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# Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. *Radiata*

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**Abstract**

The present study describes the chemical composition, antibacterial and antifungal activities of essential oils from *Bidens pilosa*, a traditional medicinal plant widely distributed in the subtropics and tropics. The essential oils from the fresh leaves and flowers of *B. pilosa* were analyzed by GC–MS. Forty-four components were identified, of which β-caryophyllene (10.9% and 5.1%) and τ-cadinene (7.82% and 6.13%) were the main compounds in leaves and flowers, respectively. The oils and aqueous extracts of leaves and flowers were subjected to screening for their possible antioxidant activities by using 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene bleaching methods. In the former case, the essential oils from leaves and flowers were found to be superior to all aqueous extracts tested with an $IC_{50}$ value of 47.5 and 49.7 μg/ml, respectively, whereas all extracts and essential oils seemed to inhibit the oxidation of linoleic acid in the latter case. The oils from *B. pilosa* exerted significant antibacterial and antifungal activities against six bacteria and three fungal strains. The inhibitory activity of the flower essential oils in Gram-negative bacteria was significantly higher than in Gram-positive. Our findings demonstrate that the essential oils and aqueous extracts of *B. pilosa* possess antioxidant and antimicrobial activities that might be a natural potential source of preservative used in food and other allied industries.


*Keywords:* Essential oils; Antifungal activity; *Bidens pilosa*; Antibacterial activity

## 1. Introduction

The food industry at present is facing a tremendous pressure from consumers for using chemical preservatives to prevent the growth of food borne and spoiling microbes. To reduce or eliminate chemically synthesized additives from foods is a current demand worldwide. A new approach to prevent the proliferation of microorganism or protect food from oxidation is the use of essential oils as preservatives. Essential oils of plants are of growing interest both in the industry and scientific research because of their antibacterial, antifungal, and antioxidant properties and make them useful as natural additives in foods (Pattnaik, Subramanyam, Bapaji, & Kole, 1997). Free radical oxidation of the lipid components in food due to the chain reaction of lipid peroxidation is a major strategic problem for food manufacturers. Due to undesirable influences of oxidized lipids on the human organisms, it is essential to decrease lipid peroxidation products in food (Karpińska, Borowski, & Danowska-Oziewicz, 2001). Reactive oxygen species are reported to be a causative agent of various diseases such as arthritis, asthma, dementia, mongolism, carcinoma and Parkinson's disease (Perry et al., 2000). Plant essential oils and their extracts have had a great usage in folk medicine, food flavoring, fragrance, and pharmaceutical industries (Kusmenoglu, Baser, & Ozek, 1995).

*Bidens pilosa* Linn. var. *Radiata* (family Asteraceae) is widely distributed in the subtropical and tropical regions of the world. It is 30–100 cm in height with yellow flowers and is well known as hairy beggar ticks, sticks tights, and Spanish needles. The plant is used in various folk medicines

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such as anti-inflammatory, antiseptic, liver-protective, blood-pressure lowering, and hypoglycemic effects (Dimo et al., 2002). Phenylpropanoid glucosides, polyacetylenes, a diterpenes, flavonoids, and flavone glycosides have been identified as bioactive components from this plant (Chiang et al., 2004). These compounds were suggested to be involved in the antioxidant (Chiang et al., 2004), antibacterial and antimicrobial activities (Rabe & Staden, 1997). The plant has been widely used in Taiwan as a traditional medicine and as a major ingredient of herbal tea, which is believed to prevent inflammation and cancer (Yang et al., 2006).

To the best of our knowledge, there are no available reports on chemical composition and biological activities of the essential oils from *B. pilosa*. Therefore, the aim of the present work was carried out to study *in vitro* antioxidant, antibacterial, and antifungal activities of the essential oils as well as the water extract of *B. pilosa* in addition to evaluate the component of essential oils by GC–MS. Essential oils obtained by steam-distillation were analyzed for their possible antioxidant activities by two complementary methods, namely DPPH free radical-scavenging and β-carotene bleaching methods due to identify all possible mechanisms characterizing an antioxidant.

## 2. Materials and methods

### 2.1. Plant material, extraction of essential oils, and preparation of the water extract

The fresh leaves and flowers of *B. pilosa* at vegetable stage were collected from nature around the campus of University of the Ryukyus, Okinawa, Japan, in April 2006. Fresh leaves or flowers (each 400 g) of *B. pilosa* were steam-distilled for 4 h. The distillates of each were extracted with 200 ml diethyl ether and conducted twice. The solvent was carefully removed under vacuum at 30 °C. The essential oils thus obtained were stored at 4 °C for testing and analyzing. After completion of steam-distillation, the aqueous mixtures of leaves or flowers were filtered to collect water extract which was evaporated to dryness on a rotary evaporator at 40 °C.

### 2.2. Identification by GC–MS

An aliquot of 1 μl oils dissolved in diethyl ether and adjusted to 1000 ppm was injected into GC–MS (QP-2010, Shimadzu Co., Kyoto, Japan). The DB-5MS column was 30 m in length, 0.25 mm i.d., and 0.25 μm in thickness (Agilent Technologies, J&W Scientific Products, Folsom, CA). The carrier gas was helium. The operating condition of GC oven temperature was maintained as: initial temperature 50 °C for 5 min, programmed rate 5 °C/min up to final temperature 280 °C with isotherm for 5 min. The injector and detector temperatures were set at 250 and 280 °C, respectively. The essential oil components were identified by comparing their retention times and mass fragmentation pattern with those of standards and MS library (Shimadzu's GCMS solution software, version 2.4). The quantity of oil components was compared using peak area measurements.

### 2.3. Antioxidant activity

#### 2.3.1. DPPH assay

In this assay, antioxidant activity of essential oils was evaluated by measuring the bleaching of the purple-colored ethanol solution of DPPH (Burits & Bucar, 2000). The radical scavenging ability was determined according to the method described by Abe, Murata, and Hirota (1998). One milliliters from a 0.5 mM ethanol solution of the DPPH radical was mixed to 2.0 ml of different concentrations of essential oils from leaves and flowers and was added 2 ml of 0.1 M sodium acetate buffer (pH 5.5). The mixtures were well shaken and kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a Shimadzu UV–Vis spectrophotometer mini 1240, Kyoto (Japan). The authentic α-tocopherol and butyl hydroxyl toluene (BHT) were used as a positive control while ethanol was as negative one. Inhibition ($I\%$) of DPPH radical was calculated using the equation:

$$I\% = (\mathrm{Ao} - \mathrm{As}/\mathrm{Ao}) \times 100$$

Where Ao is the absorbance of the control (containing all reagents except the test compound), and As is the absorbance of the test compound. The $IC_{50}$ value represented the concentration of the essential oils that caused 50% inhibition.

#### 2.3.2. β-Carotene bleaching assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius, Venskutonis, Van Beek, & Linseen, 1998). Antioxidant activity was carried out according to the β-carotene bleaching method (Siddhuraju & Becker, 2003) with minor modifications. β-Carotene (2.0) mg was dissolved in 10 ml chloroform. Linoleic acid (20 μl) and Tween-40 (200 mg) were mixed with 1 ml of the chloroform solution. The chloroform was evaporated under vacuum at 45 °C, then 50 ml oxygenated water was added, and the mixture was vigorously shaken. The emulsion obtained was freshly prepared before each experiment. The β-carotene-linoleic acid emulsion (250 μl) was transferred to a 96-well microplate. Ethanolic solutions (30 μl) of the sample extracts, BHT and α-tocopherol at 1000 ppm were added onto plate. An equal amount of ethanol was used as control. Absorbance was taken at 492 nm after incubation for every 15 min until 180 min at 45 °C using a microplate reader (Benchmark plus microplate 170-6930j1, BIO-RAD Company).

### 2.4. Antibacterial activities

All bacterial strains have been kindly provided by Laboratory of Microbiology, University of the Ryukyus, Oki-