

Semen Vaccariae

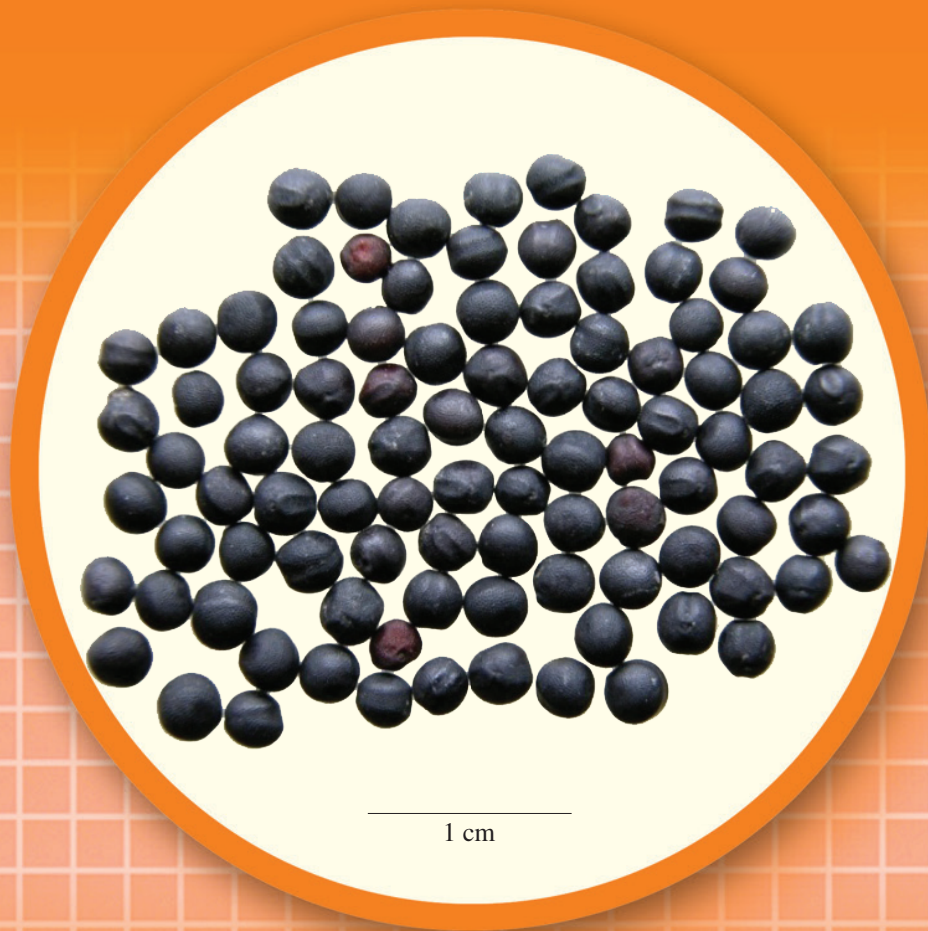


Figure 1 A photograph of Semen Vaccariae

1. NAMES

Official Name: Semen Vaccariae

Chinese Name: 王不留行

Chinese Phonetic Name: Wangbuliuxing

2. SOURCE

Semen Vaccariae is the dried seed of the ripe fruit of *Vaccaria segetalis* (Neck.) Garcke (Caryophyllaceae). The plant is collected in summer when the fruit is ripe but before dehiscing, dried under the sun, the seeds gathered and dried again under the sun, then foreign matter removed to obtain Semen Vaccariae.

3. DESCRIPTION

Spheroidal, 1.4-2.4 mm in diameter. Externally black or chestnut-brown, slightly lustrous. Fine, dense and evenly distributed granular protuberances visible on testa surface under 10× magnification; hilum suborbicular and dented, with a shallow taenioid furrow on one side. Texture hard; milky white endosperm exposed when cracked open; embryo curved like a ring; cotyledons 2. Odour slight; taste slightly astringent and bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Epidermal cells of testa are arranged in 1 row, reddish-brown, outer wall distinctly thickened with nipple-like protuberances, striations barely perceptible; inner epidermis of testa shrunken, yellowish-brown to brown. Endosperm consists of parenchyma cells containing aleurone granules. Cotyledons and radicle composed of parenchyma cells (Fig. 2).

Powder

Colour pale greyish-brown. Epidermal cells of testa reddish-brown or yellowish-brown, stellate in shape, 48-135 μm in diameter, outer wall striations thickened; inner epidermis of testa pale yellowish-brown, subsquare, rectangular to polygonal, anticlinal wall densely beaded, striations visible on the surface. Endosperm cells polygonal, subsquare or subrectangular, lumina full of aleurone grains. Cotyledon cells contain fatty oil droplets (Fig. 3).

4.2 Thin-Layer Chromatographic Identification [Appendix IV(A)]

Standard solution

Segetalin A standard solution

Weigh 1.0 mg of segetalin A CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of dichloromethane and methanol (10:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 10.0 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of a mixture of dichloromethane and methanol (4:1, v/v). Sonicate (240 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately segetalin A standard solution and the test solution (6 µL each) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. Develop over a path of about 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the plate evenly with the spray reagent and heat at about 105°C for about 1 min. Examine the plate under UV light (366 nm). Calculate the *R_f* value by using the equation as indicated in Appendix IV(A).

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the *R_f* value, corresponding to that of segetalin A.

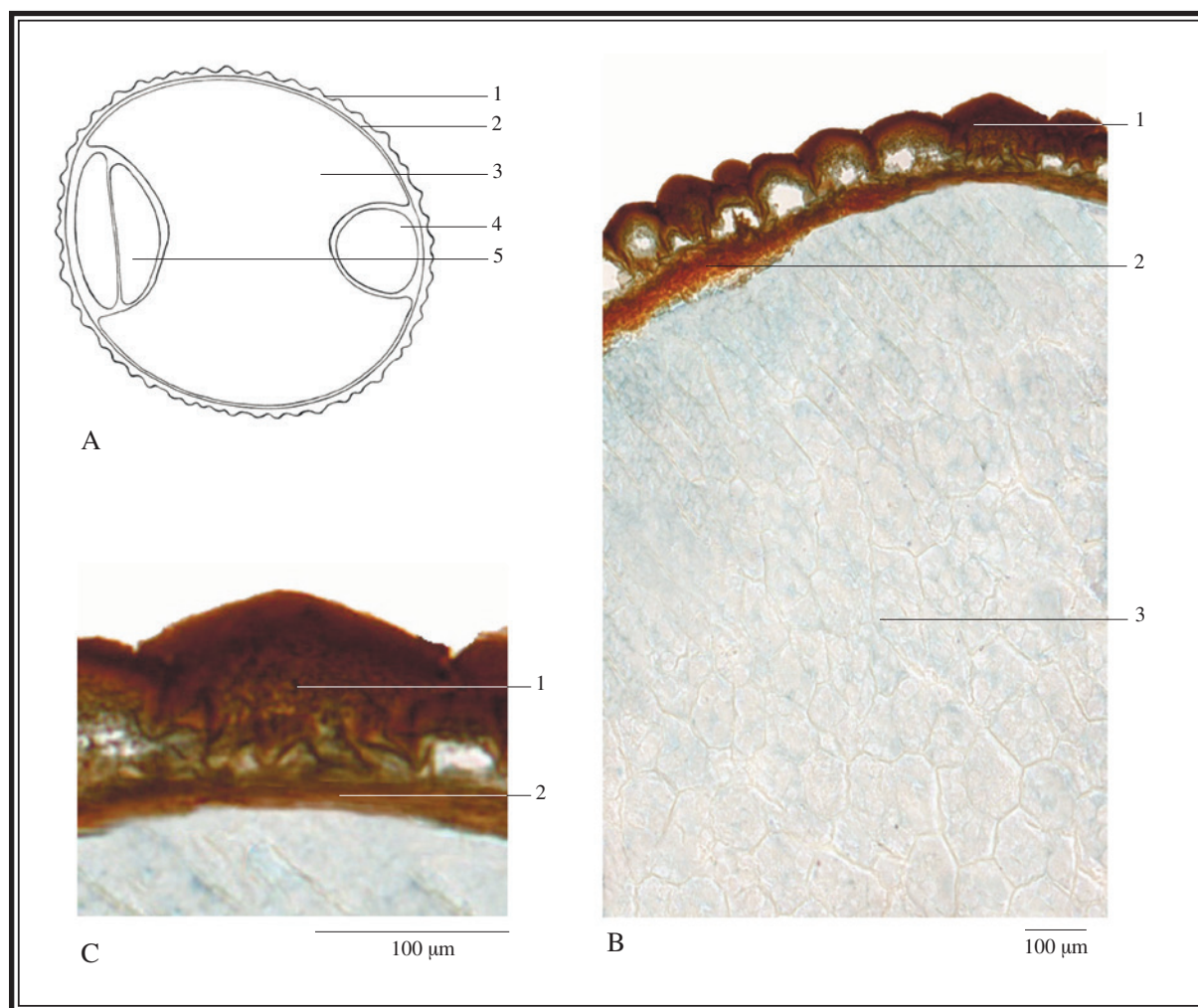


Figure 2 Microscopic features of transverse section of Semen Vaccariae

A. Sketch B. Section illustration C. Epidermal cell of testa

1. Epidermis of testa 2. Inner epidermis of testa 3. Endosperm
4. Radicle 5. Cotyledon

Note: The radicle and cotyledon are composed of parenchyma cells and contain no characteristic features for authentication. As they are located at two sides of the seed, they were not shown in detail.

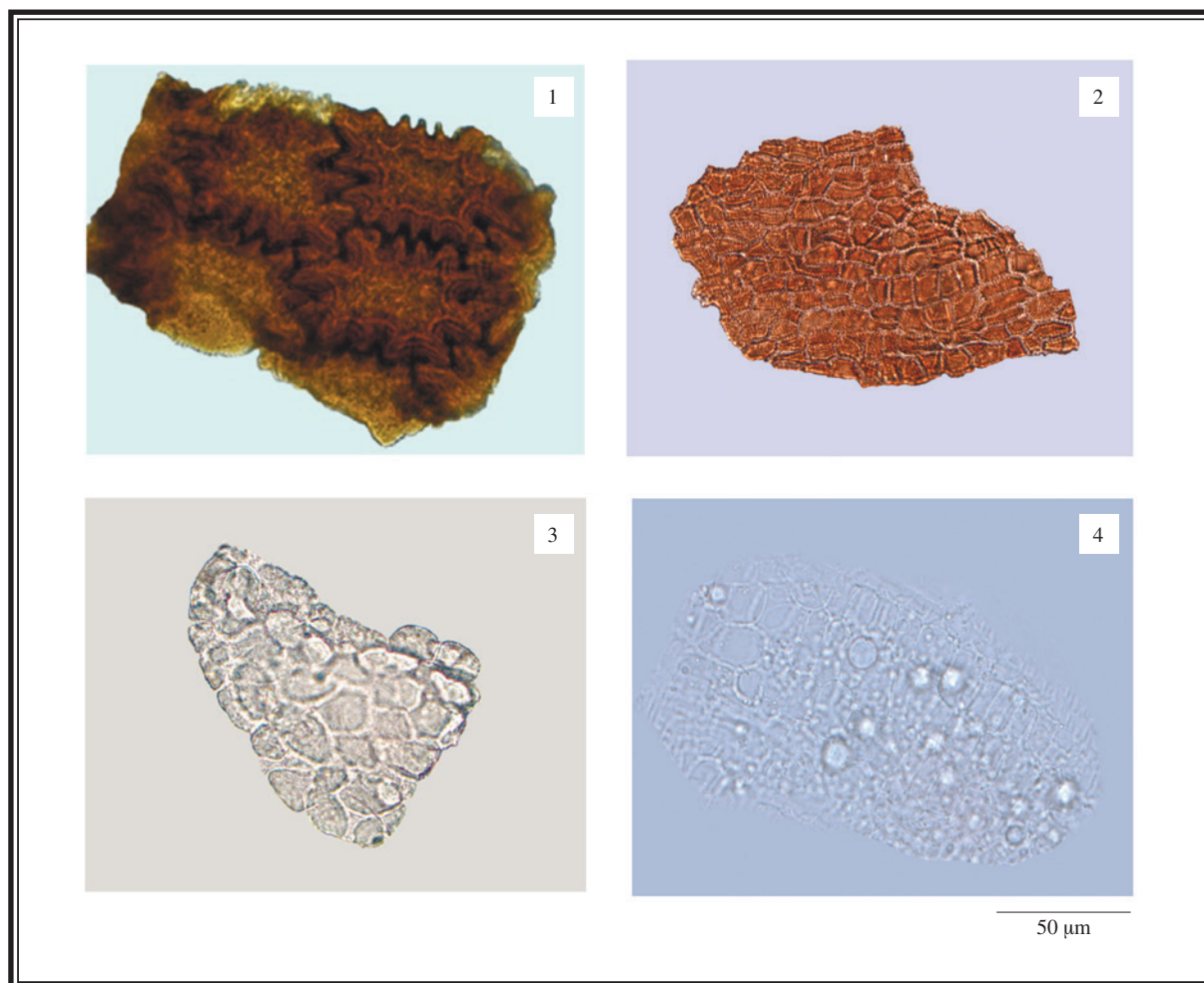


Figure 3 Microscopic features of powder of Semen Vaccariae (under the light microscope)

1. Epidermal cells of testa
2. Inner epidermal cells of testa
3. Endosperm cells
4. Cotyledon cells

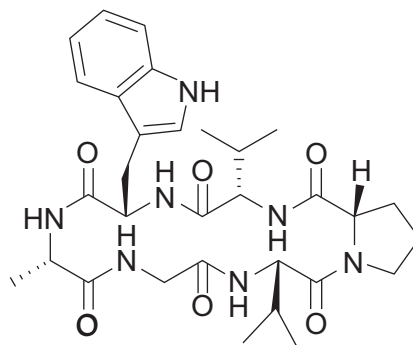


Figure 4 Chemical structure of segetalin A

4.3 High-Performance Liquid Chromatographic Fingerprinting (*Appendix XII*)

Standard solution

Segetalin A standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 1.0 mg of segetalin A CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (240 W) the mixture for 30 min. Centrifuge at about $5000 \times g$ for 5 min. Filter through a 0.45- μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (219 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 35°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (% v/v)	Acetonitrile (% v/v)	Elution
0–20	90→85	10→15	linear gradient
20–40	85→70	15→30	linear gradient
40–60	70→30	30→70	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μ L of segetalin A Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of segetalin A should not be more than 5.0%; the RSD of the retention time of segetalin A peak should not be more than 2.0%; the column efficiency determined from segetalin A peak should not be less than 150000 theoretical plates.

The *R* value between peak 6 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject segetalin A Std-FP and the test solution (20 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of segetalin A peak in the chromatogram of segetalin A Std-FP and the retention times of the six characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify segetalin A peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of segetalin A Std-FP. The retention times of segetalin A peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the six characteristic peaks of Semen Vaccariae extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Semen Vaccariae extract

Peak No.	RRT	Acceptable Range
1 (Hypaphorine)	0.24	± 0.03
2	0.44	± 0.03
3	0.59	± 0.03
4	0.71	± 0.03
5	0.92	± 0.03
6 (marker, segetalin A)	1.00	-

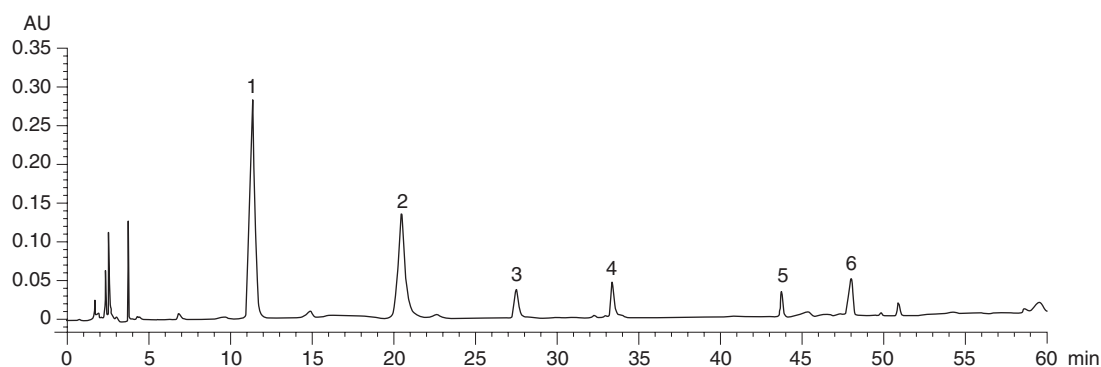


Figure 5 A reference fingerprint chromatogram of Semen Vaccariae extract

For positive identification, the sample must give the above six characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 5).

5. TESTS

- 5.1 Heavy Metals** (*Appendix V*): meet the requirements.
- 5.2 Pesticide Residues** (*Appendix VI*): meet the requirements.
- 5.3 Mycotoxins – Aflatoxins** (*Appendix VII*): meet the requirements.
- 5.4 Sulphur Dioxide Residues** (*Appendix XV*): meet the requirements.
- 5.5 Foreign Matter** (*Appendix VIII*): not more than 1.0%.
- 5.6 Ash** (*Appendix IX*)
 - Total ash: not more than 4.0%.
 - Acid-insoluble ash: not more than 1.0%.
- 5.7 Water Content** (*Appendix X*): not more than 15.0%.

6. EXTRACTIVES (*Appendix XI*)

- Water-soluble extractives (cold extraction method): not less than 9.0%.
- Ethanol-soluble extractives (hot extraction method): not less than 6.0%.

7. ASSAY

Carry out the method as directed in Appendix IV(B).

Standard solution

Segetalin A standard stock solution, Std-Stock (200 mg/L)

Weigh accurately 2.0 mg of segetalin A CRS and dissolve in 10 mL of methanol.

Segetalin A standard solution for assay, Std-AS

Measure accurately the volume of the segetalin A Std-Stock, dilute with methanol to produce a series of solutions of 8, 20, 60, 100, 160 mg/L for segetalin A.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol. Sonicate (240 W) the mixture for 30 min. Centrifuge at about $5000 \times g$ for 5 min. Transfer the supernatant to a 200-mL round-bottomed flask. Repeat the extraction for two more times. Combine the extracts. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 2-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- μ m PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (219 nm) and a column (4.6 \times 250 mm) packed with ODS bonded silica gel (5 μ m particle size). The column temperature is maintained at 35°C during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of methanol and water (50:50, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μ L of segetalin A Std-AS (60 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of segetalin A should not be more than 5.0%; the RSD of the retention time of segetalin A peak should not be more than 2.0%; the column efficiency determined from segetalin A peak should not be less than 1500 theoretical plates.

The *R* value between segetalin A peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of segetalin A Std-AS (20 μ L each) into the HPLC system and record the chromatograms. Plot the peak areas of segetalin A against the corresponding concentrations of segetalin A Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

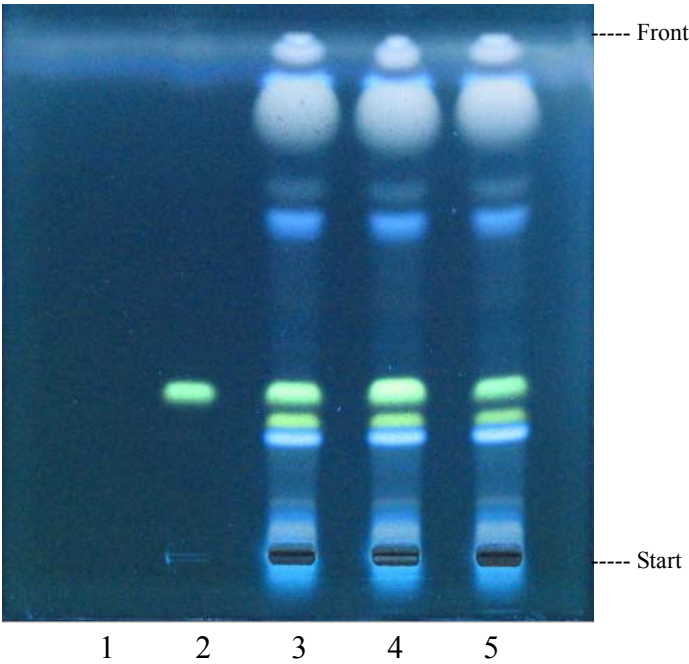
Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify segetalin A peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of segetalin A Std-AS. The retention times of segetalin A peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of segetalin A in the test solution, and calculate the percentage content of segetalin A in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.028% of segetalin A (C₃₁H₄₃N₇O₆), calculated with reference to the dried substance.

Semen Vaccariae (王不留行)



Lane	Sample	Results
1	Blank (Methanol)	Negative
2	Standard (segetalin A)	Segetalin A positive
3	Sample (Semen Vaccariae)	Segetalin A positive
4	Spiked sample (Sample plus segetalin A)	Segetalin A positive
5	Sample duplicate (Semen Vaccariae)	Segetalin A positive

Figure 1 TLC results of Semen Vaccariae extract observed under UV light (366 nm) after staining