigna Radiata*

INTRODUCTION

In the battle against diseases two approaches were attempted by humans. These are Empirical and Speculative. Recent technology has changed these methods. Even after advancement of modern technology, we still depend upon traditional method. It is called as trial and error method. It looks as if synthetic and modern drugs would make organisms independent upon plant origin drugs. But this was not occurred. Recent studies shown that majority of prescriptions in US and other European countries were from natural origin. In modern system of medicine, plants are playing very crucial role. They play important role in separation and identification of phytochemicals or active principles of plants and study its therapeutic activities. These principles can be used instead of crude extracts and active principles can apply for treatment of different signs and symptoms. Many important categories of medicines are obtained from the plants. Plants are considered as a largest segment of biodiversity. Due to plants gene expressions and variation in environment, each plant becomes unique. It is unique for its medicinal value due to its chemical composition. Plants are source of medicine as it contains active constituents. One species or plant can be used for different disorders **[1]**. Bioactive compounds can have an influence on health. Plants and animals possess bioactive compounds. These are classified according to different criteria like pharmacological or toxicological effects or chemical nature. Based on their chemical nature these are classified as glycosides, tannins, flavonoids, proanthocyanidins, terpenoids, resins, lignans, alkaloids, coumarins etc. **[2]**. Food is obtained from the plants. Foods can be potential source of medicine or ex active principles with pharmacological activity. Food consists of nutritional constituents and non-nutritional compounds. These components or secondary metabolites are biomolecules which possess

the capacity to produce health benefits and maintains health of well-being. These are also known as bioactive compounds and available in small concentrations. Example includes phenolic compounds, flavonoids, organophosphorus compounds, phytoestrogens, lycopene's, sterols, soluble dietary fibres etc. **[3]**.

In many parts of the world legumes and pulses are playing crucial role in food stuffs. Legumes are also providing carbohydrates, several water soluble vitamins, and minerals to human nutrition other than proteins **[4]**. Examples of legumes are Mung bean, adzuki bean, rice bean, black gram, etc. **[5]**. Legumes are rich in bioactive compounds like phytoestrogens, flavonoids and other. Reported literature suggested that legume phytoestrogens and saponins have anticancer and hypocholesterolemic action. Saponins found to be effective in colon cancers. Application of food in treatment of diseases will improve in the future. Currently many scientists are in search of novel compounds having pharmacological potential. These compounds could be helpful for development new therapeutic agents or pharmaceutical excipients or ingredients of dietary supplements derived from natural products **[6]**.

It is well known that natural products derived from plant and animals worked as source of compounds with good pharmacological properties. Large population (80%) still relies on medicines derived from plant based products for primary health care. Knowledge related to traditional plant based medicine encouraged new investigations of medicinal plants as a source of potential medicines and led to development of potent medicinal preparations. There is substantial scientific and commercial interest developed in the discovery of new drugs from natural sources. Legumes are very important group of plant food stuffs, particularly in the developing world. Legume seeds are valuable sources of proteins and nutrients for the majority of the world population. Legumes are also providing carbohydrates, several water soluble vitamins, and minerals, to human nutrition other than proteins. Legumes are good source of nutritional and antinutritional compounds with numerous health benefits. Legumes, or pulses, generally belong to the Leguminosae family. Vigna is one of the important genus of leguminosae family. Recently, pulses and legumes have been gaining interest because they are excellent source of bioactive compounds. Theses bioactive compounds may have good biological activities. Plant species belonging to the genus vigna are easily available and it is commonly used as a food. It is considered as poor man's meal. Report suggested that

legumes contain greater varieties of toxic constituents than any other plant family. Therefore this genus is selected for the study. Detailed review of literature suggested that polyphenols, flavonoids, trypsin inhibitors etc. are responsible for various therapeutic activities. Review of bioactive compounds of black gram and green gram suggested the presence of phytoconstituents like flavonoids, phenolic compounds, trypsin inhibitors etc. which is having therapeutic properties. Therefore two legumes grown and consumed in India on large scale are green gram (*Vigna radiata*) and black gram (*Vigna mungo*) was selected for this study. Aim of the present research work is to extract and characterize potential secondary metabolite compounds from the selected plant materials.

METHODOLOGY

Collection and authentication of Plant Material

Two plant species from the genus vigna was selected for the study. Plant material was collected from the farms of local farmers situated at Post Markal, Dist. Pune, Maharashtra, India. Voucher specimens of plant species were prepared and submitted to Botanical Survey of India, Pune for authentication.

Extraction of Plant Material

Seeds of the *Vigna mungo* and *Vigna radiata* were taken for the phytochemical analysis. Selected coarse powder material was defatted by using Petroleum ether in Soxhlet extractor. Defatted plant material was air dried and it was then subjected to further extraction. Seed material was extracted by using ethanol and water as a solvent in Soxhlet extractor. Materials were extracted till the colorless liquid appears in the extractor. The mixture of solvent and extracted material was subjected to a rotary vacuum evaporator for separation of the solvent and extract. The solvent was evaporated. The extract was collected and dried in the Vacuum oven at a lowest temperature condition. Dried extract was then kept in the desiccator. Extract was designated as ethanolic extract. (EE)

Separation of compounds from the extracts:

Column chromatography method was used for separation. Glass column was taken and placed on the stand. A glass wool was plugged at the bottom of column. With the help of glass rod wool was firmly lodged at the bottom of column. Thin layer of sand was placed above the glass wool. Slurry of silica gel was prepared in hexane. Slurry was gently poured in the column using a funnel. Sand that adhered to the wall of column was

removed by addition of hexane using pipette. For uniform packing and removal of air bubbles column was tapped gently. Thin layer of sand was placed on the top of silica gel. Semisolid extract of the sample was loaded on the top of sand. With the help of pipette 4-5 ml of hexane was added ensuring the uniformity of the sample extract. Remaining hexane was added very slowly using funnel. Stopcock was open and allowed solvent to drain very slowly. Eluted solvent was collected in conical flask. Initially n hexane was used then n hexane and chloroform in different ratios (70:30, 10:90) were added. Column was eluted with chloroform (100%). Further solvent polarity was changed using Chloroform and methanol in different ratios (80:20, 50:50, 30:70, 100). Different fractions were collected and labeled for the further analysis. During separation colored compounds were separated by visual identification. Colorless compounds were identified by UV and thin layer chromatography. Fractions with similar properties were mixed together and evaporated in rotary vacuum evaporator to isolate fraction.

Preliminary phytochemical screening:

Fractions isolated from the extract were subjected to the preliminary phytochemical analysis. These tests were performed to identify phytoconstituents present in the extracts and fractions

Quantitative analysis of Phenolic compounds by using LC-DAD-ESI-MS/MS analysis

Quantitative analysis of phenolic compounds from the isolated fractions was achieved by LC-DAD-ESI-MS/MS technique by using ten different standards. Instrument used was TOF/Q-TOF Mass Spectrometer (G6560A). Marker compounds used were Gallic acid, catechin, caffeic acid, syringic acid, rutin, cinnamic acid, quercetin, luteolin, kaempferol and apigenin. Solvent system was used in different combinations of Water, Acetonitrile and formic acid. Wavelengths used for scanning were 280nm, 344nm and 400nm. Mass spectrum of unknown compounds was recorded. Data was compared with the online library of the compounds and it was then identified.

RESULT AND DISCUSSION

The genus Vigna is very large genus in Asia with many species distributed in 7 subgenera. The genus Vigna is important part of the diet of Asian community. About one third community is using it as a part of balanced diet. The genus vigna is attractive because it shows very high nutritional value **[7]**. Two plant species *Vigna mungo* and *Vigna radiata* from the genus vigna was selected for the

study. Both plants are easily available for the study. These plants were used traditionally for the treatment of various ailments. In recent years studies have suggested that mung beans and udid beans have more biological activities. Scientific evidence suggested the presence of number of secondary metabolites and their isolation from both plant species. Seeds of the both plant were selected for the study. Plant material was collected from the farms of local farmers situated at Post Markal, Pune Maharashtra, India. (Latitude- 18.666590, Longitude- 73.958370). Seeds of the plants were collected from the pods of the plants. Plants pods were dried and then seeds were collected.

Authentication of the Plant Material

Plants were identified by the authentication letter. Reference number of letter was BSI/WRC/IDEN.CER./2016-352 dated 23/09/2016. Plants were identified as *Vigna mungo* L Hepper and *Vigna radiata L.R.* Wilzack, both belonging to Fabaceae family. Their voucher specimen numbers were DJS05 & DJS04 respectively.

Extraction

Solvent extraction is the most common method for phenolic compound isolation. Various solvents and their combinations help to improve the extraction of phenolic compounds. Example Methanol, acetone, ethanol, ethyl acetate etc. Polar phenolic compounds could not be extracted completely with organic solvent alone and hence mixture with water is preferred method. Sample pretreatment with petroleum ether was given to improve the stability of the sample and to enhance the extraction process efficiency. Soxhlet method is popular technique and it was used for the extraction in this study. Yield of extraction of Vigna *mungo* and *Vigna radiata* was found *9.75*% & 10.54% respectively.

Column Chromatography

Column was first eluted with hexane **(Table 1)**. First fraction (F1) was isolated showing three spots on the TLC plate. It was indicating presence of non-polar constituents. It was considered as hexane wash. After hexane wash the polarity of solvent was changed by addition of chloroform (70:30). Fractions of hexane and chloroform combinations showing nothing on TLC plates. Hexane: chloroform combinations were used in the ratio of 70:30 and 10:90. After evaporation of these fractions significant concentration of fraction was not observed. As these combinations showing nothing in fractions we decided to change the polarity. Polarity of the solvent was changed by addition of methanol in ratio of 80:20.

This ratio was successful and showing single spot on plate. Fraction was light yellowish green in colour and showing RF of 0.344. All these fractions were collected in the conical flask and further it was evaporated. It was showing fraction in pure form and in considerable quantity. Hence this fraction was identified for further analysis. It was designated as VMF1. Solvent system polarity was further altered by methanol (50:50). It was showing two spots on the plate. Fraction was colorless and quantity after evaporation was less. This was not taken for further analysis. Final fraction was collected at Chloroform: methanol concentration of 30:70. This fraction was labeled as VMF2. It was showing significant quantity after evaporation and also showing single spot on plate with RF of 0.86. This fraction was yellowish cream colored.

Column after loading of the EE of *Vigna radiata* was first eluted with the hexane. **(Table 2)** Fractions of hexane wash was collected together and evaporated. It was showing two spots at 0.34 and 0.52 RF respectively. Hexane wash was greenish in the colour. It was dried and kept for the analysis. Polarity of the solvent was altered by using chloroform in various concentrations with hexane like 80:80, 50:50, 30:70 etc. All fractions isolated showing no spots on the TLC plate indicated absence of desired phytoconstituents. Hexane solvent was replaced with methanol to elute more polar constituents. It was showing promising results with methanol in concentration of 60:60 and 20:80. Both fractions were showing single spots on plate at RF value of 0.45 and 0.61 respectively. These fractions were labeled as VRF3 and VRF4.

Phytochemical analysis of the *Vigna mungo* and *Vigna radiata* EE extracts showed significant quantities of total phenolic content and total flavonoid content. Many flavonoids and phenolic compounds were isolated and reported in both plants. **[8]** Therefore it was decided to fractionate EE extract by using n-hexane-chloroform-methanol solvent system. **[9]** Flavonoids are distributed in nature with varying polarity. Extraction of flavonoids depends upon their polarity and hence solvents are selected according to their polarity. For extraction of less polar flavonoids chloroform, dichloromethane, ether, ethyl acetate etc. can be used as a solvent. Example of less polar flavonoids includes isoflavones, dihydroflavonols etc. Acetone, alcohol or their combinations can be used as solvent system for extraction of polar flavonoids. Different chromatographic techniques are available for flavonoid isolation but column chromatography is one of the most useful methods. It is most

suitable method for isolation of com-pounds on large scale. It is acceptable because of its simplicity and suitability. Silica gel, cellulose, polyamide etc. are commonly used adsorbents in column chromatography. For less polar flavonoids silica gel is recommended adsorbent. Some polar flavonoid glycosides may be eluted by using more polar solvents on silica gel adsorbent **[10, 11]**.

Preliminary phytochemical screening:

Preliminary phytochemical analysis of the isolated fractions and extracts were performed according to the tests mentioned in Pharmacognosy practical handbook. In the discovery and development of novel bioactive compounds from the plants preliminary phytochemical analysis is considered as very effective and important. EE of *Vigna mungo* was showing presence of carbohydrates, saponins, flavonoids, alkaloids and tannins in preliminary analysis. EE displayed negative tests for the proteins, amino acids, fats and oils, cardiac and Anthraquinone glycosides. VMF1 and VMF2 was indicating presence of the flavonoids and phenolic compounds in significant concentrations.

EE and EEH of *Vigna radiata* were showing occurrence of different phytoconstituents. Carbohydrates, saponins, flavonoids, phenolics and tannins were identified in the EE. Tests for proteins, amino acids, fats, steroids, cardiac and anthraquinone glycosides were found to be negative. Both fractions VRF3 and VRF4 of *Vigna radiata* were indicating the flavonoid and phenolic compounds.

LC-DAD-ESI-MS/MS analysis

Phenolic acids and flavonoids quantification of fractions isolated from *Vigna mungo* and *Vigna radiata* was performed by using 10 standards. Standards used are gallic acid, catechin, caffeic acid, syringic acid, rutin, cinnamic acid, quercetin, luteolin, kaempferol and apigenin. Stock solutions were prepared at 1mg/ml in ethanol. 10 µL of the standard and sample was taken and analyzed in the HPLC-DAD system.

Calibration curve of mass concentration vs. peak area was prepared and concentration of standard was calculated from that curve. R2 value and slope of each standard curve was calculated. Following equation was used to quantify phenolic acids and flavonoids

Amount of phenolic acids/flavonoids (g/g) = Amount s × Amount sc × IRF sc/ Area s

IRF= internal response factor
IRF = Area s × Amount sc/Amount s × Area sc
s = internal standard; sc = separated phenolic acids and flavonoids from fractions (Kuppusamy *et al.*, 2018)

HPLC analysis allows instantaneous separation and identification of phenolics and flavonoids. We found good peak resolution

by using binary solvent system water and Acetonitrile. It helped us to identify both phenolic and flavonoids in fractions. Solvents are important for extraction efficacy and yield. Highest yield of extraction was obtained by using ethanol and water system. Numonov et al. (2015) have reported that 80% ethanol extraction brought the highest extraction yield due to its functional groups **[12]**.

Formic acid has good buffering capacity in pH range of 2-4 and hence it is used as a good volatile mobile phase. It is suitable for separation of Polyphenol compounds if used in 0.1% concentration **[13]**. Acetonitrile and acidified water mobile phase (with acetic acid) can leads to better peak resolution. It is better than phosphoric acid and methanol solvent system. It also gives sharp and symmetrical peaks with minimum noise.

Extracted ion chromatogram of *Vigna mungo* VMF1 shows presence of rutin and quercetin in the concentration of 84.04 µg/10 mg and 0.299 µg/10 mg of extract. VM fraction 2 shows rutin (0.072 µg/10 mg) and quercetin (0.017 µg/10 mg). Gallic acid, syringic acid, quercetin and kaempferol are present in *Vigna radiata* (VR) fraction 3. Syringic acid was found to be present in higher concentration. Fraction 4 of *Vigna radiata* shows presence of syringic acid, cinnamic acid and quercetin. Catechin, caffeic acid, luteolin and apigenin were not quantified in all four fractions. Among ten standard phenolic acid and flavonoid rutin was present as a largest mass fraction followed by syringic acid, and kaempferol **(Table 3)**.

Thin layer chromatography and paper chromatographic techniques were used earlier for the separation of flavonoids and phenolic acids **[14]**. These methods are time consuming methods. Their separation power is insufficient. Gas chromatography is advanced technique for the separation with excellent resolution. However derivatisation in GC-MS makes this method lengthy **[15]**. Capillary zone electrophoresis is another method with convincing results. Small volume of solvent is required in this method, still it shows lower resolution when complex samples are applied. HPLC-MS proved to be sensitive method even in very complex samples. **[16]**. LC is capable of separating complex compounds and therefore it becomes best method in separation of complex mixtures. LC method is not suitable to determine structural information of the samples. Structural information related to the compound can be obtained by using MS. It requires highly purified samples for better results. So for better results combination of LC-MS is necessary. In this technique LC is

used for the separation and MS for the detection of spectrum. This method provides accurate results for qualitative and quantitative analysis **[17]**. Molecules eluted from the LC column are under pressure. Mass spectrometer unable to detect the molecules when they are in continuous flow. MS operates in vacuum and it need to be free from liquid. To remove liquid/mobile phase interface is used. Many interfaces are available. Most commonly applied interfaces include electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) systems, etc. In this interfaces samples is finely nebulized and converted into ions and then passed into the MS detector. Reports suggested that for highly accurate analysis of known and unknown samples LC-MS/MS is better than LC-MS. Combination of DAD with LC–MS can provide information of individual peak in chromatogram at very low concentrations of the sample with short time for analysis **[18]**. For quantification of individual constituents HPLC-DAD is suitable method for rapid and accurate analysis. Normally binary solvent system and reverse phase C18 column is used for quantification. Water is a polar solvent and Acetonitrile or methanol is used as non-polar solvent. Separation of various phenolic and flavonoids need different and specific conditions as per nature of the compounds **[19]**. Therefore this method was selected for the analysis of isolated fractions

LC chromatogram of VMF1 fraction mixture recorded at 366nm is shown in figure no 6.27. Out of many peaks, peak at 10.140 min showing presence of flavonoid and after MS interpretation it was identified as a rutin. LC-MS/MS of VMF1 separated different fractions at retention time of 1.527, 8.053, 10.140, 11.433, 16.160 and 19.733 **(Figure 1)**.

In the LC-MS/MS studies fraction VMF2 shows RT of 1.613, 9.227, 11.00, 16.120, 20.247 and 22.127. These fractions after separation were analyzed by using mass spectrometry **(Figure 2)**.

VMF1 showed various compounds after MS/MS. Different RT for VMF1 were 1.613, 8.053, 10.140, 11.433, 16.160 and 19.733. VM F1 fraction shows presence of rutin and quercetin. Rutin is present in conc. of 84.05 µg/10mg of fraction. It is confirmed by mass spectra of sample at 10.140 min retention time. While quercetin spectra was observed at 13.027 min and its concentration was found as 0.299 µg/10mg. VM F2 fraction shows presence of Kaempferol-3-O-rutinoside at RT of 9.227 min. VR fraction 3 was showing RT of 1.533, 3.003, 4.200, 6.033, 8.347, 9.347, 10.887, 12.707, 16.227,

19.093 and 22.100. VR F3 fraction shows presence of gallic acid, syringic acid, kaempferol and quercetin. Syringic acid is present in higher concentration (2.62782082 µg/10mg) than other compounds.

Kaempferol is also present in higher concentration 1.15413231 µg/10mg were identified by NIST library search in VR F3. Syringic acid, cinnamic acid and quercetin were quantified in VR F4. Syringic acid was present in higher concentration 0.70004337 µg/10mg.

Table 1: Fractionation of *Vigna mungo* EE extract in Column chromatography

Sr. No	Mobile Phase	Ratio	Fraction	No of spots	Description	Rf value
1	n-hexane	100:0	F1	3	Green color	0.72, 0.21, 0.35
2	n hexane: chloroform	70:30	0	---	---	---
3	n hexane: chloroform	10:90	0	---	---	---
4	Chloroform	100:0	0	---	---	---
5	Chloroform: MeOH	80:20	F2	1	Light yellowish/ greenish	0.344
6	Chloroform: MeOH	50:50	F3	2	Colorless	0.56, 0.81
7	Chloroform: MeOH	30:70	F4	1	Yellowish/cream colored	0.86
8	MeOH	100:0	0	0	---	---

Table 2: Fractionation of *Vigna radiata* EE extracts in Column chromatography

Sr. No.	Mobile Phase	Ratio	Fraction	No of spots	Description	Rf value
1	n-hexane	100:0	F1	2	Greenish	0.52, 0.34
2	n hexane: chloroform	80:20	0	---	---	---
3	n hexane: chloroform	50:50	0	---	---	---
4	n hexane: chloroform	30:70	0	---	---	---
5	Chloroform	100:0	0	---	---	---
6	Chloroform: MeOH	90:10	0	---	---	---
7	Chloroform: MeOH	60:40	F2	1	Whitish yellow	0.45
8	Chloroform: MeOH	20:80	F3	1	Yellowish cream colored	0.61
9	MeOH	100:0	0	0	---	---

Table 3: XIC (extracted ion chromatogram) based quantification

Sample	Gallic acid (1)	Catechin (2)	Caffeic acid (3)	Syringic acid (4)	Rutin (5)	Cinnamic acid (6)	Quercetin (7)	Luteolin (8)	Kaempferol (9)	Apigenin (10)
Linear regression equation	y = 5328.134876 * x + 1793.599894 R^2 = 0.98295553	y = 201154.126207 * x + 66493.348348 R^2 = 0.98557848	y = 120988.027884 * x + 43203.951510 R^2 = 0.97672555	y = 15529.723093 * x + 5344.992705 R^2 = 0.96307932	y = 399238.267843 * x + 13645.804320 R^2 = 0.99873887	y = 83865.584800 * x + 1364.431239 R^2 = 0.99695275	y = 598535.526711 * x - 7444.746954 R^2 = 0.99876026	y = 2211347.548497 * x + 205915.600845 R^2 = 0.99104043	y = 646593.486559 * x + 19912.802868 R^2 = 0.99663512	y = 933256.498603 * x + 2592120.382510 R^2 = 0.91837327
F1	NQ	NQ	NQ	NQ	84.0449825	0	0.29962985	NQ	NQ	NQ
F2	NQ	NQ	NQ	0	0.07151655	NQ	0.01667754	NQ	NQ	NQ
F3	0.03788599	NQ	NQ	2.62782082	NQ	NQ	0.02381961	NQ	1.15413231	NQ
F4	0	NQ	NQ	0.70004337	NQ	0.01696732	0.01657982	NQ	NQ	NQ

Table 4: Identification of compounds by LC-MS/MS data

RT (Min)	Molecular formula	[M − H]−	Fragment	Prediction	Fraction
10.140	$C_{27}H_{30}O_{16}$	608.9	300.028, 271.025, 255.030, 151.002	Rutin	VMF1
13.027	$C_{15}H_{10}O_7$	300.8	273.042, 243.028, 178.998, 151.004, 121.030	Quercetin	VMF1
9.227	$C_{27}H_{30}O_{15}$	285.41	285.041, 255.031, 227.035, 151.003	Kaempferol-3-O-rutinoside	VMF2
4.604	$C_7H_6O_5$	169.5	169, 125	Gallic acid	VRF3
8.226	$C_9H_{10}O_5$	198.17	197, 182 , 167 , 153	Syringic acid	VRF3

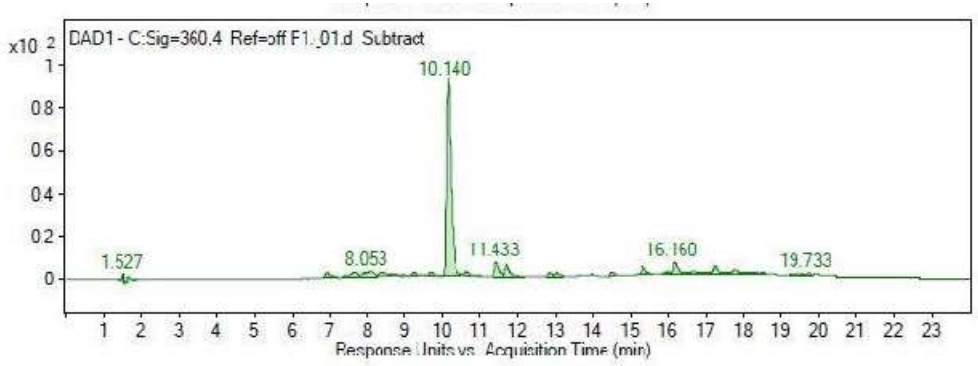

Figure 1: LC-DAD chromatogram of VMF1

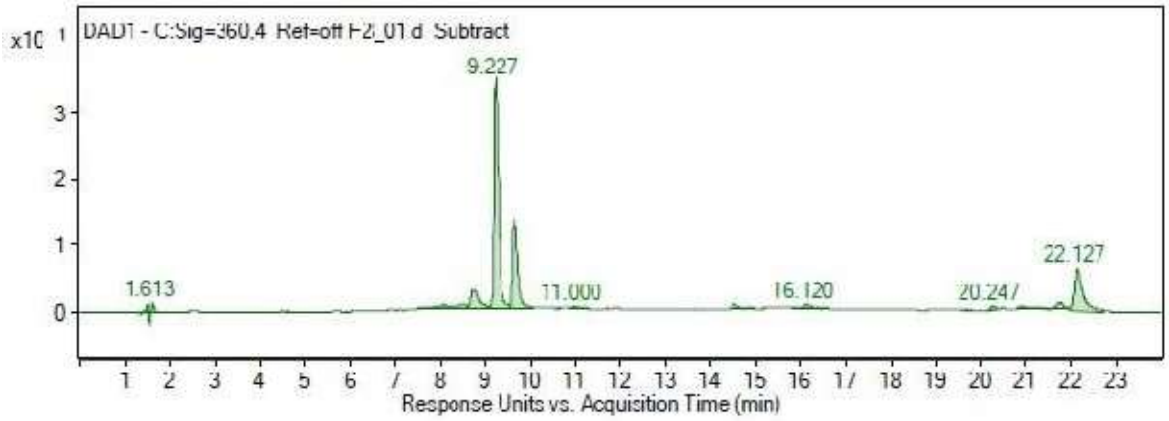

Figure 2: LC-DAD chromatograph for VMF2

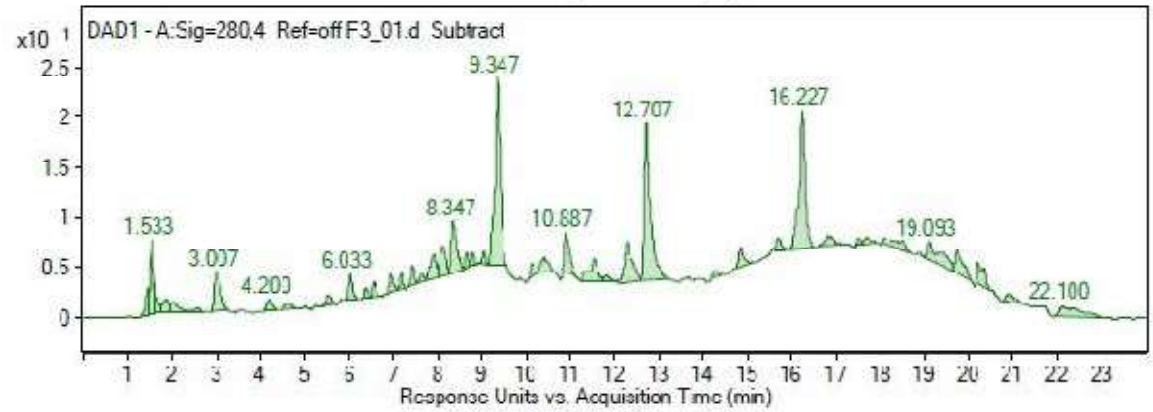

Figure 3: LC-DAD chromatograph for VRF3

SUMMARY AND CONCLUSION

VMF1 and VMF2 was indicating presence of the flavonoids and phenolic com-pounds in significant concentrations. VRF3 and VRF4 of *Vigna radiata* were indicating the flavonoid and phenolic compounds. Extracted ion chromatogram of *Vigna mungo* VMF1 shows presence of rutin and quercetin.VM fraction 2 shows rutin and quercetin. Gallic acid, syringic acid, quercetin and kaempferol are present in *Vigna radiata* fraction 3. Syringic acid was found to be present in higher concentration. Fraction 4 of *Vigna radiata* shows presence of syringic acid, cinnamic acid and quercetin.

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