Anti-Porcine Epidemic Diarrhea Virus (PEDV) Activity and Antimicrobial Activities of *Artemisia dubia* Essential Oil

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The chemical composition, anti-porcine epidemic diarrhea virus (PEDV) activity and antimicrobial activity of *Artemisia dubia* essential oil were evaluated in this study. Fifty eight compounds from *A. dubia* essential oil were identified through analysis by gas chromatography-mass spectrometry (GC-MS). The major constituents of the oil were camphor (17.18%), germacrene-D (15.70%), trans (β-) racaryophyllene (6.79%), ene thujones (6.57%), 1, 8-cineole (5.94%) and camphene (5.08%). The essential oil was evaluated for antiviral activity against PEDV in Vero cells using a cytopathic effect (CPE) reduction method. The oils actively inhibited PEDV replication with a 50% inhibitory concentration (IC$_{50}$) of 43.7 µg/mL. The 50% cytotoxicity concentration (CC$_{50}$) of the oils was over 100 µg/mL and the derived therapeutic index was >2.3. Similar analysis of the ribavirin revealed that they have a relatively weaker efficacy when compared to the oils. The antimicrobial activity of the essential oil against 5 microorganisms was evaluated by the disc diffusion method. The essential oil exhibited antimicrobial activity against 5 tested microorganisms with a clear zone of 8-22 mm. Among the tested microorganisms, *Streptococcus pyogenes* was the most sensitive and *Candida albicans* the least. Therefore, in can be concluded that essential oils of *A. dubia* may have interesting applications for microbial control or the control of PEDV-derived diseases.

**Key words:** *Artemisia dubia*, essential oil, GC-MS, antimicrobial, anti-PEDV

**Introduction**

Essential oils possess a broad range of biological activities and are widely used in food, perfumes, pharmaceuticals and in aromatherapy. The major properties of essential oils are antibacterial, antifungal and antioxidant properties [8]. The versatile composition of plant essential oils and the wide antimicrobial spectrum, associated with their low toxicity, make them potential natural agents for food preservation [7].

Porcine epidemic diarrhoea virus (PEDV), a member of the *Coronaviridae* family, causes an enteric disease that is especially severe in piglets, among which mortality can reach up to 90% [20]. Live vaccines for PEDV are used in some countries but the efficacy of commercially available vaccines is limited in field conditions, and the protective immunity induced is insufficient [26]. *Artemisia dubia* Wall. ex Besser (family; compositae) is widely distributed throughout Nepal in between the altitude of 1,200 and 3,400 m and it from government of Nepal described sixteen species of *Artemisia* from Nepal [25]. Many *Artemisia* species have a characteristic scent or taste caused by monoterpenes and sesquiterpenes, which in many cases is the reason for their application in folk medicine [13]. There have been reports on the analysis of the essential oil compositions and their biological activities from various species of *Artemisia* [2, 12, 16, 17, 21]. Although a variety of pharmacological activities associated with chemicals in *A. dubia* essential oil has been demonstrated, antiviral activities against PEDV and antimicrobial effect have not been reported. The aim of this study is to examine the anti-PEDV and antimicrobial activity of *A. dubia* essential oil.

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Materials and Methods

Extraction of the essential oil

Fresh leaves of *A. dubia* were purchased from UNIQ F&F Co., Ltd. (Seoul, Korea). Five hundred g of leaves were hydro distillated for 4 hours, to give 0.2% yield. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration stored at 4°C until analyzed. The oil has pale yellow color with powerful aroma.

Gas chromatography (GC)

The instrumental analysis was performed by Aglient 6890 (Palo Alto, CA, USA). Helium as carrier gas was used at a flow rate of 1.0 ml/min. The amount of the samples injected was 0.2 µL in split mode (400 : 1). The injector temperature was set at 270°C. The GC column was DB-5MS stationary phase (60 m × 0.32 mm i.d., 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA). The GC oven temperature was initially maintained at 60°C for 2 min and then programmed to 5°C/min to 300°C and maintained for 5 min. Sample (0.2 µL) dissolved in CH₂Cl₂ (1:100 v/v) was injected. Essential oil samples were analyzed and the relative peak areas for individual constituents averaged. Quantification was computed as the percentage contribution of each compound to the total amount present. The percentage composition of the oils was computed by the normalization method from the GC peak areas.

Gas chromatography- mass spectrometry (GC-MS)

GC-MS analysis of the essential oil was performed under the same conditions with GC (column, oven temperature, flow rate of the carrier gas) by Aglient 6890 Plus gas chromatograph (Palo Alto, CA, USA) equipped with a 5973 mass selective detector quadrupole mass spectrometer. The mass spectrometer was run in the electron impact (EI) mode with electron energy at 70 eV. The mass spectrometer was operated in full scan mode between 35 and 700 amu. The components of essential oil were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley 2001 library data of the GC-MS system and literature data [1].

Virus, cell and reagents

Vero cell (african green monkey kidney cell line; ATCC CCR-81) was kindly provided by ATCC (American Type Culture Collection, Manassas, VA, USA). PEDV CV 777 (porcine epidemic diarrhea virus) was obtained from national veterinary research & quarantine service in Korea. Vero cell lines were maintained in minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 0.01% antibiotic-antimycotic. Antibiotic-antimycotic, trypsin-EDTA, FBS and MEM were supplied by Gibco BRL (Grand Island, NY, USA). The tissue culture plates were purchased from Falcon (BD Biosciences, NJ, USA). Sulforhodamine B (SRB) was purchased Sigma-Aldrich (St. Louis, MO). All other chemicals were of reagent grade.

Assays of antiviral activity and cytotoxicity

Assays of antiviral activity and cytotoxicity were evaluated by the SRB method using cytopathic effect (CPE) reduction recently reported [6]. Ribavirin was used as positive, and DMSO was used as negative control. PEDV containing CCID₅₀ (50% cell culture infective dose) of the virus stock to produce a appropriate cytopathic effects within 2 days. The inhibitory concentrations of the samples required to inhibit 50% of the viral growth was calculated from the mean dose-response (IC₅₀). The cytotoxic concentration is the concentration of the extract that inhibited actively replicating cells by 50% of control (CC₅₀). The therapeutic index was defined as CC₅₀/IC₅₀.

Antimicrobial activity

*Staphylococcus aureus* (KCTC 1927), *Staphylococcus epidermidis* (KCTC 1917), *Streptococcus pyogenes* (KCTC 3096), *Propionibacterium acnes* (KCTC 3314), *Candida albicans* (KCTC 7965) were provided by Korean Collection for Type Cultures, Seoul, Korea. Nutrient Agar, Brain Heart Infusion broth, Brain Heart Infusion Agar and Potato Dextrose Agar were bought from Difco USA. *P. acnes* was incubated in anaerobic jar at 37°C for 48-72 h and other organisms were in aerobic condition at 37°C for 24 h.

The antimicrobial activity of the essential oil from *A. dubia* was carried out by the disk diffusion Method [19] using 100 µl of suspension containing 10⁹ CFU/ml of bacteria and 10⁶ CFU/ml of yeast spread on nutrient agar or brain heart infusion agar and potato dextrose agar medium, respectively. The disks (Whatman, 8 mm in diameter) which impregnated with 19 mg of essential oil were placed on the inoculated agar. Control disk containing only 19 mg of ethanol employed to dissolve the essential oil showed no