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INVESTIGATION INTO EXTRACTION PROCESS OF PHENOLIC COMPOUNDS FROM THREE SPECIES: *RHEUM TANGUTICUM* MAXIM. EX BALF, *RUMEX TRISETIFER* STOKES, *SENNA ALATA* (L.) ROXB., AND BIOLOGICAL ACTIVITY AGAINST PHYTOPATHOGENIC MICROORGANISMS

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PREFACE

1. The urgency of the thesis

Vietnam is a tropical agricultural with a total agricultural and forestry land area of about 20 million hectares. Approximately 14 million hectares are under cultivation in Vietnam at the moment.. The year-round high temperatures, high humidity and amounts of rain are favorable conditions for plants to grow, but they also invite the growth of weeds, fungi, and insects.

Among the pests that damage plants, plant diseases caused by fungi account for up to 80%. Fungal diseases caused by *Botrytis cineria*, *Erysiphe graminis*, *Phytophthora infestans* and *Rhizoctonia solani* are popular and severely harm a wide range of crops.

Chemical pesticides are synthetic organic compounds commonly used to protect crops from insect pests and microorganisms. However, several chemical pesticides were observed to cause environmental pollution of land, water, and air with long residual periods, and to affect the agricultural ecosystems and public health. Many chemically synthesized pesticides have been reported to show the potential to cause cancer, and genetic mutations, and affect human health. In fact, some highly toxic synthetic pesticides such as 2,4 D, carbendazim, tubeconazole, cabofuran, and azodrin are banned to use in modern agriculture. Therefore, attention must be paid to the discovery of new active ingredients from microbial and herbal resources that are less toxic to humans and the environment.

Originating from the urgency in real life, it is necessary to create biopesticides, the author chose the topic of the thesis "**Investigation into extraction process of phenolic compounds**

from three species: *Rheum tanguticum* Maxim. ex Balf, *Rumex trisetifer* Stokes, *Senna alata* (L.) Roxb., and biological activity against phytopathogenic microorganisms".

2. Research aims of the thesis

- To study the extraction processes and chemical constituents in the extracts of three species *Rheum tanguticum* Maxim. ex Balf, *Rumex trisetifer* Stokes, and *Senna alata* (L.) Roxb.

- *In vivo* evaluation of biological activity of extracts and isolated constituents from *R. tanguticum*, *R. trisetifer*, *S. alata* against various phytopathogenic fungi

- *In vitro* evaluation of biological activity of extracts and isolated constituents from *R. tanguticum*, *R. trisetifer*, *S. alata* against plant pathogenic bacteria

- Optimization of extraction processes for raw materials from *R. tanguticum*, and *S. alata*.

3. Research contents of the thesis

- Investigation into extraction and determination of the chemical structures of isolated compounds in the extracts from three species *Rheum tanguticum*, *R. trisetifer*, and *S. alata*: extract and prepare the plant extracts, isolate compounds by using chromatography methods, determine the chemical structures of isolated compounds by using spectroscopic and physical chemistry methods, and design experimental processes and optimize the extraction of the plant extracts.

- In vitro and in vivo evaluation of the extracts and their isolated compounds against phytopathogenic fungi and bacteria.

CHAPTER 1. OVERVIEW

The overview summarized studies on the following: chemical

constituents and biological activities *Rheum tanguticum* species; *Rumex* genus, *Senna alata* species (Part 1.1-1.3).

Introduction of response surface methodology (RMS) and optimization of extraction processes (Part 1.4)

Introduction of biopesticides (Part 1.5).

CHAPTER 2. RESEARCH MATERIALS AND METHODS

2.1. Plant samples

In the thesis, the materials used for the study were *Rheum tanguticum* Maxim. ex Balf, *Rumex trisetifer* Stokes, and *Senna alata* (L.) Roxb collected in Vietnam.

2.2. Methods

2.2.1. Preparation, extraction, isolation

2.2.2. Determination of physicochemical properties and the chemical structures of isolated compounds.

2.2.3. Evaluation of biological activity

2.2.3.1. In vivo evaluation of biological activity against various phytopathogenic fungi

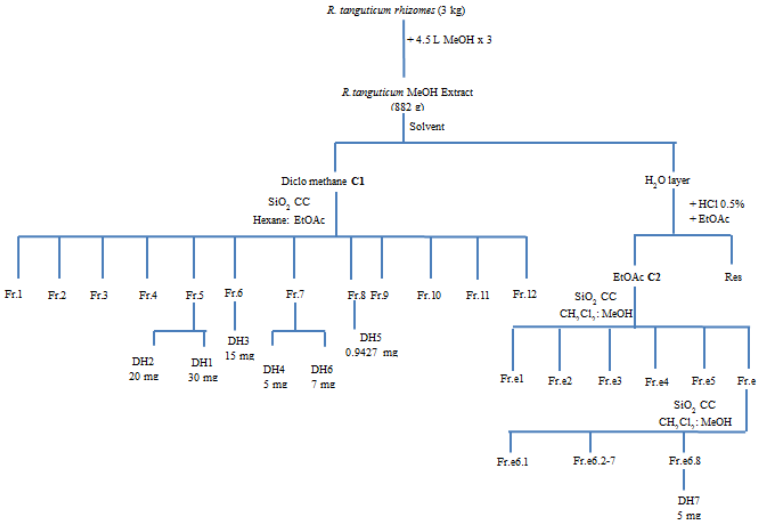
2.2.3.2. In vitro evaluation of biological activity against plant pathogenic bacteria

2.2.4. Experimental design and optimization of extraction processes

CHAPTER 3. EXPERIMENTS

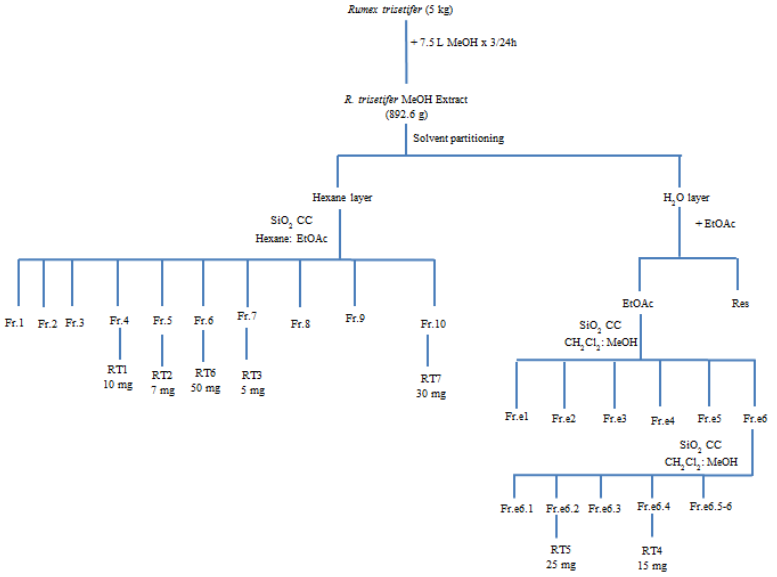
3.1. Isolated compounds from – *R. tanguticum*.

Figure 3.1



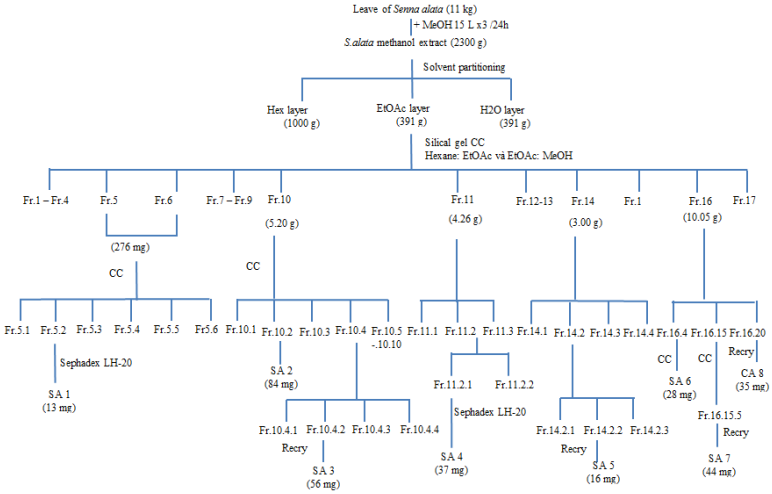
3.2. Isolated compounds from – *R.trisetifer*

Figure 3.2



3.3. Isolated compounds from – *S. alata*

Figure 3.1



CHAPTER 4. RESULTS AND DISCUSSION

4.1. Determination of the structure of compounds isolated from *R. tanguticum*.

*4.1.1. Compound **DH1** (chrysophanol)*

Orange needles

^{1}H-NMR ($CDCl_3$, 500 MHz): 12.00 (O<u>H</u>-1), 12.11 (O<u>H</u>-8), 7.82 (1H, d, *J*= 7.0 Hz, H-5), 7.68 (1H, m, H-6), 7.64 (1H, brs, H-4), 7.29 (1H, d, *J*=8.5 Hz, H-7), 7.09 (1H, brs, H-2), 2.47 (3H, s, CH_3-3).

^{13}C-NMR ($CDCl_3$ 125 MHz): 192.5 (C-9), 182.0 (C-10), 162.7 (C-1), 162.4 (C-8), 149.3 (C-3), 136.9 (C-6), 133.6 (C-10a), 133.1 (C-4a), 124.5 (C-7), 124.3 (C-2), 121.3 (C-4), 119.9 (C-5), 115.9 (C-8a), 113.7 (C-9a).

*4.1.2. Compound **DH2** (physcion)*

Orange needles

^{1}H-NMR ($CDCl_3$, 500 MHz): 12.30 (O<u>H</u>-8), 12.10 (O<u>H</u>-1), 7.61 (1H, brs, H-5), 7.35 (1H, d, *J*= 1.5 Hz, H-4), 7.07 (1H, brs, H-7), 6.67 (1H, d, *J*= 1.5 Hz, H-2), 3.93 (3H, s, OCH_3), 2.44 (3H, s, 3-CH_3).

^{13}C-NMR ($CDCl_3$ 125 MHz): 190.80 (C-9), 182.01 (C-10), 166.56 (C-3), 165.20 (C-1), 162.52 (C-8), 148.45 (C-6), 135.27 (C-10a), 133.23 (C-4a), 124.51 (C-7), 121.29 (C-5), 113.69 (C-9a), 110.27 (C-8a), 108.22 (C-4), 106.78 (C-2), 56.09 (-OCH_3), 22.17 (3-CH_3).

*4.1.3. Compound **DH3** (emodin)*

Orange needles

^{1}H-NMR (DMSO-d_6, 500 MHz): 12.09 (O<u>H</u>-1), 12.02 (O<u>H</u>-8), 7.49 (1H, brs, H-5), 7.17 (1H, brs, H-7), 7.12 (1H, d, *J*= 2.0 Hz, H-4), 6.59 (1H, d, *J*= 2.0 Hz, H-2) 2.47 (3H, s, 3-CH_3).

^{13}C-NMR (DMSO-d_6,125 MHz): 190.18 (C-9), 181.88 (C-10), 166.08 (C-3), 164.93 (C-1), 161.89 (C-8), 148.72 (C-6), 135.60 (C-10a), 133.31 (C-4a), 124.61 (C-7), 120.95 (C-5), 113.86 (C-9a), 109.42 (C-8a), 109.27 (C-4), 108.41 (C-2), 21.99 (3-CH_3).

*4.1.4. Compound **DH4** (aloe-emodin)*

Orange needles

ESI-MS: m/z 269 $[M-H]^-$

^{1}H-NMR (Aceton-d_6, 500 MHz): .781 (1H, t-like, *J*= 8.0, 7.5 Hz, H-6), 7.77 (1H, d, *J*= 6 br Hz, H-5), 7.76 (1H, s, H-4), 7.34 (1H, d, *J*= 7.5 Hz, H-7), 7.33(1H, s, H-2), 4.75 (2H, s, –CH_2–).

*4.1.5. Compound **DH5** (rhapotigenin)*

Brown colorless crystals.

^{1}H-NMR (500MHz, CD_3OD), δ (ppm): 6.99 (1H, d, *J* = 1.5 Hz, H-2'), 6.90 (1H, d, *J* =14.5 Hz, H-*α*), 6.87 (2H, m, H-5', H-6'), 6.78 (1H, d, *J* = 16.0 Hz, H-*β*), 6.44 (2H, d, *J* = 2.0 Hz, H-2, 6), 6.16 (1H,

d, *J* = 2.0 Hz, H-4), 3.83 (3H, s, 4'-OCH_3).

^{13}C-NMR (125 MHz, CD_3OD), δ (ppm): 158.3 (C-3, 5), 147.6 (C-4'), 146.3 (C-3'), 139.1 (C-1), 130.8 (C-1'), 127.9 (C-*α*), 126.5 (C-*β*), 118.7 (C-2), 112.2 (C-5'), 111.3 (C-6'), 104.5 (C-2, 6), 101.4 (C-4), 55.0 (4'-OCH_3).

*4.1.6. Compound **DH6** (deoxyrhapotigenin)*

Brown solid.

^{1}H-NMR (500 MHz, CD_3OD), δ (ppm): 7.43 (1H, d, *J* = 8.5 Hz, H-2',6') 6.98 (1H, d, *J* = 16.5 Hz, H-*α*), 6.89 (2H, d, *J* = 9.0 Hz, H-3',5'), 6.83 (1H, d, *J* = 16.5 Hz, H-*β*), 6.45 (2H, d, *J* = 2.5 Hz, H-2,6), 6.16 (1H, t, *J* = 2.0 Hz, H-4), 3.80 (3H, s, 4'-OCH_3),

^{13}C-NMR (125 MHz, CD_3OD), δ (ppm): 160.8 (C-4'), 159.7 (C-3,5), 141.2 (C-1), 131.5 (C-1'), 129.1 (C-*α*), 128.8 (C-2',6'), 127.8 (C-*β*), 115.1 (C-3',5'), 105.8 (C-2,6), 102.8 (C-4),55.7(4'-OCH_3).

*4.1.7. Compound **DH7** (rhaponticin and isorhapontin)*

Brown solid.

See Table. 4.2.7

4.2.* Determination of the structure of compounds isolated from *R. trisetifer

*4.2.1. Compound **RT1** (chrysophanol)*

See **DH1**

*4.2.2. Compound **RT2** (physcion)*

See **DH2**

*4.2.3. Compound **RT3** (emodin)*

See **DH3**

*4.2.4. Compound **RT4** (emodin-8-O-β-D-glucoside)*

Orange powder

^{1}H-NMR (500MHz, DMSO-d_6), δ_H (ppm): 7.46 (1H, brs, H-4), 7.28

(1H, d, *J* = 1.5 Hz, H-5), 7.16 (1H, brs, H-7), 7.00 (1H, brs, H-2), 5.10 (1H, d, *J* = 7.5 Hz, H-1'), 3.17-3.75 (5H, m, H-2'-6'), 2.41 (3H, s, 3-CH_3).

^{13}C-NMR (125MHz, DMSO-d_6), δ_C (ppm): 186.39 (C-9), 182.08 (C-10), 164.15 (C-6), 161.54 (C-8), 161.03 (C-1), 146.86 (C-4), 136.47 (C-10a), 132.06 (C-4a), 124.14 (C-2), 119.21 (C-4), 114.43 (C-9a), 113.31 (C-8a), 108.29 (C-7,5), 100.81 (C-1'), 77.27 (C-2"), 76.36 (C-3'), 73.24 (C-2"), 69.45 (C-4'), 60.57 (C-6'), 21.35 (3H, s, 3-CH_3).

*4.2.5. Compound **RT5** (chrysophanol -8-O-β-D-glucoside and physcion -8-O-β-D-glucoside)*

Orange powder

See Table 4.3.5

*4.2.6. Compound **RT6** (β-sitosterol)*

White powder

^{1}H-NMR (500MHz, $CDCl_3$), δ_H (ppm): 5.31 (1H, m, H-6), 3.51 (1H, m, H-3), 1.01 (3H, s, CH_3-19) 0.92 (3H, d, *J*=6.2 CH_3-21), 0.84 (3H, t, J=7.0 CH_3-29), 0.83 (3H, d, *J*=6.5 CH_3-26), 0.81 (3H, d, *J*=6.5 CH_3-27), 0.68 (3H, s, CH_3-18).

*4.2.7. Compound **RT7** (daucosterol)*

White powder

^{1}H-NMR (500MHz, DMSO-d_6), δ_H (ppm): 5.83 (1H, m, H-6), 5.03 (1H, d, *J*=7.0 Hz, H-1'), 4.05-4.60 (5H, m, H-2'-H-6'). 3.93 (1H, m, H-3), 3.98 (1H, m, H-5'), 1.00 (3H, d, *J*=6.5 CH_3-21), 0.94 (3H, s, CH_3-19), 0.90 (3H, t, *J*=7.0 CH_3-29), 0.89 (3H, d, *J*=6.5 CH_3-26), 0.89 (3H, d, *J*=6.5 CH_3-27), 0.67 (3H, s, CH_3-18).

^{13}C-NMR (125MHz, DMSO-d_6), δ_C (ppm): 140.6 (C-5), 121.3 (C-6), 100.9 (C-1'), 77.1 (C-3'), 76.8 (C-5'), 76.8 (C-3), 73.6 (C-2'), 70.2

(C-4'), 61.2 (C-6'), 56.3 (C-140), 55.5 (C-17), 50.7 (C-9), 49.7 (C-24), 45.2 (C-13), 38.4 (C-4), 36.9 (C-12), 36.3 (C-1), 35.6 (C10), 33.4 (C-20), 31.5 (C-8), 29.4 (C-7), 27.7 (C-16), 28.8 (C-23), 27.9 (C-2), 25.5 (C-25), 24.0 (C-15), 22.7 (C-28), 22.10 (C-11), 20.7 (C-27), 19.8 (C-19), 19.0 (C-26), 18.9 (C-21), 12.2 (C-29). 11.9 (C-18).

4.3. **Determination of the structure of compounds isolated from *S.alata L***

4.3.1 Compound ***SA1***

Yellow powder

^{1}H-NMR (500MHz, $(CD_3)_2CO$), δ (ppm)): 9.99 (2H, s, 2-OH, 6-OH), 9.93 (4-OH), 5.93 (2H, s, H-3, H-6), 4.04 (3H, brs, $-CH_3$),

^{13}C-NMR (125MHz, $(CD_3)_2CO$), δ(ppm): 170.22 (-COO-), 162.90 (C-4), 164.75 (C-2, C-6), 95.42 (C-3, C-5), 93.23 (C-1), 52.05($-CH_3$).

4.3.2 Compound ***SA2 (****kaempferol)*

Yellow crystalline solid.

^{1}H-NMR (500MHz, CD_3OD), δ (ppm): 8.08 (2H, d, *J*=7.0 Hz, H-2', -6',), 6.91 (2H, d, *J*=7.0 Hz, H-3',-5'), 6.40 (1H, d, *J*=2.5 Hz , H-8), 6.18 (1H, d, *J*=2.0 Hz, H-6).

^{13}C-NMR (125MHz, CD_3OD), δ (ppm): 177.39 (C-4), 165.58 (C-7), 162.53 (C-5), 160.57 (C-4'), 158.28 (C-8a), 148.06 (C-2), 137.14 (C-3), 130.68 (C-2', C-6'), 123.74 (C-1'), 116.31 (C-3', C-5'), 104.56 (C-4a), 99.26 (C-6), 94.46 (C-8).

4.3.3 Compound ***SA3 ((-)****epiafzelechin)*

White powder

^{1}H-NMR (500MHz, $(CD_3)_2CO$), δ_H (ppm): 8.28 (1H, brs, 4'-OH), 8.16 (1H, brs, 5-OH), 7.99 (1H, brs, 7-OH), 7.38 (2H, d, *J*= 8.5 Hz, H-2',-6'), 6.83 (2H, d, *J* =8.5 Hz, H-3',-5'), 6.04 (1H, d, *J*= 2.5 Hz,

H-6), 5.94 (1H, d, *J*= 2.5 Hz, H-8), 4.95 (1H, s, H-2), 4.24 (1H, m, H-3), 3.65 (1H, d, *J*=5.5 Hz, 3-OH), 2.89 (1H, dd, *J*= 16.5, 4.5 Hz, H-4β), 2.76 (1H, dd, *J*= 16.5, 3.0 Hz, H-4α).

^{13}C-NMR (125MHz, $(CD_3)_2CO$), δ_C (ppm): 157.63 (C-4'), 157.62 (C-7), 157.57 (C-5), 157.19 (C-8a), 131.49 (C-1'), 129.13 (C-2', C-6'), 115.46 (C-3', C-5'), 99.74 (C-4a) , 96.19 (C-6), 95.72 (C-8), 79.47(C-2), 66.85 (C-3), 29.13 (C-4).

*4.3.4 Compound **SA4**(rhein)*

Orange powder

^{1}H-NMR (500MHz, DMSO-d_6), δ_H (ppm): 8.11 (1H, brs, H-4), 7.78 (1H, dd, *J*=8.0,7.5Hz, H-6), 7.71 (1H, brs, H-2), 7.69 (1H, d, *J*=8.0Hz, H-5), 7.37 (1H, d, *J*=8.0 Hz, H-7).

^{13}C-NMR (125MHz, DMSO-d_6), δ_C (ppm): 191.23 (C-9), 181.27 (C-10), 166.27 (3-COOH), 161.46 (C-1,8), 137.33 (C-6,3), 133.31 (C-4a, 10a), 124.20 (C-2,7), 119.23 (C-5,4), 117.52(C-9a) ,116.14 (C-8a).

*4.3.5 Compound **SA5***

See **DH4**

*4.3.6 Compound **SA6 (**aloe-emodin-8-O-glucoside)*

Orange powder

^{1}H-NMR (500MHz, DMSO-d_6), δ_H (ppm): 7.89 (1H, dd, *J*= 8.5, 1.5 Hz, H-5), 7.85 (1H, t, *J*= 8.0Hz, H-6), 7.71 (1H, dd, *J*= 8.5, 1.5 Hz, H-7), 7.67 (1H, d, *J*= 1.5 Hz, H-4), 7.29 (1H, d, *J*= 1.5 Hz, H-2), 5.56 (1H, d, *J*=6.0 Hz, H-1'), 4.60 (2H, m, 3 CH_2OH).

^{13}C-NMR (125MHz, DMSO-d_6), δ_C (ppm): 187.58 (C-9), 182.11 (C-10), 161.62 (C-1), 158.23 (C-8), 152.26 (C-3), 135.95 (C-6), 134.48 (C-4a), 132.26 (C-10a), 122.44 (C-7), 120.72 (C-2), 120.59 (C-5), 116.01 (C-4), 115.47 (C -8a,9a), 100.49 (C-1'), 77.26 (C-5'), 76.52

(C-3'), 73.28 (C-2'), 69.51 (C-4'), 62.0 (3-CH_2OH), 60.06 (C-6').

*4.3.7 Compound **SA7** (kaempferol-3-O-glucoside)*

Orange powder

^{1}H-NMR (500MHz, CD_3OD), δ (ppm): 8.07 (2H, d, *J*=8.0 Hz, H-2', H-6'), 6.90 (2H, d, *J*=8.0 Hz, H-3',-5'), 6.42 (1H, d, *J*=2.0 Hz, H-8), 6.26 (1H, d, *J*=2.0 Hz, H-6), 5.27 (1H, d, *J*=7.5 Hz, H-1''), 3.70 (1H, dd, *J*= 12.0, 2.0, H-6"$_a$), 3.52 (1H, dd, *J*= 12.0, 2.0, H-6"$_b$), 3.44 (1H, m, H-2"), 3.41 (1H, m, H-3"), 3.20 (1H, m, H-5").

^{13}C-NMR (125MHz, CD_3OD), δ (ppm): 179.53 (C-4), 166.26 (C-7), 163.11 (C-5), 161.59 (C-4'), 159.07 (C-8a), 158.56 (C-2), 135.47 (C-3), 132.28 (C-2', C-6'), 122.83 (C-1'), 116.32 (C-3', C-5'), 105.70 (C-4a), 104.11 (C-1"), 99.97 (C-6), 94.80 (C-8), 78.44 (C-5"), 78.07 (C-3"), 75.75 (C-2"), 71.38 (C-4"), 62.65 (C-6").

*4.3.8 Compound **SA8 (**kaempferol-3-O-gentiobioside)*

Orange powder

^{1}H-NMR (500MHz, CD_3OD), δ (ppm): 8.11 (2H, d, *J*=8.5 Hz, H2', H-6'), 6.90 (1H, d, *J*=8.5 Hz, H-3', H-5'), 6.42 (1H, d, *J*=2.0 Hz, H-8), 6.21 (1H, d, *J*=2.0 Hz, H-6), 5.25 (1H, d, *J*=7.4, H-1"), 4.16 (1H, d, *J*= 7.5 Hz, H-1"'), 3.96 (1H, dd, *J*= 12.0, 1.5 Hz, H-6"$_b$), 3.65 (dd *J*=12.0, 7.0, H-6"$_a$) 3.74 (1H, dd *J*= 12.0, 2.0, H-6"'$_b$), 3.58 (dd *J*=, 12.0, 5.5, H-6"'$_a$), 3.49 – 3.37 (4H, m, H-2",H-3",H-4",H-5"), 3.25 (1H, dd, *J*= 9.0, 9.0 Hz, H-4"'), 3.20 (1H, t, *J*=9.0 Hz, H-3"'), 3.09 (1H, dd, *J*= 8.0, 8.5 Hz, H-2"'), 3.04 (1H , ddd, *J*= 9.5,7.0,3.5 H-5"')

^{13}C-NMR (125MHz, CD_3OD), δ (ppm): 179.41 (C-4), 166.20 (C-7), 161.56 (C-5), 161.56 (C-4'), 158.55 (C-8a), 158.99 (C-2),135.52 (C-3), 132.37 (C-2', C-6'), 122.70 (C-1'), 116.23 (C-3', C-5'), 105.75 (C-4a), 104.56 (C-1"'), 104.08 (C-1"), 100.05 (C-6), 94.93 (C-8), 77.93 (C-5"), 77.81 (C-3"'), 77.78 (C-3"), 77.64 (C-5"'), 75.74 (C-

2”'), 75.08 (C-2”), 71.25 (C-4”), 71.35 (C-4”'), 69.53 (C-6”), 62.54 (C-6”').

4.4. Evaluation of the biological activity of extract and isolated constituents

4.4.1. Evaluation of the biological activity of extract and isolated constituents from R. tanguticum.

4.4.1.1. Evaluation of the biological activity of extracts and isolated constituents from R. tanguticum against various phytopathogenic fungi

4.4.1.1.a. *In vivo* evaluation of the biological activity of extracts from *R. tanguticum* against various phytopathogenic fungi

Table. 4.17. Disease control efficacy of ethyl acetate and dichloromethane soluble extracts derived from *R. tanguticum* rhizomes against seven plant pathogenic fungi

Extract	Con. (μg/mL)	Control value[b] (%)						
		RCB[c]	RSB	TGM	TLB	WLR	BPM	PAN
EtOAc[a]	500	25 ± 0	11 ± 10	0	7 ± 7.1	3 ± 3.3	95 ± 1.7	0
	1000	75 ± 0	16 ± 15	21 ± 7.1	21 ± 7.1	43 ± 10	100 ± 0	93 ± 2.5
	3000	91 ± 3.7	0	14 ± 0.7	96 ± 3.3	83 ± 0	100 ± 0	92 ± 0
DCM	500	50 ± 12.5	11 ± 0.5	7 ± 7.1	7 ± 7.1	3 ± 3.3	97 ± 0	64 ± 3.3
	1000	88 ± 0	16 ± 12.5	29 ± 0	64 ± 7.1	60 ± 0	100 ± 0	94 ± 3.3
	3000	93 ± 0	5 ± 5	21 ± 7.1	100 ± 0	100 ± 0	100 ± 0	96 ± 0.8
Blasticidin-S	50	100 ± 0	-[d]	–	–	–	–	–
	1	90 ± 2.5	–	–	–	–	–	–
Validamycin	50	–	100 ± 0	–	–	–	–	–
	5	–	85 ± 5	–	–	–	–	–
Fludioxonil	50	–	–	100 ± 0	–	–	–	–
	5	–	–	88 ± 2.1	–	–	–	–
Dimethomorph	10	–	–	–	100 ± 0	–	–	–
	2	–	–	–	88 ± 2.1	–	–	–
Flusilazole	10	–	–	–	–	87 ± 0	–	–
	2	–	–	–	–	43 ± 10	–	–
Benomyl	100	–	–	–	–	–	100 ± 0	–
	1	–	–	–	–	–	87 ± 2.3	–
Dithianon	50	–	–	–	–	–	–	83 ± 3
	10	–	–	–	–	–	–	20 ± 0

4.4.1.1.b. *In vivo* evaluation of the biological activity of isolated constituents from *R. tanguticum* against various phytopathogenic fungi (Table)

4.4.1.1.c. *In vitro* evaluation of the biological activity of isolated constituents from *R. tanguticum* against various phytopathogenic fungi

Anthraquinone	Con. (ppm)	*Rhzoctonia solani*		*Botrytis cinera*		*Phytophthora infestans*		*Colletotrichum gloesporioides*	
		Opp	Eff %	Opp	Eff %	Opp	Eff %	Opp	Eff %
DH1 (chrysophanol)	100	5.7	34.5	5.3	19.7	6.3	14.9	5.7	(Ctrl)
	ĐC	8.7		6.6		7.4		6.8	
	200	5.1	37.8	4.6	31.3	5.8	24.7	4.6	27.0
	ĐC	8.2		6.7		7.7		6.3	
DH2 (physcion)	100	4.1	53.9	5.8	15	6.7	11.8	5.7	13.0
	ĐC	8.9		6.8		7.6		6.5	
	200	3.4	63.4	5.5	17	6.3	18.2	5.0	26.5
	ĐC	9.3		6.6		7.7		6.8	
DH3 (emodin)	200	2.6	71.1	2.2	65.1	4.6	42.5	4.1	37.9
	ĐC	9.0		6.3		8.0		6.6	
	300	1.7	79.5	0.7	88.5	2.2	71.8	2.4	67.1
	ĐC	8.3		6.1		7.8		7.3	

4.4.1.2. In vitro evaluation of the biological activity of extracts and isolated constituents from R.tanguticum against plant pathogenic bacteria

Table. 4.17. Minimum inhibitory concentration (MIC, μg/mL) of extracts and constituents derived from *R. tanguticum* rhizomes against ten pathogenic bacterial strains

Bacterial strains	MIC (μg/mL)									
	DH01[b]	DH02	DH03	DH04	DH05	DH06/07	EtOAc	DCM	SS	Chl
Acidovorax avenae subsp. *cattlyae*	18	150	–	–	38	–	125	125	nt[c]	1.25
Agrobacterium tumefaciens	–	150	–	–	75	–	–	–	nt	20
Burkholderia glumae	300	75	600	600	38	–	–	500	nt	2.5
Clavibacter michiganensis subsp. *michiganensis*	600	150	600	600	75	–	500	250	nt	2.5
Pectobacterium carotovara subsp. *carotorova*	–	300	–	–	–	–	–	–	nt	2.5
Pectobacterium chrysanthemi	–	–	–	–	–	–	–	–	nt	1.25
Pseudomonas syringae pv. *lachrymans*	–	–	–	–	–	–	–	–	nt	inhibit
Xanthomonas arboricola pruni	–	75	–	–	–	–	500	250	nt	2.5
Pseudomonas syringae pv. actinidiae KW11	–	–	–	–	38		250	500	1.25	nt
Ralstonia solanacearum	–	150	–	–	–	600	500	500	5	nt

4.4.2. Evaluation of biological activity of extract and isolated constituents from R.trisetifer

4.4.2.1. Evaluation of the biological activity of extract and isolated constituents from R.trisetifer against various phytopathogenic fungi

Table. 4.21. *In vivo* antifungal activity of methanol extract of *R.trisetifer* and hexane and ethyl acetate layers therefrom against seven phytopathogenic fungi

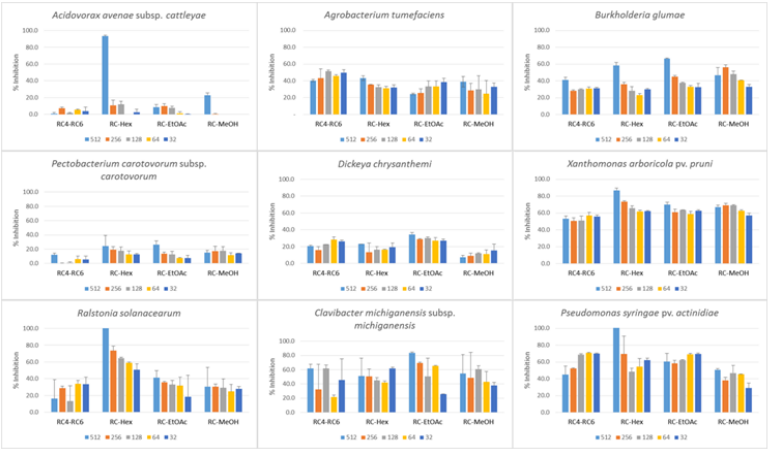
Material	Concn. (μg/mL)	Control value (%)[b]						
		RCB[c]	RSB	TGM	TLB	WLR	BPM	PAN
RC-Hex[a]	3000	61 ± 6.3	0 ± 0	90 ± 1.4	95 ± 0	90 ± 0	100 ± 0	89 ± 5.0
	1000	0 ± 0	0 ± 0	50 ± 0	43 ± 13.5	53 ± 7.4	100 ± 0	79 ± 5.0
	333	0 ± 0	0 ± 0	7 ± 7.1	7 ± 7.1	3 ± 3.3	95 ± 1.6	10 ± 10.0
RC-EtOAc	3000	40 ± 0	0 ± 0	7 ± 7.1	7 ± 7.1	60 ± 6.7	100 ± 0	85 ± 5.0
	1000	20 ± 0	0 ± 0	0.0 ± 0	0 ± 0	60 ± 6.7	97 ± 0	30 ± 10.0
	333	0 ± 0	0 ± 0	0.0 ± 0	0 ± 0	0 ± 0	93 ± 1.6	0 ± 0
RC-MeOH	3000	0 ± 0	0 ± 0	14 ± 0	0 ± 0	27 ± 6.7	100 ± 0	83 ± 3.0
	1000	0 ± 0	0 ± 0	7 ± 7.1	0 ± 0	0 ± 0	97 ± 0	10 ± 10
	333	0 ± 0	0 ± 0	7 ± 7.1	0 ± 0	0 ± 0	93 ± 1.6	0 ± 0
Blasticidin-S	50	100 ± 0	-[d]	–	–	–	–	–
	1	90 ± 2.5	–	–	–	–	–	–
Validamycin	50	–	100 ± 0	–	–	–	–	–
	5	–	85 ± 5	–	–	–	–	–
Fludioxonil	50	–	–	100 ± 0	–	–	–	–
	5	–	–	88 ± 2.1	–	–	–	–
Dimethomorph	10	–	–	–	100 ± 0	–	–	–
	2	–	–	–	88 ± 2.1	–	–	–
Flusilazole	10	–	–	–	–	87 ± 0	–	–
	2	–	–	–	–	43 ± 10	–	–
Benomyl	100	–	–	–	–	–	100 ± 0	–
	1	–	–	–	–	–	87 ± 2.3	–
Dithianon	50	–	–	–	–	–	–	83 ± 3
	10	–	–	–	–	–	–	20 ± 0

4.4.2.2. Evaluation of the biological activity of extracts and isolated constituents from R.trisetifer against plant pathogenic bacteria

4.4.2.2.a. *In vitro* evaluation of the biological activity of extract from *R.trisetifer* against plant pathogenic bacteria

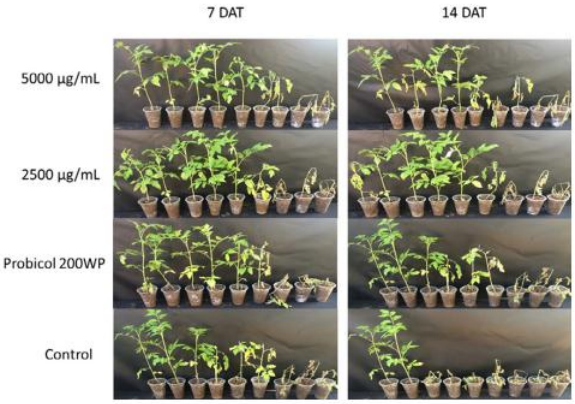
4.4.2.2.b. *In vitro* evaluation of the biological activity of isolated constituents from *R.trisetifer* against plant pathogenic bacteria

Figure 4.25. Inhibitory activity of botanical materials from *R.trisetifer* against the growth of nine phytopathogenic bacteria.



4.4.2.2.c. *In vivo* evaluation of the biological activity of n-hexane extract from *R.trisetifer* against plant pathogenic bacteria

Figure 4.25. Control efficacy of the hexane layer from the aerial parts of *R.trisetifer* against tomato bacterial wilt caused by phytopathogenic bacterial strains *Ralstonia solanacearum (BVTV)* on four-week tomato seedlings *(Lycopesium esculentum)* in greenhouse



4.4.3. Evaluation of the biological activity of extract and isolated constituents from S. alata

4.4.3.1. Evaluation of the biological activity of extract and isolated constituents from S. alata against various phytopathogenic fungi

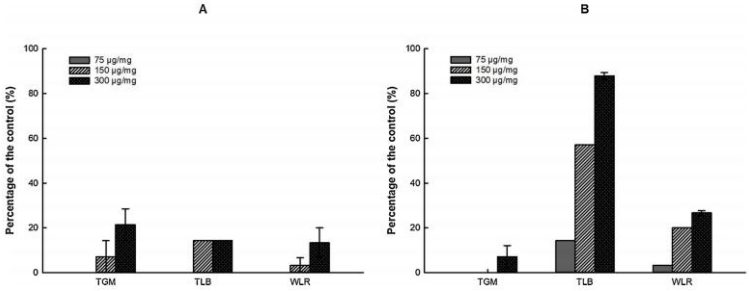
4.4.3.1.a. *In vivo* evaluation of biological activity of extract from ***S. alata*** against plant pathogenic bacteria

Table. 4.23 Disease control efficacy of ethyl acetate soluble extracts derived from *S. alata* against seven plant pathogenic fungi

Material	RCB	RSB	TGM	TLB	WLR	BPM	PAN
n-hexane extract	25	0	0	64.29	26.67	0	58.33
Ethyl acetate extract	93.75	0	28.57	95.71	90.00	0	93.33

4.4.3.1.b. *In vivo* evaluation of the biological activity of isolated constituents from ***S. alata*** against plant pathogenic bacteria

Figure 4.29. In vivo control effiacy of compounds SA4 **(rhein)** (A) and SA5 **(aloe-emodin)** (B) isolated from *S. alata* leaves against fungal diseases.



4.4.3.1.c. *In vitro evaluation of the biological activity of ethyl acetate extract and isolated constituents from S. alata against plant pathogenic bacteria*

Table. 4.24. *In vitro* inhibitory activity of ethyl acetate-soluble extract and the pure constituents isolated from the leaves of *S. alata*

Sample	*Phytophthora* sp. SK5		*Phytophthora capsici* TVH		*Magnaporthe oryzae*	
	IC_{50} (µg/mL)	IC_{90} (µg/mL)	IC_{50} (µg/mL)	IC_{90} (µg/mL)	IC_{50} (µg/mL)	IC_{90} (µg/mL)
**EtOAc[$] **	675.9 ± 56.3	1215 ± 56.1	< 250	> 2000	737.1 ± 94.0	1305.6 ± 255.2
SA1 (methyl 2,4,6-trihydroxybenzoate)	nt	nt	297.4 ± 34.2	> 300	nt	nt
SA2 (kaempferol)	196.0 ± 57.9	473.4 ± 64.1	341.8 ± 39.3	> 600	> 600	> 600
SA3 ((-)epiafzelechin)	189.5 ± 71.0	479.2 ± 79.9	350.5 ± 58.7	> 600	455.5 ± 50.9	> 600
SA4 (rhein)	85.1 ± 17.4	233.1 ± 19.8	127.5 ± 21.3	329.2 ± 27.7	> 600	> 600
SA5 (aloe- emodin)	413.7 ± 89.4	> 600	> 600	> 600	nt	nt
SA6 (aloe-emodin-8 -*O*-*β*-*D*-glucoside).	77.0 ± 26.6	194.3 ± 33.8	137.7 ± 26.0	> 200	nt	nt
SA7 (kaempferol-3 -*O*-glycoside)	> 600	> 600	> 600	> 600	nt	nt
SA8 (kaempferol-3 -*O*-gentiobiside)	359.2 ± 38.3	> 600	> 600	> 600	nt	nt

4.4.3.2. *In vitro evaluation of the biological activity of extracts and isolated constituents from S. alata against plant pathogenic bacteria*

Table. 4.25 Minimum inhibitory concentration (MIC) of Hex- and EtOAc-soluble extracts and the pure constituents derived from *S. alata* against ten phytopathogenic bacteria

	Bacterial strains	MIC[$] (µg/mL)											
		SA1 (methyl 2,4,6-trihydroxybenzoate)	SA2 (kaempferol)	SA3 ((-) epiafzelechin)	SA4 (rhein)	SA5 (aloe-emodin)	SA6 (aloe-emodin-8-O-β-D-glucoside)	SA7 (kaempferol-3-O-glycoside)	SA8 (kaempferol-3-O-gentiobiside)	Hex	EtOAc	SS	Chl
1	*Acidovorax avenae* subsp. *cattleyae*	nt	> 512	nt	<19	150	> 512	> 512	> 512	–	125	nt	1.25
2	*Agrobacterium tumefaciens*	nt	> 512	nt	–	–	> 512	> 512	> 512	–	–	nt	20
3	*Burkholderia glumae*	nt	> 512	nt	–	> 512	> 512	> 512	> 512	2000	500	nt	2.5
4	*Clavibacter michiganensis* subsp. *michiganensis*	nt	> 512	nt	> 512	> 512	> 512	512	> 512	500	500	nt	2.5
5	*Pectobacterium carotovora* subsp. *carotovora*	nt	> 512	nt	–	–	> 512	> 512	> 512	–	–	nt	2.5
6	*Dickeya chrysanthemi*	nt	> 512	nt	nt	nt	> 512	> 512	> 512	nt	nt	nt	nt
7	*Pseudomonas syringae* pv. *lachrymans*	nt	> 512	nt	–	–	> 512	> 512	> 512	–	–	nt	nt
8	*Xanthomonas arboricola pruni*	nt	> 512	nt	512	–	> 512	> 512	> 512	–	250	nt	2.5
9	*Pseudomonas syringae* pv. *actinidiae KW11*	nt	> 512	nt	–	–	> 512	> 512	> 512	–	500	1.25	nt
10	*Ralstonia solanacearum*	nt	> 512	nt	> 512	512	> 512	> 512	> 512	–	500	5	nt

4.5. Quantitative analysis of biological constituents of the extract by HPLC

4.5.1. Quantitative analysis of active constituents of R.tanguticum extracts by HPLC

Table. 4.28. Active constituents quantified (mg/mg) extract of *R.tanguticum*

	DH3 (emodin)	**DH5** (rhapontigenin)	**DH2** (physcion)	**DH1** (chrysophanol)	**DH6** (desoxyrhapontin/rin)	Total
Retention time	16,554	3,350	27,504	18,985	4,246	
Conc. (mg/mL)	0,0318-0,318	0,0266-0,26	0,0294-0,392	0,0344-0,344	0,013-0,13	
Linear mode	27213,2374x-14,327702	2640,28289x+ 286,23187	8153,71966x + 15,125033	28245,4253x+ 687,48645	8672,59332x+ 5,0837478	
Peak area	14,327702	286,23187	15,125033	687,48645	5,0837478	
Concentration of standar	27213,2374	2640,28289	8153,71966	28245,4253	8672,59332	
Regression coefficien	0,99999	0,99992	0,99933	0,99316	0,99995	
Content of substances in dichloromethane extract (mg/g)	27,92	12	260,06	386,030	36,7	
Content of substances in dichloromethane extract (%)	2,792		26,006	38,6030		67,4019

4.5.2. Quantitative analysis of active constituents of R.trisetifer extracts by HPLC

Table. 4.29. Anthraquinone derivatives identified and quantified (mg/mg) in the solvent layers and extract of *R.trisetifer*

	RT1 (chrysophanol)	**RT2** (physcion)	**RT3** (emodin)	**RT4** (emodin-8-*O*-*β*-D-glucoside),	**RT5a** (chrysophanol-8-*O*-*β*-D-glucoside)	**RT5b** (physcion-8-*O*-*β*-D-glucoside)	Total
Retention time	40,231 ± 0,003	41,827 ± 0,006	39,158 ± 0,018	27,244 ± 0,023	28,713 ± 0,018	29,239 ± 0,008	
Linear mode	y = 2,7214x + 8,8142	y = 0,4018x - 1,0714	y = 2,8208x + 51,459	y = 1,2238x + 74,778	y = 0,8519x + 18,033	y = 0,8524x + 4,515	
Peak area	8,8142	1,0714	51,459	74,778	18,033	4,515	
Concentration of standar	2,7214	0,4018	2,8208	1,2238	0,8519	0,8524	
Regression coefficien	0,9998	0,9998	0,9934	0,9945	0,9982	0,9982	
RT-Hex (µg/mg)	66,86	119,62	11,72	-	-	-	
Content of substances in RT-Hex (%)	6,68	11,96	1,72				20,36
RT-EA(µg/mg)	32,83	113,36	81,57	125,789	168,22	93,888	
Content of substances in RT-EA (%)	3,28	11,13	8,15	12,58	16,82	9,39	61,66
RT-MeOH(µg/mg)	11,85	31,06	11,25	14,204	20,26	13,745	
Content of substances in RT-MeOH (%)	1,18	3,10	1,12	1,42	2,20	1,37	10,39

4.5.3. Quantitative analysis of active constituents of S. alata extracts by HPLC

Table. 4.29. HPLC quantifiation of anthraquinones in the EtOAc-soluble extract and methanol extract of ***S. alata*** leaves

Tham số	**SA4** (rhein)	**SA5** (aloe-emodin)	**SA6** (aloe-emodin glucoside)	Total
Retention time	34,661	30,748	27,377	
Con mg/mL	0,25-4 mg/mL	0,5-6 mg/mL	0,5-16	
Linear mode	y = 2287,2x – 81,406	y = 2287,2x – 81,406	y = 3431,9x + 9,75	
Peak area	81,406	81,406	9,75	
Concentration of standar	2287,2	2287,2	3431,9	
Regression coefficien	R^2 = 0.9995	R^2=0,999	R^2 = 0.9998	
DCM extract	6.38	10.97	32.75	50.10
EA extract	5.77	12.51	55.98	74.26
MeOH extract	0.90	2.40	9.37	12.67

4. 6. Experimental design and optimization of extraction processes

4.6.1. Experimental design and optimization of extraction processes for R. tanguticum.

4.6.1.1. Effect of extraction solvent

4.6.1.2. Effect of extraction temperature

4.6.1.3. Effect of extraction time

4.6.1.4. Effect of ratio of solvent and herbal raw material (mL/g)

4.6.1.5. Optimization of experimental design

4.6.1.6. Optimization

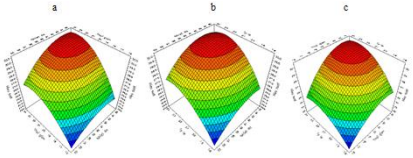


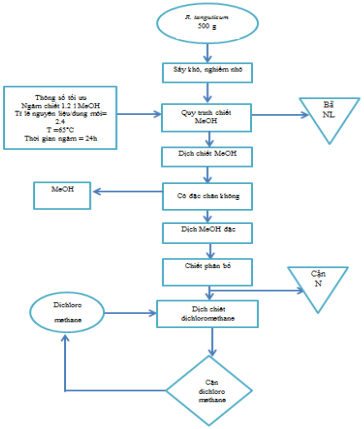
Figure 4.38 Response surface methodology (RMS) representing the relationship between the yield variables a: extraction time/ temperature; b: ratio of solvent and herbal raw material/temperature c: ratio of solvent and herbal raw material/extraction time

The optimal conditions for the extraction were: extraction temperature of 65˚C, ratio of solvent and medicinal herbs of 2.4/1, extraction time of 24h.

4.6.1.7. Model verification

4.6.1.8.Present of the extraction process

4.6.2. Extraction process for producing dichloromethane extract of R. tanguticum



4.6.3. Experimental design and optimization of extraction processes for S. alata

4.6.3.1. Effect of extraction solvent

4.6.3.2. Effect of extraction temperature

4.6.3.3. Effect of extraction time

4.6.3.4. *Effect of ratio of solvent and herbal raw material (mL/g)*

4.6.3.5. Optimization of experimental design

4.6.3.6. Optimization

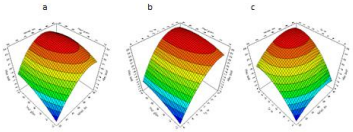


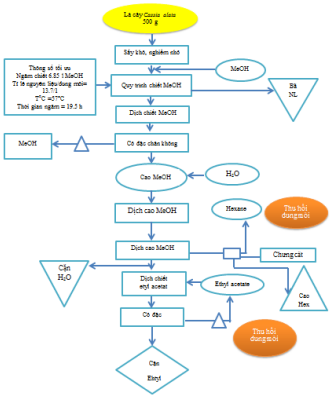
Figure 4.39 Response surface methodology (RMS) representing the relationship between the yield variables a: extraction time/ temperature; b: ratio of solvent and herbal raw material/temperature c: ratio of solvent and herbal raw material/extraction time

The optimal conditions for the extraction were: extraction temperature of 57˚C, ratio of solvent and medicinal herbs of 13.7/1, extraction time of 19h

4.6.3.7. Model verification

4.6.3.8. Present of extraction process

4.6.4. Extraction process for producing ethyl acetate extract of S.alata



CONCLUSIONS

1. Results of the study on chemical constituents from the investigated plant extracts

- Seven compounds from ethyl acetate extract and dichloromethane extract of *R. tanguticum* were isolated and determined to be 4 anthraquinones **DH1** (chrysophanol), **DH2** (physcion), **DH3** (emodin), **DH4** (alo-emodin), 2 stilbenes **DH5** (rhapotigenin), **DH6** (deoxyrhapotigenin) from and a mixture of 2 stilbene **DH7** (rhaponticin and isorhapontin).

- Seven compounds from n-hexane and ethyl acetate extracts of *R. trisetifer* including 3 anthraquinone: **RT1** (chrysophanol), **RT2** (physcion), **RT3** (emodin), 2 sterols: **RT6** (*β*-sitosterol), **RT7** (daucosterol) and 2 anthraquinone glycoside **RT4** (emodin-8-*O*-*β*-D-glucoside), and a mixture of anthraquinone glucoiside **RT5** (chrysophanol -8-*O*-*β*-D-glucoside and physcion -8-*O*-*β*-D-glucoside).

- Eight compounds from ethyl acetate extract of *S. alata* were isolated and determined to be 2 anthraquinone **SA4** (rhein), **SA5** (aloe-emdodin) and 1 anthraquinone glycoiside **SA6** (aloe-emodin-8-*O*-glucoside), 3 phenolic compounds **SA1** (methyl 2,4,6-trihydroxybenzoate), **SA2** (kaempferol), **SA3** (-) epiafzelechin) and 2 phenolic glycosides **SA7** (kaempferol-3-*O*-glucoside), **SA8** (kaempferol-3-*O*-gentiobioside).

2. The results for biological activity against phytopathogenic fungi and bacteria

+ Dichloromethane and ethyl acetate soluble extracts derived from *R. tanguticum* rhizomes effectively controlled plant diseases caused by five fungi *M. oryzae* (**RCB**), *P. infestans* (**TLB**), *P. recondita*

(**WLR**), *B.graminis* f.sp. *hordei* (**BPM**) and *C. coccodes* (**PAN**) in *in vivo* whole plant bioassay at a concentration of 3000 μg/mL. These extracts showed strong inhibitory activity against *B.graminis* f.sp. *hordei* (**BPM**) with control values from 95 to 97% at a concentration of 500 μg/mL.

These extracts showed strong *in vitro* inhibitory activity against the bacterial growth of *Acidovorax avenae* subsp. *cattlyae* (Aac), *Clavibacter michiganensis* subsp. *michiganensis*, *Xanthomonas arboricola* pv. *pruni* (Xap), and *Pseudomonas syringae* pv. *actinidiae* (Psa) with MIC values ranging from 125 to 250 μg/mL. In particular, for Aac, these extracts had IC_{90} values of 63.4 μg/mL and 118.6 μg/mL (respectively); and IC_{50} values of 30.8 μg/mL and 46.0 μg/mL respectively.

DH1 (chrysophanol), **DH2 (**physcion) and **DH3 (**emodin) were significantly effective in suppressing the development of *B. cinerea*, *C. gloesporioides*, *R. solani*, *P. infestans* at concentrations ranging from 75 to 300 μg/mL. Inhibitory activity of **DH3 (**emodin) were effectively increased from 65-88% comparing to controls and depend different concentrations.

+ The methanol extract of aerial part of *R. trisetifer* effectively controlled plant diseases caused by two fungi *B.graminis* f.sp. *hordei* (**BPM**) and *C. coccodes* (**PAN**) in *in vivo* whole plant bioassay with control values from 93 to 100% at concentration 3000 μg/mL.

n-Hexane layers showed inhibitory activity against the bacterial growth of *Acidovorax avenae* subsp. *cattlyae* (with control values 92.0%), *X. pruni* (86.0%), *R. solanacearum* (100%) and *P. actinidiae* (100%) at concentration 512 μg/mL.

+ Ethyl acetate soluble extracts derived from *S. alata* effectively controlled plant diseases caused by four fungi *M. grisea* (**RCB**), *P. infestans* (**TLB**), *P. recondita* (**WLR**), *C. gloeosporioides* (**PAN**) in *in vivo* for whole plant bioassay with control values more than 90% at concentrations 3000 µg/mL.

These extracts showed strong inhibitory activity against the bacterial growth of *A. avenae* subsp. *cattlyae*, *B. glumae*, *C. michiganensis* subsp. *michiganensis*, *P. syringae* pv. *actinidiae*, *R. solanacearum* and *X. arboricola pruni* with MIC values ranging from 125 to 600 µg/mL. In particular, for Aac, these extracts have strong *in vitro* inhibitory activity at concentration MIC 125 µg/mL.

CA4 (rhein) and **CA5** (aloe-emodin) showed *in vitro* inhibition for the bacterial growth of *Acidovorax avenae* subsp. *cattlvae*. In particular, **CA4** showed excellent *in vitro* inhibition with at concentration 10 µg/mL (MIC <19 µg/mL)

3. Optimization and proposed technological processes

- Research on optimization of technological processes making extract of rhubarb *R.tanguticum*: the optimal conditions for the extraction were: extraction temperature 65˚C, ratio of solvent and medicinal herbs 2.4/1, extraction time 24h.

- Proposed technological process making dichloromethane extract of rhubarb *R.tanguticum*.

- Research on optimization of technological processes making extract of *S. alata*: the optimal conditions for the extraction were: extraction temperature 57˚C, ratio of solvent and medicinal herbs 13.7/1, extraction time 19h.

- Proposed technological process making ethyl acetate extract of *S.alata*.

RECOMMENDATION

- Structure–activity relationship study on phenolic compounds with *in vivo* biological activity against various phytopathogenic fungi and *in vitro* against plant pathogenic bacteria.
- Continue to research on optimization of extraction technological processes using ultrsonic or microwave for *R. tanguticum.*
- Continue to research on optimization of extraction technological processes using ultrsonic or microwave for *S. alata.*

NEW CONTRIBUTIONS OF THE THESIS

- This is the first publication on new biological activity of inhibition against various phytopathogenic fungi and plant pathogenic bacteria of constituents of three species, including *R. tanguticum, S. alata*, *R. trisetifer*, indicating structure of main ingredients showed strong inhibitory and could be potentially used for developing biopesticides environmentally friendly.
- The biologically active content of the plant extracts was quantitatively analyzed by HPLC, thus clarifies scientifis evidence on the effectiveness of the plant extracts against phytopathogenic microorganisms.
- Thesis report on extracts processes were investigated by using response surface methodology (RMS). Two model were constructed for *R. tanguticum* and *S. alata*. The value of optimal were indicated.

LIST OF PUBLISHED ARTICLES

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https://doi.org/10.1016/j.indcrop.2021.113465

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Antimicrobial efficacy of extracts and constituents fractionated from *Rheum tanguticum* Maxim. ex Balf. rhizomes against phytopathogenic fungi and bacteria. Industrial Crops & Products 108 (2017) 442–450.
https://doi.org/10.1016/j.indcrop.2017.06.067

Isolation and identification of phenolic compounds from the leaf extract of *Cassia alata* L. Vietnam Journal of Chemistry, International Edition, 55(5): 589-594, 2017.
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