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ANTIMICROBIAL AND ANTIMALARIAL ACTIVITIES OF EXTRACTS ISOLATED FROM HERBAL PLANT *POLYGONUM HYDROPIPER*

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ABSTRACT

In the present article, we examined the antimicrobial, antifungal and antimalarial activities of two crude extracts 1, 4 (ethanol and methanol) and two fractions 2, 3 (dichloromethane and ethyl acetate) what isolated from *Polygonum hydropiper*. Antimicrobial activity was tested against *Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Cryptococcus neoformans, Aspergillus fumigatus, Klebsiella pneumonia* and methicillin resistant *Staphylococcus aureus (MRSa)*. Samples 1 and 3 were effective against *A. fumigates*. Sample 1 was mild active against *K. pneumoniae* with IC_{50} of 9.0 µg/mL. Sample 2 demonstrated mild activity against *E. coli and C. neoformans* with IC_{50} of 10.0 and 13.0 µg/mL, respectively.

Key words: *Polygonum hydropiper*, flavonoids, extractive substances, antibacterial, antifungal and antimalarial activity, *P. falciparum*.

INTRODUCTION

Polygonum hydropiper (L.) belongs to the family of *Polygonaceae*. The species is commonly known as marsh-pepper smartweed, marsh-pepper knotweed, smartweed, or water pepper [1, 2]. The plant generally grows in wet areas at watersides and in marshes and is usually predominant in agricultural fields. It is also commonly distributed to highland sites with highly organic, moist, or silty areas [3].

P. hydropiper has a strong peppery taste and is commonly used as a hot-tasting spice, food flavor, and garnish for a variety of traditional dishes. The Japanese people use the young shoot as spice and garnish with raw fish such as “sashimi” for its pungent taste. In Southeast Asia, the Chinese and Malays use the leaves in traditional dishes [2]. *P. hydropiper* also has a wide range of traditional uses for medicinal purposes. *P. hydropiper* and other species of *Polygonaceae* are used to treat fever, chill, joint pain, oedema and infectious diseases for more than 300 years in Chinese folk medicine [4].

Domestically the plant is used as anti-inflammatory, carminative, astringent, diuretic, CNS stimulant, diaphoretic, stomachic, emmenagogue, anthelmintic, in bleeding disorders and in diarrhea [5]. Moreover it is also used to treat rheumatoid arthritis [6], Prostate gland inflammation, diarrhea [7], insomnia, kidney diseases, hemorrhoids [8], hypertension, angina and other cardiovascular diseases [9, 10].

Antibacterial and antifungal activities of individual compounds isolated from *P. hydropiper* are studied earlier. Confertifolin isolated from the leaf essential oil showes strong/good antibacterial activity against *Enterococcus faecalis* (MIC 31.25 μg/mL) [11], *Bacillus subtilis* (minimum bactericidal concentration, MBC 100 μg/mL), *Staphylococcus aureus* (MBC 100 μg/mL), *Escherichia coli* (MBC 100 μg/mL), and *Salmonella choleraesuis* (MBC 50 μg/mL) [12]. Moreover, confertifolin is also found to have potent antifungal activity against *Epidermophyton floccosum*, *Curvularia lunata,* and *Scopulariopsis* sp. (MIC 7.81 μg/mL) and moderate activity against *Aspergillus niger*, *Botrytis cinerea*, *Magnaporthe grisea*, *Trichophyton mentagrophytes*, *Trichophyton rubrum* (MTCC 296 and clinical isolate) and *Trichophyton simii* (MIC 16.62–125 μg/mL) as compared to fluconazole (MIC < 12.5 – 100 μg/mL) and ketoconazole (MIC < 12.5 μg/mL) [11]. Polygodial is also reported to inhibit *Candida albicans*, *C. utilis*, *C. krusei*, *Cryptococcus neoformans*, *Saccharomyces cerevisiae*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, *T. rubrum*, and *Penicillium marneffei* [13, 14]. It showes potent fungicidal activity against *C. albicans* [13]. In another studies, polygodial isolated from *Warburgia* species and *P. hydropiper* show fungicidal activity against *S. cerevisiae* [15, 16].

In the present article, we examine the antimicrobial, antimalarial and antifungal activities of some extracts of *P. hydropiper*.

MATERIALS AND METHODS

Phytochemical investigation

Plant material *P. hydropiper* was bought at Pharmaceutical company "TES" (Almaty, Kazakhstan). Qualitative and quantitative phytochemical analysis of the plant extracts was carried out for the existence of flavonoids, tannins, coumarins, saponins, aminoacids, phenolic acids, alkaloids and polysaccharides using the methods reported previously [17].

Aluminum chloride test was used for the detection of flavonoids. Briefly ethanol extract of *P. hydropiper* was mixed with few drops of 10% lead acetate solution followed by heating. Formulation of flaky precipitate indicates presence of tanidium lead. For the detection of coumarins lactone test was used. Test solution was added a few drops of 10% alcohol solution KOH followed by heating. Formated yellow color of solution was disappear after added some distilled water. Then, 10% HCI was added for an acidic reaction, white precipitation was observed, this was for the presence of coumarins in extract. Presence of triterpene saponins was detected based on the formation more resistant foam in HCl solution than in KOH solution (steroid saponins). Formation of violet color of solution indicates presence of aminoacids. Ferric chloride solution was used for the detection of polyphenolic acids. The presence of alkaloids was determined using Dragendorff's reagent. Polysaccharides were detected by boiling the test sample with ethanol followed by cooling and appearance of white precipitate.

Determination of ES and total flavonoid contents

The plant material (10 g) was extracted percolating 10, 30, 50, 70%, absolute ethanol and methanol (100 ml) for 1 h at ambient temperature then all extracts were heated for 2 h at 76-78°C. Each liquid extract was filtered. 10 mL aliquot of extract was taken and dried under vacuum. The dry residues in extracts were determined according the method of the Pharmacopoeia of Republic of Kazakhstan [18]. Each analysis was repeated three times.

The total flavonoid content was determined according as the aluminum chloride colorimetric method [19]. 2 mL of each of these solutions with addition of 1 ml of $AlCl_3$ 1% were diluted to 25 ml with 95% ethanol. The absorption of these sample solutions was measured at 430 nm after 20 min. The calibration curve was prepared by preparing extract solutions (2 mL) was diluted to 25 mL with 95% ethanol.

Extraction and isolation

The dried material (1800 g), which was extracted with 50% ethanol after maceration with hitting for 1 hour at 78-80°C using water bath and constant shaking. Extraction with ethanol was repeated three times, added to original extract and filtered through muslin cloth and then through filter paper. The filtrate was concentrated using rotary evaporator (IKA RV 10 digital, IKA®-Werke GmbH & Co. KG, Germany) under reduced pressure at 40°C resulting in 150 g (8.31%) of dark brown semisolid mass. Crude ethanolic extract (1) 65 g of *P. hydropiper* was suspended in 500 ml of 50% methanol and consequently partitioned with dichloromethane (4×300 ml), ethyl acetate (4×300 ml), n-butanol (4×300 ml) and finally aqueous fraction was left. Extraction yield were 5.5 g (8.5%) for DCM fraction (2), 4.6 g (7.07%) for ethyl acetate fraction (3), 11 g (16.9%) for *n*-butanol fraction. The material (5.24) was soaked in methanol (50 ml) in a conical flask for 24 hours with frequent shaking. Methanol extract (4) was concentrated under reduced pressure.

Anti-microbial assay

All organisms were obtained from the American Type Culture Collection (Manassas, VA) and included the fungi *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906, the methicillin-resistant bacterium *Staphylococcus aureus* ATCC 43300 (MRS), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 13883. Susceptibility testing was performed using a modified version of the CLSI (formerly NCCLS) method [20]. Samples were serially-diluted in 20% DMSO/saline and transferred in duplicate to 96 well flat bottom microplates. Microbial inocula were prepared by correcting the OD630 of microbe suspensions in incubation broth to afford final target inocula. Drug controls [Ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and Amphotericin B (ICN Biomedicals, Ohio) for fungi] were included in each assay. All organisms were read at either 630 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Vermont) or 544ex/590em, (*A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Germany) prior to and after incubation. Minimum bactericidal concentrations (MBCs) were determined by removing 5μL from each clear well, transferring to agar and incubating. The MBC is defined as the lowest test concentration that kills the organism (allows no growth on agar). Percent growth was plotted versus test concentration to afford the IC_{50}.

***In vitro* antimalarial assay**

The *in vitro* antimalarial activity was determined against two strains of *Plasmodium falciparum*, D6 (chloroquine sensitive) and W2 (chloroquine resistant). The assay is based on the determination of plasmodial LDH activity. For the assay, a suspension of red blood cells infected with D6 or W2 strains of *P. falciparum* (200 μL, with 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 μg/mL Amikacin) was added to the wells of a 96well plate containing 10 μL of test samples diluted in medium at various concentrations. The plate was placed in

a modular incubation chamber (Billups-Rothenberg, CA) flushed with a gas mixture of 90% N_2, 5% O_2, and 5% CO_2, and incubated at 37°C, for 72 h. Parasitic LDH activity was determined by using Malstat™ reagent (Flow Inc., Portland, OR) according to the procedure of Makler and Hinrichs. Briefly, 20 μL of the incubation mixture was mixed with 100 μL of the Malstat™ reagent and incubated at room temperature for 30 min. Twenty microliters of a 1:1 mixture of NBT/ PES (Sigma, St. Louis, MO) was then added and the plate is further incubated in the dark for 1 h. The reaction was then stopped by the addition of 100 μL of a 5% acetic acid solution. The plate was read at 650 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Vermont). IC_{50} values were computed from the dose–response curves. Artemisinin and chloroquine were included in each assay as the drug controls. DMSO (0.25%) was used as vehicle control.

RESULTS AND DISCUSSION

P. hydropiper was tested positive for the presence of flavonoids, tannins, coumarins, saponins, aminoacids, phenolic acids and polysaccharides while tested negative for the presence of alkaloids (table 1).

Table 1. Phytochemical constituents in crude extract of *P. hydropiper*

BAS	Test	Observations	Results	Quantity, %
Flavonoids	Aluminum chloride test	Formation of yellow color which changed to colorless on acid addition	+	2.96
Tanins	10% lead acetate solution	Formulation of flaky precipitate	+	2.11
Coumarins	Lactone test	Appearance of white precipitate	+	0.42
Saponins	Foam test	Resistant foam in HCl solution (Triterpene saponins)	+	2.89
Aminoacids	Ninhydrin solution	Appearance of violet color	+	7.72
Phenolic acids	Ferric chloride	Formation of yellow-orange color	+	1.01
Alkaloids	Dragendorff's Test	No changes	-	-
Polysaccharides	Precipitation by alcohol	Appearance of white precipitate	+	4.15

Solvent extractions are the most commonly used procedures to prepare extracts from plant materials. Water, aqueous mixtures of ethanol, methanol, and acetone are commonly used to extract antioxidants from plants [21]. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities. Selecting the right solvent affects the amount and rate of polyphenols extracted.

Table 2 shows the total extractive substances (dry residues) and the total flavonoids in samples where a non linear correlation between the alcohol degree of the solutions and the total flavonoid contents can be observed. The most important differences are observed between 50% ethanol and 96% ethanol and methanol liquid extracts, whereas water and 10% ethanol show less important differences in the flavonoid content.

Table 2. Initial content of extractive substances of the plant materials and concentration of flavonoids of the extracts for different extracting solvents

Extracting solvent	Initial content of ES, %	Concentration of flavonoids, %
Water	18.00±0.38	2.55±0.18
Methanol	22.65±0.13	2.72±0.08
10% ethanol	22.30±0.16	2.56±0.11
30% ethanol	24.03±0.62	2.65±0.09
50% ethanol	26.80±0.38	2.96±0.22
70% ethanol	21.60±0.11	2.92±0.19
96% ethanol	16.72±0.25	2.95±0.14

The antifungal activities were evaluated against a panel of pathogenic fungi (*C. albicans, Cryptococcus neoformans* and *Aspergillus fumigatus*) associated with opportunistic infections. Fraction 2 showed growth inhibition activity against *C. neoformans* and *E. coli* with IC_{50} values of 13.0 and 10.0 μ/mL, respectively as shown in table 3. However, extract 1 showed comparatively stronger activity against *A. fumigates* and *K. pneumoniae* with an active concentration of 6.0 and 9.0 μg/mL.

Table 3. Antibacterial activity of some extracts of *P. hydropiper*, IC_{50} [a] (μg/mL)

S. No	*C. albicans*	*A. fumigatus*	*C. neofor mans*	*MRS*	*E. coli*	*P. aerugi nosa*	*K. pneumo niae*
1	18	6	59	na	20	na	9
2	43	na*	13	na	10	na	na
3	17	6	89	na	20	na	17
4	28	na	24	na	14	na	na
Ciprofloxacin	nt[b]	nt	nt	0.10	nt	nt	nt
Amphoterecin B	0.3	nt	1.5	nt	nt	nt	nt

Notes: *na – not active at 20 μg/mL; [a]IC_{50} – the concentration that affords 50% inhibition of bacterial/fungal growth; [b]nt – not tested.

Samples 1-4 were inactive against *MRS. Aureus* and *P. aeruginosa* . Samples 1-4 showed weak antifungal activity against *E. coli* (IC_{50} = 20.0, 10.0, 20.0 and 14.0 μg/mL, respectively) and *C. albicans* (IC_{50} = 18.0, 43.0, 17.0 and 28.0 μg/mL, respectively). Fraction 3 exhibited antifungal activity with an IC_{50} value of 6.0 μg/mL against *A. fumigatus* and IC_{50} value of 17.0 μg/mL against *K. pneumonia*. While fraction 2 and extract 4 are inactive against *A. fumigatus* and *K. pneumonia*.

The results of the antimalarial screening showed that fractions 2 and 3 demonstrated low activity against *P. falciparum* D6 clone (IC_{50} = 15113.8 μg/mL and 34774.3 μg/mL) and W2 clone (IC_{50} = 8934.9 μg/mL and 37637.7 μg/mL) with selectivity index ranging from > 3.1 to > 5.3 (table 4).

Table 4. Antimalarial activity of fractions 2 and 3 of *P. hydropiper*

S. No	*P. falciparum* (D_6 clone)		*P. falciparum* (W2 clone)	
	IC_{50}	SI	IC_{50}	SI
2	15113.8	>3.1	8934.9	>5.3
3	34774.3	>1.4	37637.7	>1.3
Artemisinin	0.016	14.0	0.12	7.5
Chloroquine	0.014	13.5	0.0095	140
Notes: SI = Selectivity Index = IC_{50} Vero cells/IC_{50} *P. falciparum*.				

CONCLUSION

DCM and EtOAc fractions isolated from *P. hydropiper* weak exhibited antimalarial activity. Ethanol extract and EtOAc fraction exhibited antimicrobial activity against *A. fumigatus*. EtOAc fraction was isolated from ethanol extract and showed the same antimicrobial activity against *A. fumigates* with the first one.

REFERENCES

1. Furuta T., Fukuyama Y., Asakawa Y. Polygonolide, an isocoumarin from *Polygonum hydropiper* possessing anti-inflammatory activity. *Phytochemistry*, 1986, vol. 25, pp. 517-520.
2. Huq A.K.M., Jamal J.A., Stanslas J. Ethnobotanical, phytochemical, pharmacological, and toxicological aspects of Persicaria hydropiper (L.) delarbre. *Evidence-Based Complementary and Alternative Medicine*, 2014.
3. Miyazawa M., Tamura N. Inhibitory compound of tyrosinase activity from the sprout of *Polygonum hydropiper* L. (Benitade). *Biological and Pharmaceutical Bulletin*, 2007, vol. 30, no. 3, pp. 595-597.
4. Wang K.W., Zhu J.R., Shen L.Q. A new lignan with anti-tumour activity from Polygonum perfoliatum L. *Natural Product Research*, 2013, vol. 27, no. 6, pp. 568-573.
5. Peng Z.F., Strack D., Baumert A., Subramaniam R., Goh N.K., Chi T.F., Tan S.N., Chi L.S. Antioxidant flavonoids from leaves of Polygonum hydropiper L. *Phytochemistry*, 2003, vol. 62, pp. 219-228.
6. Yang Y., Yu T., Jang H.-J., Byeon S.E., Song S.-Y., Lee B.-H., Rhee M.H., Kim T.W., Lee J., Hong S. In vitro and in vivo anti-inflammatory activities of Polygonum hydropiper methanol extract. *J. Ethnopharmacol.*, 2012, vol. 139, no. 2, pp. 616-625.
7. Redzic S.S. The ecological aspect of ethnobotany and ethnopharmacology of population in Bosnia and Herzegovina. *Collegium Antropologicum*, 2007, vol. 31, no. 3, pp. 869-890.
8. Popovic Z., Smiljanic M., Kostićb M., Nikić P., Janković S. Wild flora and its usage in traditional phytotherapy (Deliblato Sands, Serbia, South East Europe). *Indian Journal of Traditional Knowledge*, 2014, vol. 13, no. 1, pp. 9-35.
9. Qureshi R., Ghufran M., Sultana K., Ashraf M., Khan A. Ethnomedicinal studies of medicinal plants of Gilgit District and surrounding areas. *Ethnobotany Research and Applications*, 2008, vol. 5, pp. 115-122.

10. Choudhary R.K., Oh S., Lee J. An ethnomedicinal inventory of knotweeds of Indian Himalaya. *Journal of. Medicinal Plants Research.,* 2011, vol. 5, no. 10, pp. 2095-2103.

11. Duraipandiyan V., Indwar F., Ignacimuthu S.. Antimicrobial activity of confertifolin from *Polygonum hydropiper*. *Pharmaceutical Biology,* 2010, vol. 48, no. 2, pp. 187-190.

12. Kubo I., Fujita K.-I., Lee S.H., Ha T.J. Antibacterial activity of polygodial. *Phytotherapy Research.,* 2005, vol. 19, no. 12, pp. 1013-1017.

13. Malheiros A., Filho V.C., Schmitt C.B. Antifungal activity of drimane sesquiterpenes from *Drimys brasiliensis* using bioassay-guided fractionation. *Journal of Pharmacy and Pharmaceutical Sciences,* 2005, vol. 8, no. 2, pp. 335-339.

14. Lee S.H., Lee J.R., Lunde C.S., Kubo I. *In vitro* antifungal susceptibilities of Candida albicans and other fungal pathogens to polygodial, a sesquiterpene dialdehyde. *Planta Medica,* 1999, vol. 65, no. 3, pp. 204-208.

15. Kubo I., Taniguchi M. Polygodial, an antifungal potentiator. *Journal of Natural Products,* 1988, vol. 51, no. 1, pp. 22-29.

16. Taniguchi M., Yano Y., Tada E. Mode of action of polygodial, an antifungal sesquiterpene dialdehyde. *Agricultural and Biological Chemistry,* 1988, vol. 52, no. 6, pp. 1409-1414.

17. Muzychkina R.A., Korulkin D.Yu., Abilov Zh.A. Qualitative and quantitative analysis of the main BAS groups in medicinal plant raw materials and phytopreparations. Almaty, Kazakh University, 2004, 288 p. [Russian].

18. State Pharmacopeia of the Republic of Kazakhstan. Astana, Zhibek Zholy, 2015, 592 p. [in Russian].

19. Murav'eva D.A., Samylina I.A., Yakovlev G.P. Pharmacognosy. Moscow, Meditsina, 2007, 656 p. [in Russian].

20. Radwan M.M., Manly S.P., Ross S.A. Two new sulfated sterols from the marine sponge Lendenfeldia dendyi. *Natural Product Communications,* 2007, vol. 2, no. 9, pp. 901-904.

21. Xu B.J., Chang S.K.C. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of food science,* 2007, vol. 72, no. 2, pp. 159-166.