DOI:https://doi.org/10.31351/vol29iss2pp37-47

Phytochemical Investigation and Anti-angiogenic Examination of Iraqi *Vigna radiata* L. Seeds and Sprouts

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Abstract

The plant ***Vigna radiata* L.** which belongs to Fabaceae family and known mung bean with characteristic greenish seeds. It was cultivated in Iraq in Al-Nasiriya city. Literature survey available so far revealed there was no studies about Iraqi *Vigna radiata* plant and its antiangiogenic activity, therefore the objective of this study was to investigate the phytochemical constituents of two different parts of *Vigna radiata* (seeds and sprouts), and identify their antiangiogenic activity .The flavonoids were isolated by preparative layer chromatography and subjected to different physio-chemical and spectral analytical techniques to identify their chemical structure; rat aorta anti-angiogenesis assay was conducted for both n-butanol fraction of seeds and sprouts. The results showed that two flavonoids (vitexin and isovitexin) were isolated in pure form, both n-butanol fraction of seeds and sprouts have antiangiogenic activity, but sprout extract showed highest blood vessels inhibition in comparison with seeds extract. In conclusion, the difference of Antiangiogenic activity may be related to variation of concentration of bioactive constituents or appearance of new bioactive constituents during germination.

Keywords: *Vigna radiata*, vitexin, isovitexin, high performance thin layer chromatography HPTLC.

دراسة كيميائية نباتية وفحص الفعالية المثبطة للأوعية الدموية لبذور وابواغ نبات الماش في العراق

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الخلاصة

نبات الماش هو نوع من النباتات التي تنتمي الى العائلة البقولية ويعرف بالماش او اللوبيا الشـــعاعية مع بذور ذات اللون الأخضـــر التي يتميز بها. وهو نبات مســتزرع بالعراق في جنوب العراق وتحديدا في مدينة الناصـــرية. ونظرا لعدم وجود دراســات حتى الوقت الحالي عن نبات الماش العراقي وفعاليته المثبطة لتوليد اوعية دموية جديدة لذا أصـبح الهدف من الدراســة هو دراســة تحليلية كيميائية لجزئي البذور والابواغ لنبات الماش العراقي مع عمل دراســـة مقارنة لدرجة تأثير كل من الابواغ والبذور في تثبيط عملية تطوير اوعية دموية جديدة تم عزل فلافونويدات من جزء البيوتانول باستخدام كروماتوغرافيا الطبقة الرقيقة التحضـيري وقد استخدمت التقنيات الفيزيائية والكيميائية والتحليلية الطيفية لتحديد تركيبها الكيميائي وايضـــا تم اختبار فحص تثبيط الاوعية الدموية لكلا المســـتخلص البيوتانولي لبذور وابواغ نبات الماش.النتيجة تشـــير الى عزل مادتي الفتكسين والايزوفتكسين بشكل نقي , كلا البذور والابواغ لها تأثير مثبط للأوعية الدموية لكن المستخلص البيوتانولي لأبواغ نبات الماش يملك نسبة تثبيط الاوعية الدموية اعلى من المستخلص البيوتانولي لبذور نبات الماش . الاختلاف في نسبة تثبيطها للأوعية الدموية يكون نتيجة الاختلاف في تركيز المواد الفعالة او ظهور مواد جديدة اثناء عملية الانبات.

الكلمات المفتاحية: نبات الماش، الفتكسين الايزوفتكسين، كروماتوغرافيا الطبقة الرقيقة ذات الاداء العالي.

Introduction

Natural compounds provide a large of antioxidants which act as radical scavengers and help in converting the radicals to less reactive species. *Vigna radiata* L. plant also known mung bean. Mash is shrub, belong to Fabaceae family and is native in China, India Bangladesh, South East and Western countries [(1,2)]. Mung beans contain balanced nutrients, including protein and dietary fibers, and a large number of bioactive phytochemical constituents. These bioactive phytochemical constituents, thought to be the main contributor factors to the antimicrobial, antioxidant, antitumor activity [(4,5)]. Flavonoids are the important metabolites found in mung bean [(6,7)]. Germination of mung bean seeds improves the antioxidant activity and elucidate important metabolites level for better usage. Germinating (sprouting) of mung bean by breeding or usage some hormones [(8)].

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Received:17 / 9/ 2019
Accepted: 8/3 /2020

Iraqi Journal of Pharmaceutical Science

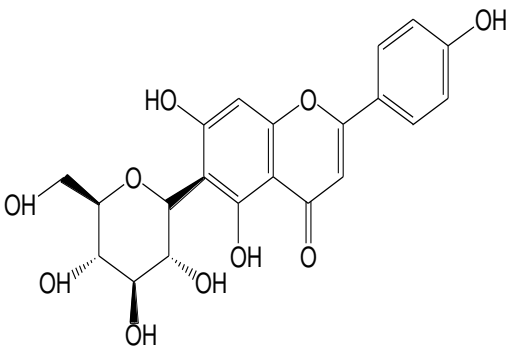
Figure 1. Iraqi *Vigna radiata* L.plant [3]

Germination of mung bean seeds improves the antioxidant activity and elucidate important metabolites level for better usage. Germinating (sprouting) of mung bean by breeding or usage some hormones [8].

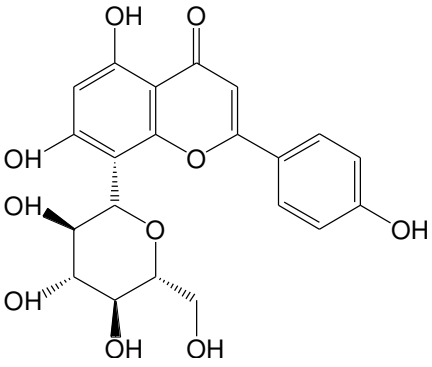
Vigna radiata contains large numbers of proteins [9], during germination proteolytic cleavage of proteins lead to elevate levels of amino acids. Cinnamic acid, p-hydroxy benzoic acid and gentistic acid are the main phenolic acids that reformed during germination. [10,11]

Mash have been used in Asia countries as traditional herbal medicine which have a large variety of beneficial effects, mash is known for its detoxification effects and is used to regulate gastrointestinal upset, to moisturize the skin, and to minimize the swelling in the summer [12]

Angiogenesis is a process of new blood vessel formation from pre-existing one, regulatory by a variety of endogenous cytokines and several regulating factors including growth factor. Angiogenesis plays a vital role in the growth and metastasis of tumor and several chronic anti-inflammatory diseases such as rheumatoid arthritis and proliferative diabetic retinopathy. Vitexin (apigenin – 8 – C– β glucopyranoside) and isovitexin (apigenin – 6 – C – β glucopyranoside) have been reported the important flavonoids that present in *Vigna radiata* seeds [13,14] as shown in Figure (2) .Vitexin has been reported to exhibit anti angiogenic activity by its effect on hypoxia-inducible factor -1α HIF-1α in rat pheochromacytoma PC12 [15,16].



(a)



(b)

Figure 2. Structures of isovitexin (a) and vitexin (b) [17]

Materials and Methods

Plant material

Vigna radiata seeds were collected from a farm in Nasiriya city during October 2018. The plant was identified and authenticated by Dr. Khansaa AL. Joboury in Iraq Natural History Research Centre and Museum / University of Baghdad.

Five hundred grams of seeds were cleaned from unwanted materials, washed in deionized water then left to dry in air for two days, following sample preparation for biochemical analysis for two parts:

Part A: seeds of mung bean were grinded in electric grinder to provide fine powder

Part B: seeds of mung bean were soaked in water over night. On next day these seeds were tied in muslin cloth for 3 days. Cloth containing soaked seeds were kept moist by spraying water every day in interval of 6 hours to germinate mung bean [18].

Extraction method

1. Grounded powder of seeds of 250 grams were defatted by maceration with hexane for 3 days then allowed to dry at room temperature. The defatted plant materials were extracted by soxhlet apparatus using aqueous ethanol 80% as a solvent for extraction for 18 hrs. [19] . Extract of seeds was filtered and the solvent was evaporated under reduced pressure using

rotary evaporator to make a dry extract of seeds (part A), then the residue was suspended in 250 ml of deionized water and partitioned successively with petroleum ether, chloroform, ethyl acetate and n-butanol (3 x 200) ml for each fraction. The first three fractions were dries over anhydrous sodium sulfate, filtered and evaporated to dryness.

2. The same procedure was applied to the sprouts, the dry extract obtained named part B.

Hydrolysis of n-butanol fraction of seeds of Vigna radiata L-plant

One gram of n-butanol fraction of seeds was hydrolyzed in 150 ml of 7% HCl for 6 hr. under reflux, cooled and partitioned with 150 ml x 3 Ethyl acetate, the organic layers were combined then concentrated to dryness over anhydrous sodium sulfate then evaporated by rotary evaporator, weighted and subjected for identification of these compounds by TLC and HPTLC.

Thin layer chromatography examination of extracts obtained from seeds and sprouts of Vigna radiata plant

In this qualitative identification using a ready-made aluminum plates of silica gel GF_{254} and using 3 different developing solvent systems for detection the plant flavonoid in fractions of ethyl acetate and n-butanol for seeds and sprouts

Comparing with flavonoids standards and detection under UV 254, 366 nm, and they are listed in the Tables (1 and 2):

Table 1. Developing solvent systems were used in the identification of expected Flavonoids in above fraction.

NO.	Composition	References
Sk_4	Ethyl acetate: chloroform: formic acid: water (8:1 :1:1)	20
Sk_5	Ethyl acetate: acetic acid: formic acid: water (84:4:4:10)	21
Sk_6	Ethyl acetate: formic acid: acetic acid: water (100:11:11:27)	22

Table 2. Thin layer chromatography for separated spots:

Mobile phase	Standard name	R_f value of standard	R_f value of matched flavonoid	Compound name
Sk_4	Isovitexin	0.33	0.32	R1
	Vitexin	0.51	0.5	R2
Mobile phase	Standard name	R_f value of standard	R_f value of matched flavonoid	Compound name
Sk_5	Isovitexin	0.3	0.29	R1
	Vitexin	O.41	0.41	R2
Mobile phase	Standard name	Value R_f of standard	R_f value of matched flavonoid	Compound name
Sk_6	Isovitexin	0.62	0.62	R1
	Vitexin	0.73	0.74	R2

Identification of vitexin and isovitexin by HPTLC

Ethyl acetate and n-butanol before and after hydrolysis fractions for the seeds, and ethyl acetate and n-butanol fractions of sprouts were analyzed also for its flavonoids, coumarin and phenolic acid contents utilizing HPTLC (Eike Reich/CAMAG Laboratory, Switzerland), using silica gel GF254 plates developed in a mobile phase composed of ethyl acetate: formic acid: acetic acid: water (84:4:4:10 V/V) examined at 254 and 366 nm wavelength.

The HPTLC results revealed the presence of vitexin and isovitexin in different Fractions ethyl acetate, n-butanol before and after hydrolysis fraction of seeds, ethyl acetate and n-butanol fractions of sprouts of *Vigna radiata* all these steps were done in a Baghdad - College of Pharmacy.

Isolation and purification of vitexin and isovitexin by preparative thin layer chromatography (PTLC)

Vitexin and isovitexin were isolated fraction from n-butanol fraction of seeds by preparative layer chromatography (PLC) using (1 mm) thickness plate (20 x 20 cm) and 100 ml of mobile phase (ethyl acetate: acetic acid: Formic acid: water) in the volume ratio of (84: 4: 4: 10) V / V.

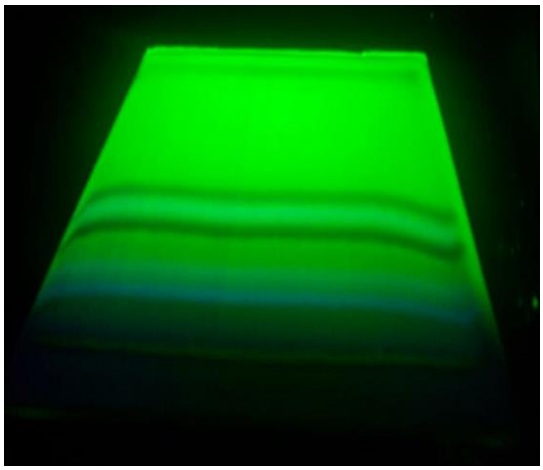


Figure 3. Preparative layer chromatography chromatogram of flavonoids isolation observed at 254 wave length

The purity of each isolated compound was examined using the analytical thin layer chromatography until obtaining single spot on TLC plate and detection was recorded under UV light 254 and 366 nm. All these steps were done at College of Pharmacy / University of Baghdad. The isolated compound was recrystallized using hot methanol and compared with vitexin and isovitexin standards by different identification methods, including:

1. Thin layer chromatography (TLC) using the best mobile phase system. Ethyl acetate: Formic acid: acetic acid: water (84: 4: 4: 10)
2. Fourier transforms infrared Spectroscopy (FTIR) in KBr disk.
3. High performance thin layer chromatography HPTLC using the previously mobile phase.

Rat aorta anti-angiogenesis assay

The angiogenesis assay conducted via rat aorta angiogenesis [23] Aortic tissue sample were acquired from 12-14 weeks old male rat, where obtained from animal house of institute for diagnosis and reproductive technique in AL-Nahrain University. The animal was sacrificed via cervical dislocation under anesthesia with diethyl ether. After thoracic aorta was excised and cleaned and rinsed with bank balanced salt solution containing 2.5 µg/ml amphotericin B, then sliced to 1 mm thickness aortic ring. The assay was performed in 48 well tissue cultured plates,5 mg/ml of fibrinogen in serum free M199 growth medium and 3 mg/ml of aprotinin were added to each well to prevent fibrinolysis of vessel fragment. One aorta ring was seeded in each well. 15 ml of thrombin in 0.5 ml NaCl. Bovine plasma was added to the well and mixed rapidly with fibrinogen. After embedding the vessel fragment in the fibrin gel. 0.5 ml of medium M199 supplemented with 20% heat inactivated fetal calf serum, 0.1% α- aminocaproic acid, 1% L-glutamine, 1% amphotericin, 0.6% gentamycin. Test sample extracts of seeds and sprouts were prepared by dissolving the sample in dimethyl sulphoxide (DMSO) and diluted in M199 growth medium to make the final DMSO concentration 1%. The tissue rings were incubated at 37 C^0, 5% CO_2 in humidified incubator. The DMSO (1%)v/v) and acetyl salicylic acid (Aspirin) (100 µg /ml) were used as a negative and positive control respectively. The extent of blood vessel growth was quantified using inverted microscopic on day five of experiment with aid camera software. The magnitude of blood vessel growth inhibition was determined according to technique developed by Nicosia [24]. The experiment was repeated three times using six replicate per sample. The percentage of blood vessel inhibition was determined according to the following formula

Blood vessel inhibition= (1- A_0/A) ×100

Where A_0 = distance of blood vessel growth in test substance in mm.

A= distance of blood vessel growth in the control in 1 mm.

Dose response study of n-butanol fraction for seeds and sprouts of Vigna radiata plant with Rat Aorta using anti angiogenic assays

Serial dilution of each seeds and sprouts samples were prepared in the following concentration,200, 100,50,25, 12.5,6.25, and 3.125 µg/ml. g/ml. Dried samples were dissolved in the DMSO and then diluted in M199 growth medium to make the final DMSO concentration 1%. Wells without test samples were received medium with 1% DMSO used as a negative control.

The concentration that inhibit 50% of growing blood vessel IC 50% was calculated by using linear regression equation for extract where Y= the percentage of inhibition, X=Concentration.

Results and Discussion

1. The results showed that the most two important flavonoids that present in this plant are the vitexin and isovitexin.
2. Identification of vitexin and isovitexin by HPTLC. The results indicated that HPTLC method was developed for the first time for qualitative identification from seeds of mung

bean plant in which qualitative identification was made by the comparison of maximum retardation factor (R_f) and UV spectrum of ethyl acetate fraction and n-butanol fraction of seeds and sprouts with standards of vitexin and isovitexin and isolated compounds R1 ,R2 as shown in Figures (7 -10).

3. The results showed that sprouted mung bean produced higher yield in extraction followed by seeds. As shown in Table (3)

Table 3. Differences in extract contents yield (gm/ 250 gm) in different parts of *vigna radiata* plant (seeds and sprouts) using the same extraction method:

Extracted part	Part used	Solvent	% W/W of crude extract
Part A	Seeds	80% ethanol	13 gm.
Part B	Sprouts	80% ethanol	57 gm.

4. Anti-angiogenesis results of analyzed fraction of seeds and sprouts showed that seeds and sprouts have anti angiogenic activity, but percentage of the anti-angiogenesis effect of sprouts (88%) higher than anti-angiogenesis effect of seeds (56%) at IC50% (58.8 µg/ml), (56.6 µg/ml) for sprouts and seeds respectively as shown in Figures (11-15) and Tabled (5-8).

The anti angiogenic activity may be related to the existence of flavonoids and other phenolic compounds but the variation in inhibition percentage may relate to the concentration of the bioactive constituents or appearance of new bioactive constituents during germination.

***Characterization of isolated vitexin and isovitexin*:**

1. The isolated compound appeared as a single spot having the same color and R_f value as that of standards vitexin and isovitexin as shown in Figure (4).

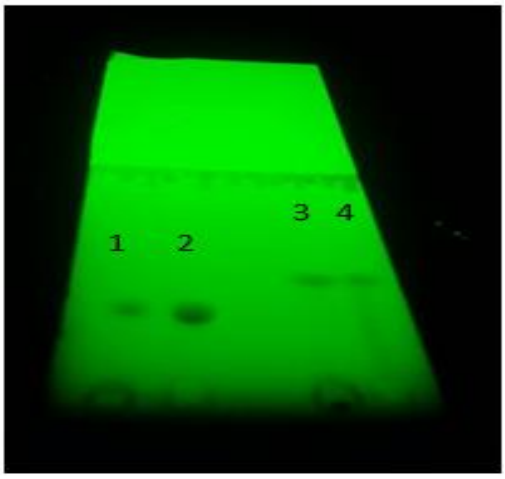


Figure 4. TLC chromatogram of isolated compounds (1 and 3) with standards (2: isovitexin,4: vitexin) respectively on silica gel GF 254 nm, developing in ethyl acetate: formic acid: acetic acid: water (84:4:4:10)

2. Fourier transforms infrared (FT– IR) spectrum: FT – IR spectroscopy is most commonly used in phytochemical studies as finger printing device. The FT – IR spectra of separated compounds was detected in the College of Sciences Al-Mustansirya University using SHIMADZU device. IR spectra of isolated compounds as shown in Figures (5 and 6) and the characteristic IR absorption bands of isolated compounds are listed in Table (4)

Table 4. Characteristic FI – IR absorption bands in (cm^{-1}) of isolated compounds:

Functional group	Group frequency wave number in cm – 1		Assignment
	Measured for isolated compound vitexin	Measured for isolated compound isovitexin	
O – H	3381 -3231	3433-3000	O-H stretching of phenol
C = C – H	3210	3285	C – H Stretching of aromatic ring
C – H	2910	2928 ,2843	Asymmetric and symmetric stretching of CH2
C = O	1651	1641	C = O stretching conjugation and H – bonding
C = C	1614,1570,1506	1608, 1568,1516	C = C stretching aromatic ring
C – H	1419	1444	C – H bending of CH2.
O-H	1384	1363	O-H bending of phenol
C-O-C	1251	1238	C-O-C stretching of ether
CH	1091 – 1066	1084 – 1072	C – H bending of aromatic (in plane)
CH	987 , 879, 758	916 , 887 , 775 ,698	C-H bending of aromatic (out of plane)

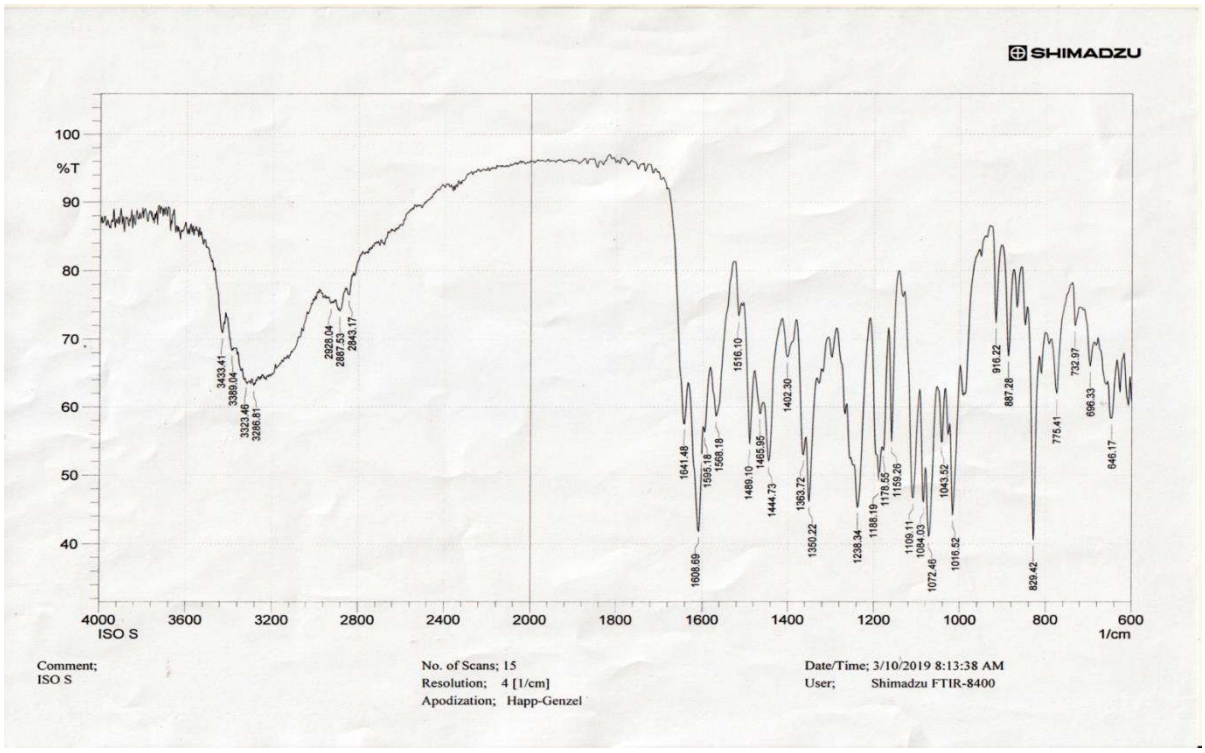


Figure 5. IR spectrum of isolated compound R1

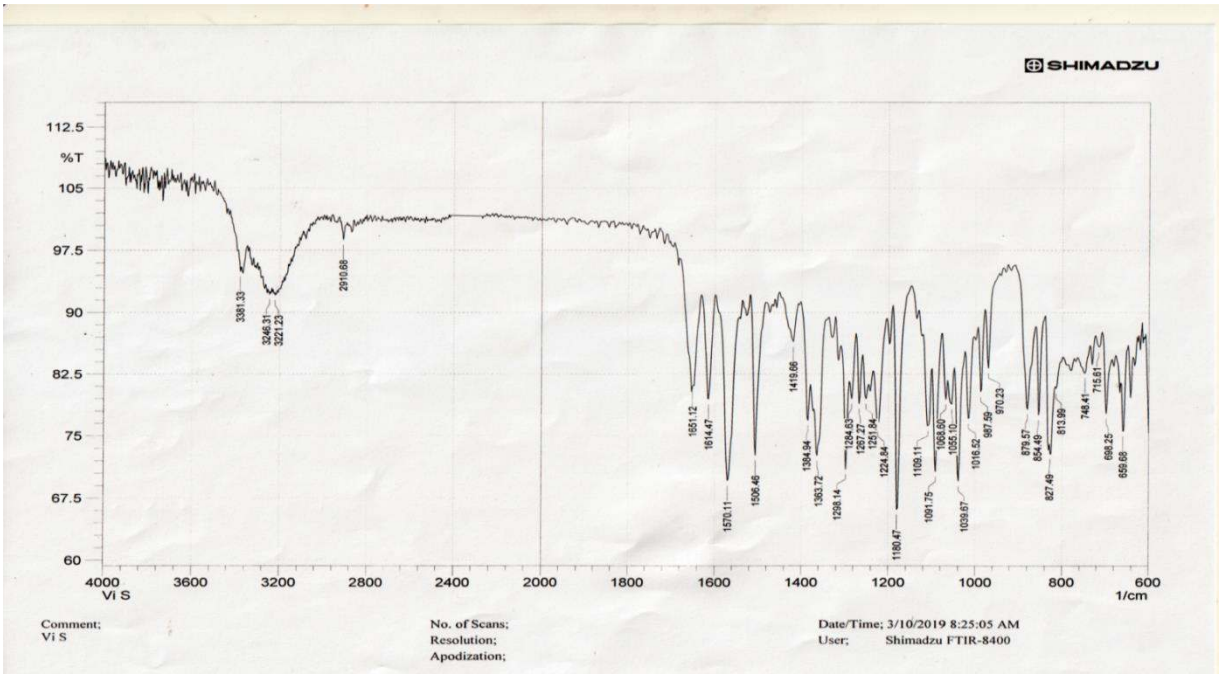


Figure 6. IR spectrum of isolated compound R2

3. HPTLC was used again for characterization and identification by comparing Max. Retardation factor R_f of isolated compounds (R2 and R1) with that of standards and respectively as shown in Figures (7-9).

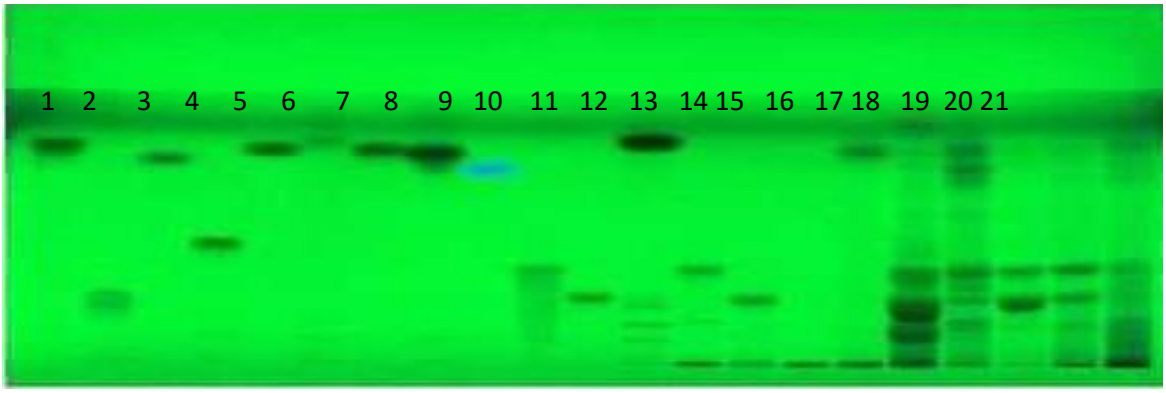
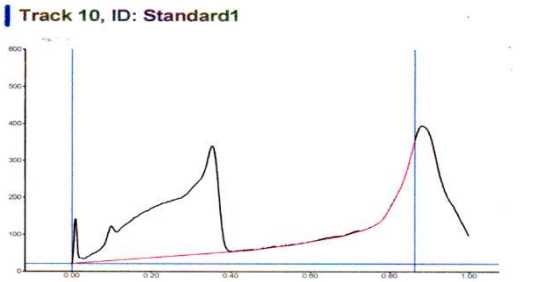
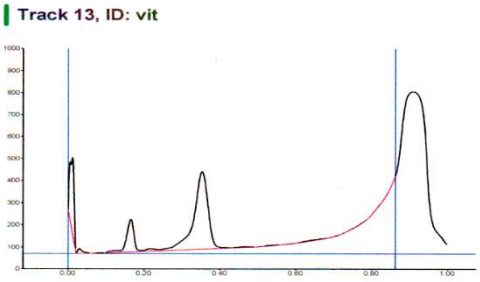


Figure 7. HPTLC plate for isolated compounds (13 ,14) and related standards (10: vitexin, 11, isovitexin) respectively and ethyl acetate and n- butanol fractions of seeds and sprouts (17,18,19,20,21) observed at 254 wave length.

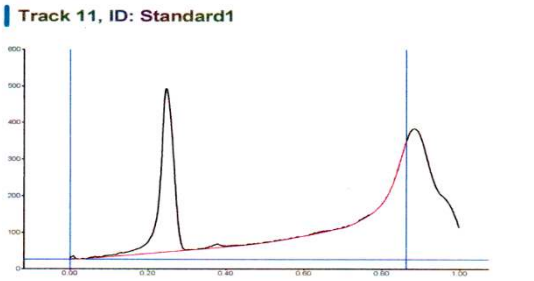


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height
1	0.01	121.5	0.01	121.5	24.04	0.03	11.1
2	0.03	11.1	0.10	93.9	18.58	0.11	76.3
3	0.11	76.6	0.35	290.0	57.38	0.40	1.7

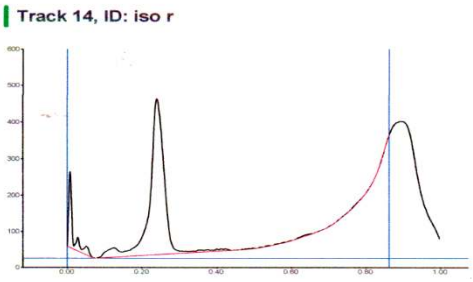


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height
1	0.01	268.2	0.01	348.6	40.15	0.02	5.3
2	0.03	13.2	0.03	23.1	2.66	0.06	0.3
3	0.14	9.0	0.17	147.5	16.99	0.19	5.1
4	0.26	10.5	0.35	349.0	40.20	0.41	3.6

Figure 8.HPTLC chromatogram for vitexin standard comparing with HPTLC chromatogram of isolated compound (R2)

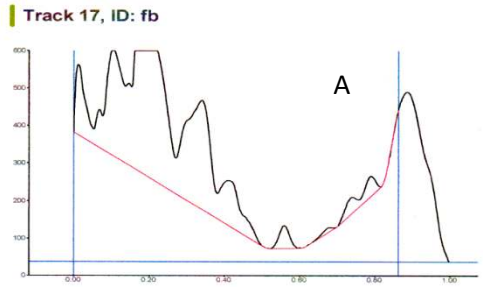


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height
1	0.15	9.2	0.25	447.0	100.00	0.30	1.0

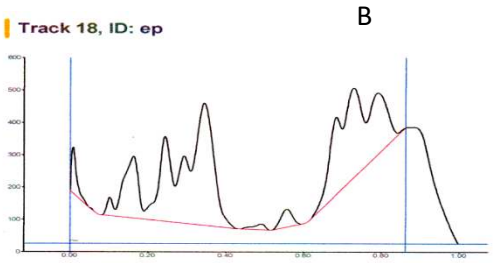


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height
1	0.02	8.1	0.03	37.3	7.27	0.04	8.9
2	0.04	9.1	0.05	23.7	4.61	0.07	0.3
3	0.08	1.3	0.13	25.9	5.04	0.15	12.7
4	0.16	15.1	0.24	426.9	83.08	0.30	6.2

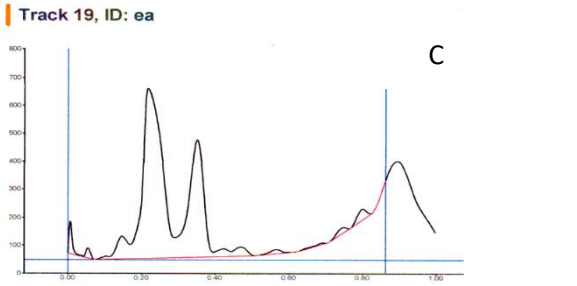
Figure 9. HPTLC chromatogram of isovitexin standard comparing with HPTLC chromatogram of isolated compound (R1)



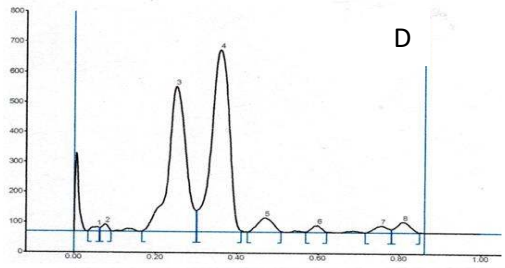
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height
1	0.01	170.6	0.01	187.6	11.84	0.05	38.7
2	0.05	40.2	0.07	101.7	6.41	0.08	89.3
3	0.08	90.0	0.11	288.4	18.19	0.14	205.9
4	0.15	214.4	0.18	420.5	26.52	0.27	94.5
5	0.27	96.0	0.34	291.8	18.41	0.38	67.8
6	0.38	67.9	0.42	122.0	7.70	0.50	3.0
7	0.53	0.3	0.56	60.1	3.79	0.60	0.4
8	0.64	1.2	0.68	13.1	0.83	0.70	1.1
9	0.70	0.9	0.74	45.9	2.90	0.76	18.5
10	0.76	18.7	0.79	54.1	3.41	0.82	0.6



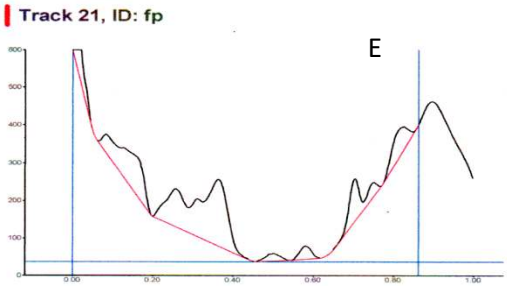
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height
1	0.01	142.2	0.01	142.2	7.02	0.05	0.1
2	0.08	0.0	0.10	57.4	2.83	0.11	20.8
3	0.12	21.7	0.16	192.6	9.51	0.19	25.3
4	0.19	26.0	0.24	262.5	12.96	0.27	111.8
5	0.27	113.5	0.29	208.7	10.30	0.31	163.5
6	0.31	165.2	0.34	378.0	18.66	0.43	0.8
7	0.44	0.5	0.49	17.5	0.86	0.51	0.3
8	0.52	0.5	0.56	55.9	2.76	0.60	0.0
9	0.62	0.1	0.69	240.4	11.87	0.70	185.1
10	0.70	185.2	0.73	278.5	13.75	0.76	133.7
11	0.76	133.7	0.79	192.0	9.48	0.85	4.3



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height
1	0.04	2.1	0.06	35.4	2.75	0.07	0.5
2	0.07	0.1	0.10	11.6	0.90	0.11	9.6
3	0.11	9.7	0.15	81.2	6.31	0.17	49.9
4	0.17	50.6	0.22	606.6	47.09	0.29	71.7
5	0.29	71.7	0.35	417.6	32.42	0.40	15.7
6	0.40	15.9	0.43	27.6	2.14	0.45	17.0
7	0.45	17.4	0.47	32.0	2.49	0.50	2.4
8	0.53	2.1	0.57	15.2	1.18	0.59	1.8
9	0.72	2.6	0.74	21.5	1.67	0.77	0.4
10	0.77	0.4	0.80	39.4	3.05	0.83	0.2



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height
1	0.03	0.2	0.06	14.4	1.14	0.06	11.5
2	0.06	11.8	0.08	23.6	1.88	0.09	1.4
3	0.17	0.5	0.25	483.0	38.48	0.30	70.2
4	0.30	70.7	0.36	604.7	48.17	0.41	4.3
5	0.43	0.6	0.47	47.5	3.78	0.51	3.5
6	0.57	2.6	0.60	23.1	1.84	0.62	2.2
7	0.72	0.8	0.76	22.6	1.80	0.78	11.6
8	0.78	11.8	0.81	36.3	2.89	0.85	1.6



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height
1	0.01	106.7	0.01	125.6	14.04	0.05	0.1
2	0.09	51.2	0.16	104.4	11.68	0.20	0.7
3	0.20	0.4	0.26	101.4	11.34	0.29	65.9
4	0.29	66.0	0.31	100.8	11.27	0.32	98.3
5	0.32	98.3	0.37	177.0	19.80	0.43	1.1
6	0.47	4.0	0.50	20.2	2.26	0.54	0.0
7	0.55	0.3	0.58	35.2	3.93	0.61	3.0
8	0.66	0.5	0.70	113.5	12.70	0.73	21.3
9	0.73	21.4	0.75	40.4	4.51	0.77	0.1
10	0.78	0.1	0.81	75.7	8.46	0.86	0.1

Figure 10. A- HPTLC chromatogram of n-butanol before hydrolysis of seeds, B- HPTLC chromatogram of ethyl acetate fraction of sprouts , C- HPTLC chromatogram of ethyl acetate fraction of seeds , D- HPTLC chromatogram of n-butanol fraction after hydrolysis of seeds ,E- HPTLC chromatogram of n- butanol fraction of sprouts .

EX vivo rat aorta ring anti-angiogenesis assay for n-butanol fraction of seeds and n-butanol fraction of sprouts of Vigna radiata plant

Aortic rings embedded in complete growth medium have received a concentration of 100 µg/ml of each the two n-butanol fraction (seeds, sprouts) the blood vessels growth inhibition was presented as percent of inhibition as Table (5 and 6)

The results showed that two extract significantly inhibited blood vessels growth at day five of experiments, there was no significant difference in blood vessels growth inhibition among each of two extract of *Vigna radiata* of seeds and sprouts (P >0.05%). There IC50% (56.6 µg/ml,58.5 µg/ml) for seeds and sprouts respectively. Among these two extracts, the n-butanol fraction of sprouts showed the highest anti-angiogenic activity 88% (in term percentage of blood vessels inhibition) in comparison with n-butanol fraction of seeds 56%. The difference between the n-butanol fraction of seeds, n-butanol fraction of sprouts and positive control (acetylsalicylic acid) as shown in the Figures (11-13).

Table 5. The inhibition percentage of blood vessels growth produced by tested fraction of seeds, negative and positive control.

Compound	% of inhibition
Negative control "DMSO"	0
Positive control " aspirin"	90
n- butanol fraction of seeds	56

Table 6. The inhibition percentage of blood vessels growth produced by tested fraction of sprouts, negative and positive control.

Compound	% of inhibition
Negative control "DMSO"	0
Positive control " aspirin"	92
n-butanol fraction of sprouts	88

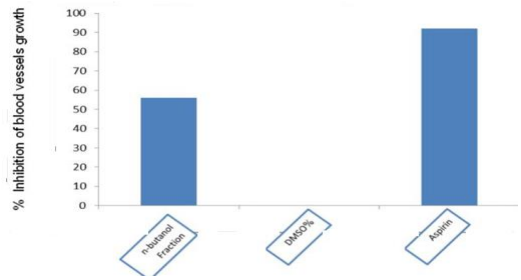


Figure 11. Anti –angiogenesis activity of 100 µg/ml of n-butanol fraction of seeds, positive and negative control in ex vivo aortic ring model

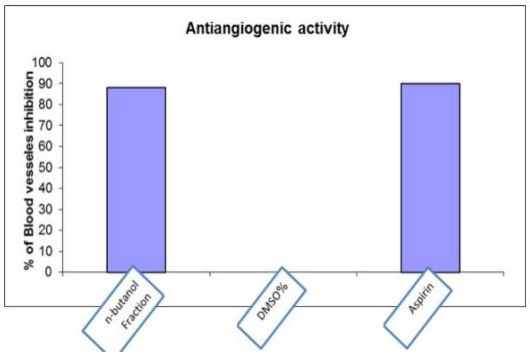
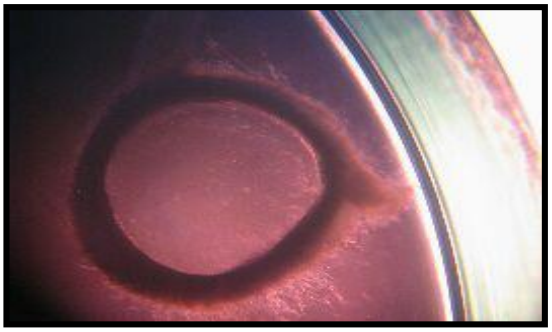
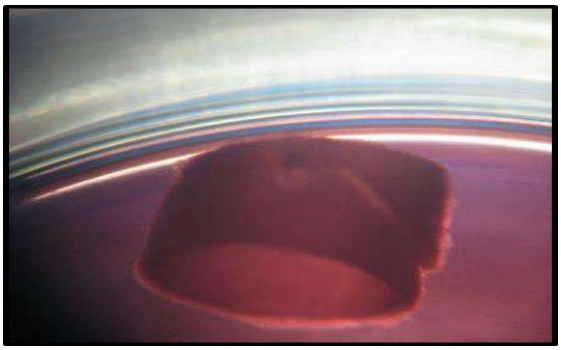


Figure 12. Anti-angiogenesis activity of 100 µg/ml of n- butanol fraction of sprouts, positive and negative control in ex vivo aortic ring model.



c



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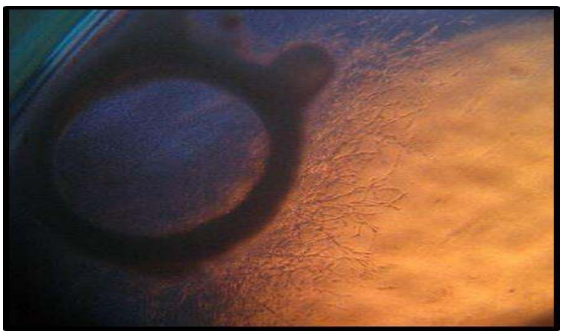

a

Figure 13. Effects of *Vigna radiata* seeds and sprouts extracts on blood vessels growth in rat aorta rings , where A,B,C represent the activity of negative control (DMSO), n-butanol fraction of sprouts and n-butanol fraction of seeds respectively.

Dose response effect of n- butanol fraction of Vigna radiata seeds and sprouts on aortic ring model.

Seven serial dilution of n- butanol fraction of each seeds and sprouts alone was prepared and added to the embedded rat aortic ring model to determine the dose response curve. n-butanol fraction of seeds and sprouts showed significant dose dependent inhibition of blood vessels growth when compared to negative control (DMSO%), at day five of experiment as shown in the Tables (7 and 8) and Figures (14 and 15).

Table 7. Serial concentration and their respective inhibition percentage for n-butanol fraction of *Vigna radiata* seeds.

Concentration (µg/ml)	% of inhibition
89	200
80	100
56	50
34	25
12	12.5
5	6.25
0	53.12

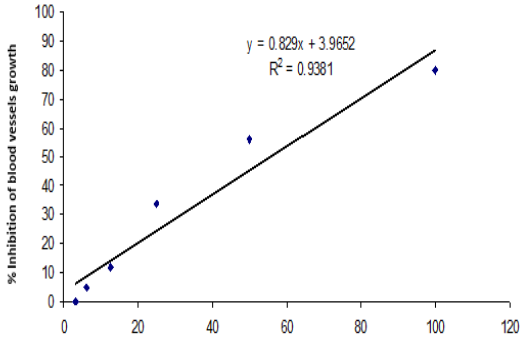

Figure 14. Dose response curve of n-butanol fraction of *Vigna radiata* seeds in rat aortic rings model.

Table 8. Serial dilution and their respective inhibition percentage of n-butanol fraction of *Vigna radiata* sprouts.

Concentration(µg/ml)	% of inhibition
200	100
100	85
50	67
25	45
12.5	22
6.25	19
3.125	8

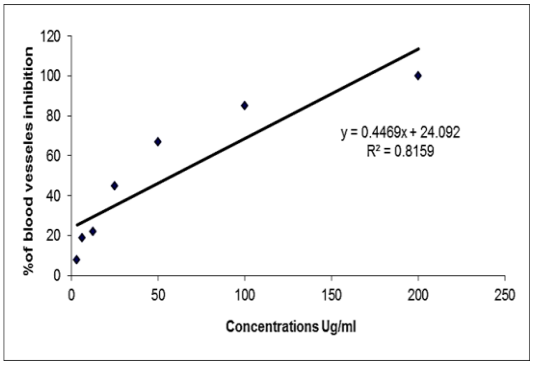

Figure 15. Dose response curve of n-butanol fraction of *Vigna radiata* sprouts in rat aortic rings model.

The IC_{50} was determined for n-butanol fraction of seeds and sprouts by linear equation as shown in Figure (14 and 15) and it was found to be: IC_{50}% of n-butanol fraction of seeds =56.6 µg/ml; $IC_{50\%}$Of n-butanol fraction of sprouts =58.8 µg/ml Where y= the percentage of inhibition of blood vessels growth, and was set at 50%; x= the concentration.

Conclusion

From the above finding, two flavonoids are isolated from *Vigna radiata* seeds (vitexin and isovitexin), On extraction of the *Vigna radiata* seeds and sprouts in the same extraction method, the percentage of yield of extract of sprouts were higher than percentage of yield of extract of seeds, which attributed to increase in phytochemical constituents thus the germination of *Vigna radiata* seeds increase in the amount of phytochemical constituents. The anti angiogenic activity may be related to the existence of flavonoids and other phenolic compounds but the variation in inhibition percentage may relate to the concentration of the bioactive constituents or appearance of new bioactive constituents during germination

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