

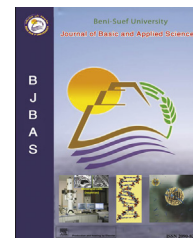
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Full Length Article

Antioxidant and antibacterial activities of various extracts of *Inula cuspidata* C.B. Clarke stem

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ABSTRACT

The objective of the present study includes estimation of total phenolic and total flavonoid contents and evaluation of antioxidant and antibacterial activities of various extracts of *Inula cuspidata* stem. *I. cuspidata* belongs to family Compositae; it is an erect shrub, distributed in western Himalaya, usually found growing on steep rocky or precipitous ground.

Total phenolic and flavonoid contents were estimated by Folin-Ciocalteu and aluminum chloride method. Antioxidant activity was performed by four methods: DPPH (1,1-diphenyl-2-picrylhydrazyl radical), ferrous chelating activity, reducing power and nitric oxide scavenging activity. These extracts were screened for antibacterial studies using macro-dilution method.

Total phenolic and flavonoid contents were found to be highest in methanol extract (69.44 ± 1.12 mg GAE/g, 12.45 ± 0.67 mg QE/g) followed by chloroform (33.53 ± 0.88 mg GAE/g, 1.27 ± 0.51 mg QE/g) and *n*-hexane (12.25 ± 1.03 mg GAE/g, 0.08 ± 0.43 mg QE/g) extracts. Methanol extract of *I. cuspidata* exhibited potent antioxidant activity in all the antioxidant assays followed by chloroform and *n*-hexane extracts. IC_{50} values of methanol, chloroform and *n*-hexane extract were found to be 43.35 ± 0.58 μ g/mL, 298.08 ± 0.62 μ g/mL, 1989.24 ± 0.71 μ g/mL for DPPH, 396.63 ± 0.73 μ g/mL, 915.29 ± 0.81 μ g/mL, 1180.56 ± 0.88 μ g/mL for ferrous chelating and 594.68 ± 0.99 μ g/mL, 930.55 ± 1.03 μ g/mL, 1959.26 ± 1.25 μ g/mL for nitric oxide scavenging assays. A strong correlation was found between total phenolic, flavonoid and $1/IC_{50}$ values obtained by different antioxidant assays. The correlation coefficient (R) value obtained was more than 0.9 which exhibits a strong correlation.

All the extracts showed significant antibacterial activities against Gram positive bacterial strains with minimum inhibitory concentration (MIC) values ranging from 187.5 to 750 μ g/mL and moderate to weak inhibition against Gram negative bacteria with MIC values ranging from 750 to 3000 μ g/mL. The present study proves the *in vitro* anti-oxidant and antibacterial activities of different extracts of *I. cuspidata* stem.

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1. Introduction

Free radicals and oxidants are of paramount importance in disease progression. They are produced either from normal cell metabolisms in situ or from external sources like pollution, cigarette smoke, radiation, and medication. Accumulation of free radicals in the body generates a phenomenon called oxidative stress, which plays a major role in the development of chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases, although the human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, but if the oxidative stress is high, the internal mechanism has to be augmented with the administration of antioxidants (PhamHuy et al., 2008).

Nowadays, natural antioxidants are gaining importance in the market as they are found to be safe, non-toxic and environmental friendly in comparison to synthetic antioxidants, which have been restricted due to their various deleterious effects (Kumaran and Karunakaran, 2007). Flavonoids, phenolic acids and tannins are considered as crucial phyto-constituents for exhibiting antioxidant activities. Redox property of hydroxyl groups present in plant phenolic is responsible for their antioxidant properties that allow them to act as hydrogen donors, reducing agents, metal chelators and free radical quenchers (Shukla et al., 2009).

In addition to free radicals developing resistance in the bacterial species to many antibiotics is another major issue in antimicrobial therapy that continuously encouraging researchers to develop novel antibiotics. Recently, most of the approved novel antimicrobials are derived from natural products or from their derivatives. According to Oshea's and Moser's analysis, out of 148 compounds 66% fall into natural product category (Brown et al., 2014). Thus, natural products are of paramount importance as a source of antibacterial agents. The antibacterial activity is mainly associated with the presence of secondary metabolites like phenolic, terpenes and alkaloids present in the plant extracts.

The genus *Inula*, a variable perennial herb distributed in Asian, African and European continents, comprises more than hundred species of the Compositae (Asteraceae) family belonging to the tribe Inuleae (Seca et al., 2014; Zhao et al., 2006). *Inula* species possess medicinal properties and are used in folk medicines as tonic, stomachic, anti-inflammatory, bactericidal, diuretic, diaphoretic, hepatoprotective, antitumor and carminative (Mathela et al., 2008). Leave extract of *Inula cuspidata* shows anti-inflammatory, antifungal and antibacterial activities (Chauhan and Saxena, 1985; Sati et al., 2011; Thapliyal et al., 2011) and stem, flower and whole plant extracts were reported to have profound anti-inflammatory and hepatoprotective activities (Kaur et al., 2014a, 2014b). Previous chemical investigations done on the *I. cuspidata* shows the presence of monoterpenoids, sesquiterpenoids, flavonoids and glycosides (Bohlmann et al., 1982; Sahai et al., 1981; Verma et al., 2014). Until today, this plant has not been explored for its quantitative property (total phenol, total flavonoid content) and antioxidant activities; thus, the aim of the study was to evaluate the phyto-quantitative property and antioxidant and

antibacterial activities of different stem extracts of *Inula cuspidata*. Results from this work will enlighten the medicinal aspects of this herb.

2. Materials and methods

2.1. Collection and authentication of plant material

Stems of *I. cuspidata* were procured from Nainital, Uttarakhand, India, in the months of August to September 2013 and were authenticated (Voucher number-114758) at Botanical Survey of India, Dehradun. The stems were shade dried, coarsely powdered and stored in an air tight container for further use.

2.2. Chemicals, reagents and instrument

L-Ascorbic acid, quercetin, gallic acid, trichloroacetic acid, DPPH, ferrozine and nutrient broth were purchased from Himedia, Mumbai, India. Sulfanilamide, EDTA and N-1-naphthyl ethylene diamine dihydrochloride were purchased from Sigma Aldrich, Mumbai, India. Folin-Ciocalteu reagent, potassium ferricyanide, ferrous chloride, and ferric chloride were procured from Merck, Mumbai, India. Aluminum chloride and dimethyl sulfoxide was procured from Molychem, Mumbai, India. All the chemicals used in the study were of analytical grade. The rotary evaporator was procured from Heidolph, Schwabach, Germany. UV-visible spectrophotometer (Shimadzu, UV-1800) was purchased from Shimadzu, Japan.

2.3. Physico-chemical evaluation

The stem powder was evaluated for physico-chemical parameters such as total ash value, acid insoluble ash value, water soluble ash value and alcohol soluble extractive value as per WHO guidelines (WHO, 1998).

2.4. Extraction of plant material

2.4.1. Maceration method

The coarsely powdered plant material (20 g) was taken in a glass-stoppered conical flask and extracted thrice with organic solvent (3 × 100 mL) in a sequential manner first with *n*-hexane followed by chloroform and methanol. The extraction was carried out in a mechanical shaker at room temperature for 6 h and then allowed to stand for 18 h. The extraction time for each solvent was about 3 × 24 h respectively. The extracts obtained were filtered and concentrated under vacuum using rotary evaporator (Heidolph, Schwabach, Germany). Yields of extracts were calculated on the basis of percentage w/w (WHO, 1998).

2.5. Preliminary phytochemical screening

The preliminary phytochemical screening was performed for identifying the presence of phyto constituents in *n*-hexane, chloroform and methanol extracts of *I. cuspidata* stem (Harborne, 1998).

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