Research Article

ISSN: 2230-7346

Available online http://WWW.JGTPS.COM

Journal of Global Trends in Pharmaceutical Sciences

Vol.2, Issue 3, pp -290-295, July -Sept 2011

PRELIMINARY PHYTOCHEMICAL, PHYSICOCHEMICAL AND ANTIMICROBIAL STUDIES OF *INULA CUSPIDATA* LEAVES

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ABSTRACT

Inula cuspidata (Asteraceae) is used for the treatment of respiratory, gastrointestinal and urinary disorder. Methanolic extract of leaves was prepared by soxhlet extraction. Preliminary phytochemical screening was performed for qualitative identification of phytoconstituents. Physicochemical parameters such as ash value, extractive value and fluorescence analysis were performed to standardize the plant material. The extract was evaluated for antibacterial activity against *S. aureus, B.subtilis, E. coli, P.aeruginosa* and antifungal activity against *C. albicans* by cup plate method. The extract showed significant antimicrobial activity against all test strains when compared with standard drugs amoxycillin and fluconazole.

Keywords: *Inula cuspidata*, Antibacterial, Antifungal, Antimicrobial, Methanolic extract.

INTRODUCTION:

Inula cuspidata is also known as jhuri. It belongs to the family Asteraceae. *Inula cappa, Inula eupatorioides, Inula racemosa* are the major known species of this genus. *Inula cuspidata* is a small or medium sized deciduous or subdeciduous shrub of India found in the western Himalaya from Kashmir to Uttarakhand [1,2]. The aerial parts of *Inula cuspidata* are used for the treatment of respiratory, gastrointestinal, urinary disorder. The essential oil of leaves possesses good anti fungal activity against plant and human pathogenic fungi [3]. The biological screening of the plant extract showed anticancer activity. Incaspitolides A,B,C,D, hydroxygermacrene, two isomeric acetylenic sulfoxides, isoquercitin, β-sitosterol and its β D glucoside, , geranyl linalool and squalene have been isolated from the aerial parts of the plants[4,5].

As not much work has been done on antibacterial activity, it was considered worthwhile to carry out this activity together with antifungal activity.

MATERIALS AND METHODS:

Collection of plant material:

Clean and fresh leaves of *inula cuspidata* were collected from Sahastradhara, Dehradun and authenticated by Dr. J.K.Tewari, Reader ,Department of Botany,H.N.B.Garhwal ,Uttarakhand.

Preparation of extract:

The fresh leaves were dried at room temprature (25-30^{o}C) for 20 days and powdered. Six hundred gram of coarse powder was extracted with methanol in soxhlet extractor for 72 hrs. The extract was vacuum dried in a rotatory vacuum evaporator (Perfit Model No. 5600 Buchi type).The yield was found to be 9.37%.

Preliminary phytochemical screening:

The qualitative chemical tests give the general idea regarding the nature of chemical constituents of crude drugs. The preliminary phytochemical screening of methanolic extract was carried for the presence of alkaloida, carbohydrates,glycosides,steroids, sterols, tannins,flavonoids using the standard procedure described by Kokate [6] and Harbone[7] .

Physico chemical constants:

Physicochemical parameters such as ash value, extractive value were performed according to the official method prescribed and the WHO guidelines [8] on the quality control methods for medicinal plant material.

Fluorescence analysis:

Fluorescences analysis was carried out according to the the method of Chase[9] and Prattand Kokoski[10].

Antimicrobial activity:

The methanolic extract of leaves was examined for antibacterial activity against Gram positive bacteria *S. aureus, B.subtilis*, Gram negative bacteria *E. coli, P.aeruginosa* and anti fungal activity against *C. albicans*. The antimicrobial screening was performed by cup plate method. A previously liquified and sterlizied Muller Hinton Agar medium(Hi-media) by autoclaving at 120^{o}C for 30 minutes was taken and poured into petri-plates and allowed to solidify. The plates were swabbed with the bacterial strains of *S. aureus, B.subtilis, E. coli, P.aeruginosa* and fungal strain of *C.albicans.* In each plate wells of 6 mm diameter were made at equal distances using sterile cork borer. Different dilutions of the extract were made having concentration of 100µg/ml, 250µg/ml, 500µg/ml, and 1000µg/ml in DMSO (dimethyl sulphoxide). 0.1 ml of each test solution and control were placed in 6 mm diameter wells. One well was filled with 0.1 ml of standard drug Amoxycillin (10 µg/disc) in case of antibacterial activity whereas standard drug Fluconazole (10 µg/disc) in antifungal activity. The petri plates were incubated at 37^{o}C for 24 hrs and 48 hrs for antibacterial and antifungal activity respectively. Diameter of zone of inhibition was measured [11]. The experiment was performed three times to minimize the error and the average values are presented. The diameter obtained for the test samples were compared with diameter produced by the standard Amoxycillin and fluconazole in antibacterial and antifungal activity.

RESULTS AND DISCUSSION:

Methanolic extract of *Inula cuspidata* leaves showed the presence of alkaloids, carbohydrates, glycoside, tannins, steroids and flavonoids (Table-1). Ash value of a drug gives an idea of the earthy matter or inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash, water soluble ash and sulphated ash are presented in Table-2.Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water soluble and alcohol soluble extractive value have been tabulated in Table-3. The result of fluorescence analysis of the drug powder is presented in Table-4.

Antimicrobial activity was carried out for methanolic extract of leaves of *Inula*

cuspidata. The data for antibacterial and antifungal activity is listed in Table-5. From the data it is evident that methanolic extract of leaves of *Inula cuspidata* showed the greater zone of inhibition against gram positive than gram negative bacteria. This shows that gram positive bacteria are more susceptible than gram negative bacteria. The extract also showed significant anti fungal activity against *C. albicans* (Table-5). The results of present study indicates that the plant extract showed both anti bacterial and anti fungal activity against the test organisms which might be due to the phyto constituents present in the leaves.

Table1: Preliminary Phytochemical screening of the Methanolic extract of *Inula cuspidata* leaves

Test	**Methanolic Extract**
Alkaloids	+
Carbohydrates	+
Proteins	-
Amino Acid	-
Glycoside	+
Steroids and Sterols	+
Flavonoids	+
Tannins	+
Triterpenoids	-
Saponin Test	-
Fixed oils	-

Table2: Ash values of the leaves powder of *Inula cuspidata*

Types of Ash	Ash Value in %w/w
Total ash	7.50
Acid insoluble ash	1.06
Water soluble ash	2.12

Table 3: Extractive values of the leaves powder of *Inula cuspidata*

Type of extractive value	Extractive value in % w/w
Alcohol soluble extractive value	8.34
Water soluble extractive value	17.58

Table4: Fluorescence analysis of the leaves powder of *Inula cuspidata*

Treatment	**Day light**	**UV light (254nm)**
Powder + 1N NaoH (Alc.)	Yellow	Yellow
Powder + Ammonia	Green	Greenish yellow
Powder + 1N HCl	Green	Light brown
Powder +1N HNO_3	Green	Reddish brown
Only powder	Green	Green

Table5: Antimicrobial activity of methanolic extract of *Inula cuspidata* leaves

Concentration	Zone of inhibition (mm) of Inula cuspidate				
	Antibacterial Activity				Antifungal Activity
	Gram positive		Gram negative		
	S.aureus	*B.subtilis*	*E. coli*	*P.aeruginosa*	*C.albicans*
100µg/ml	20	22	12	10	12
250µg/ml	24	25	13	11	14
500µg/ml	27	28	15	13	14
1000µg/ml	30	32	17	16	17
Control	…	…	…	…	…
Amoxyllin10µg/disc	13	14	11	12	…
Fluconazole10µg/disc	…	…	…	…	22

ACKNOWLEDGEMENT:

Authors are thankful to the Department of Botany and Biotechnology of SBSPG Institute of Biomedical Sciences and also thankfull to Quality Control (Q.C) department at I.M.P.C.L.Mohan Almora ,Uttarakhand..

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