Trichomonicidal Activity of Herbal Extracts Used in Traditional Medicine in Korea

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Trichomonicidal activity of thirty methanolic herbal extracts used in traditional medicine in Korea was evaluated. *Trichomonas vaginalis* was used as experimental model, and anti-*Trichomonas* activity was determined over cultures of the parasite in TYM Diamond medium. Six methanolic extracts such as Acanthopanacis Cortex, Agrimoniae Herba, Pulsatillae Radix, Sanguisorbae Radix, Sophorae Radix, and Torilidis Fructus showed more than 50% trichomonicidal activity at the concentration of 200 g/ml. These extracts were further fractionated into n-butanol soluble and aqueous phases. Except for Acanthopanacis Cortex, all of n-butanol soluble phases showed potent trichomonicidal activity, while none of aqueous phases exhibited trichomonicidal activity.

Key words : *Trichomonas vaginalis*, Anti-Trichomonas, Korean traditional medicine

Introduction

Trichomonas vaginalis commonly causes vaginitis and perhaps cervicitis in women as well as urethritis in both sexes[1]. In the pregnant women, trichomonadsare implicated in the premature rupture of membranes, premature delivery, and the delivery of low-birth weight infants[2,3]. In addition, trichomoniasis has been implicated as a risk factor of human immunodeficiency virus transmission[4]. More than 250 million people worldwide are annually infected by this parasite and the prevalence rate was recently found to be 10.4% in the area of Kuri city, Korea[5].

Oral metronidazole has been the gold standard for the treatment of trichomoniasis for over 30 years[6], and is approved by the WHO although its potential carcinogenicity in rats and mutagenicity in bacteria[7,8]. In the United States, no alternatives to metronidazole are available for the treatment of trichomoniasis. Topical vaginal medications and pessaries can be prescribed for the treatment of *T. vaginalis* in women. Modern preparations include clotrimazole, povidone-iodine, nonoxynol-9, and arsenical pessaries. These preparations provide local symptom relief, but documentation on their effectiveness as cures has been inconsistent[9]. Although oral metronidazole therapy is highly effective in treating trichomoniasis, clinical failures have been observed and clinical resistance has been reported[10,11]. In addition, metronidazole sometimes causes adverse effects e.g. nausea, vomiting, headache, insomnia, dizziness, drowsiness, rash, dry mouth, and metallic taste[12].

Therefore, in the context of the efforts to improve the therapy against *T. vaginalis*, natural products could be a source of new drugs with high activity and low toxicity. In this paper, we describe the preliminary screening for the anti-Trichomonas activity of thirty methanolic extracts used in traditional medicine in Korea.

Materials and Methods

1. Plant Materials

All screened herbal drugs were purchased from University Oriental Drugstore, Iksan, Korea in August 2004 (Table 1). They were then authenticated by matching with herbarium specimen (herbarium of the College of Pharmacy, Wonkwang University, Korea).

2. Extraction and Fractionation of the Extracts

The methanolic extracts were obtained by sonication of

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plant material (each 100 g dry weight) for 3 h with methanol (1 l). After filtration, the solvent was removed completely by evaporation in vacuo. Samples of the dry methanolic extract were suspended in distilled water (500 ml) and partitioned with equal volume of n-butanol twice to afford n-butanol soluble and aqueous phases.

Table 1. Survival rates (%) of *T. vaginalis* of the treated with methanolic herbal extracts.

Samples	Plant family	100 g/ml	200 g/ml	400 g/ml
Acanthopanacis Cortex	Araliaceae	87.8±3.2	50.0±1.9	8.0±1.6
Agrimoniae Herba	Rosaceae	30.5±6.9	12.1±1.3	2.4±1.3
Amomi tsao-ko Fructus	Zingiberaceae	98.9±1.6	76.3±4.3	69.4±1.0
Angelicae dahuricae Radix	Umbelliferae	99.3±1.1	84.9±1.8	79.6±1.3
Arecae Semen	Palmae	82.4±4.3	56.1±1.6	35.4±0.5
Artemisiae asiaticae Herba	Compositae	89.6±1.6	70.9±5.4	34.3±1.1
Cimicifugae Rhizoma	Ranunculaceae	72.6±1.8	62.4±4.8	33.2±3.5
Coptidis Rhizoma	Ranunculaceae	87.4±1.4	51.7±2.1	42.2±2.8
Evodiae Fructus	Rutaceae	97.7±2.2	93.1±2.1	45.8±0.5
Fraxini Cortex	Oleaceae	84.6±2.2	64.3±3.9	46.1±0.8
Galla Rhois	Anacardiaceae	85.4±4.3	69.0±0.5	58.7±1.1
Genkwae Flos	Thymelaceae	96.7±4.6	71.3±3.5	24.3±0.4
Gentianae scabrae Radix	Gentianaceae	98.2±2.5	89.4±1.4	78.3±2.9
Lonicerae Flos	Caprifoliaceae	100±0	90.4±1.3	60.3±1.1
Meliae Fructus	Meliaceae	75.9±4.9	70.2±2.1	65.6±4.3
Moutan Cortex Radicis	Paeoniaceae	87.6±2.2	79.3±0.9	67.6±1.7
Mume Fructus	Rosaceae	99.2±1.1	92.9±0.8	76.3±6.9
Paeoniae Radix rubra	Ranunculaceae	87.7±5.4	65.6±4.4	40.8±2.7
Patriniae Radix	Valerianaceae	69.4±3.3	57.6±0.6	50.7±1.6
Phellodendri Cortex	Rutaceae	93.1±2.1	75.3±1.3	63.3±6.5
Picrorrhizae Rhizoma	Scrophulariaceae	91.6±0.9	67.0±4.3	56.6±1.7
Polygoni multiflori Radix	Polygonaceae	81.8±6.1	79.3±4.3	50.7±1.3
Pulsatillae Radix	Ranunculaceae	42.1±1.6	11.5±5.0	7.0±5.0
Sanguisorbae Radix	Rosaceae	73.5±0.4	25.6±6.7	7.2±6.6
Scutellariae Radix	Labiatae	94.9±0.7	71.8±2.2	36.1±1.4
Sophorae Radix	Leguminosae	58.8±7.6	44.3±1.5	0.5±0.7
Sophorae subprostratae Radix	Leguminosae	88.9±0.4	80.6±0.8	59.0±3.5
Stemonae Radix	Stemonaceae	75.1±1.6	71.9±1.8	55.7±5.4
Torilidis Fructus	Umbelliferae	49.4±7.5	10.7±0.6	0±0
Zanthoxyli Fructus	Rutaceae	85.8±2.8	77.1±3.3	70.2±2.1

3. *Trichomonas vaginalis* and Tube Dilution Test

T. vaginalis strain, Korean isolate (KT4), was used in this study[13]. The trophozoites were axenically cultured in a TYM medium[14]. In order to determine the minimum inhibitory concentrations (MIC), culture tubes were inoculated with 1 $\times 10^6$ trophozoites/ml at the early log phase, and with additions of the agents. The concentration ranges of the agents tested were 100, 200, and 400 g/ml. Tubes were incubated for 24 h at 37 ℃ with 5% CO_2. Viable protozoa were assessed with trypan blue exclusion 24 h after incubation with agents using the Neubauer chamber. Metronidazole was added, as reference drug, to a batch of tubes. Assays were carried out in triplicate. Percentages of survival rate were calculated versus control and expressed as the mean ± S.D.

Table 2. Survival rates (%) of *T. vaginalis* treated with the fractions of methanolic herbal extracts.

Samples	Fractions	100 g/ml	200 g/ml	400 g/ml
Acanthopanacis Cortex	n-BuOH Fr.	94.3±4.9	59.5±2.9	0.7±0.3
	Aqueous Fr.	100±0	79.6±0.9	74.6±0
Agrimoniae Herba	n-BuOH Fr.	3.1±3.3	0.08±0.1	0±0
	Aqueous Fr.	100±0	92.1±3.5	68.8±4.1
Pulsatillae Radix	n-BuOH Fr.	7.3±2.7	4.2±1.3	1.3±1.0
	Aqueous Fr.	92.1±1.6	85.3±0.9	68.8±4.1
Sanguisorbae Radix	n-BuOH Fr.	66.1±5.8	11.6±1.2	2.1±0.5
	Aqueous Fr.	92.2±0.5	78.1±0.9	68.8±4.1
Sophorae Radix	n-BuOH Fr.	34.5±1.7	0.4±0.1	0±0
	Aqueous Fr.	91.0±5.1	78.9±6.0	58.0±3.0
Torilidis Fructus	n-BuOH Fr.	83.4±6.7	16.7±0.8	0.7±0.2
	Aqueous Fr.	100±0	84.6±2.1	66.0±4.0

Results and Discussion

In the present study aiming at the determination of anti-Trichomonas activity of Korean traditional medicine, thirty of the plant methanolic extracts were investigated. *T. vaginalis* KT4 strain was used as experimental model and anti-*Trichomonas* activity was determined over cultures of the parasite in Diamond medium.

The results of the *in vitro* anti-*Trichomonas* activity of thirty methanolic herbal extracts are summarized in Table 1. Among the tested samples, the methanolic extracts of Acanthopanacis Coertex, Agrimoniae Herba, Pulsatillae Radix, Sanguisorbae Radix, Sophorae Radix, and Torilidis Fructus showed promising anti-*Trichomonas* activity. Especially, the methanolic extracts of Toridilis Fructus, Sophorae Radix, and Agrimoniae Herba showed the complete trichomonicidal activity at the concentration of 400 g/ml. Metronidazole, a positive control, showed the 0.94 ±0.3% of viability at the concentration of 5 g/ml.

In order to define the bioactive fractions, which contain thetrichomonicidal components in each methanolic extracts, above six methanolic extracts were suspended in distilled water and partitioned with n-BuOH. As shown in Table 2, except for Acanthopanacis Cortex all of the n-BuOH soluble

fractions showed potent trichomonicidal activity. These results suggest that the anti-*Trichomonas* compound(s) in each plant extract possesses less polar characteristics. Even though, the above fractions did not show the strong trichomonicidal activity in comparison to that of metronidazole, it is important to continue the search of the new trichomonicidal compounds from their n-BuOH fractions.

In summary, the investigation of anti-*Trichomonas* activity on thirty of Korean traditional medicine led to the findings that their n-BuOH soluble fractions of methanolic extracts of Agrimoniae Herba, Pulsatillae Radix, Sanguisorbae Radix, Sophorae Radix, and Torilidis Fructus showed potent trichomonicidal activity. These results allowed us to suggest that these five Korean traditional medicines could be the sources of new compounds against *T. vaginalis*, and would be necessary to study for the isolation of their trichomonicidal constituents.

Further study would be necessary to find strong trichomonicidal component among their constituents.

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