Studies on chemical constituents and biological activity of *Pulicaria incisa* subsp. *Incisa* (Asteraceae)

Emad A. Ewais[1]*, Mohamed M. Abd El-Maboud[2] and Muhammad I. Haggag[1]

[1.] Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr city, Cairo, Egypt
[2.] Ecology and Range Management Department, Desert Research Center, Cairo, Egypt
*Ewais-E@yahoo.com

Abstract: Phytochemical screening and chemical constituents of aerial parts (flower, leaf and stem) of *Pulicaria incise* subsp. *Incisa* were investigated. Soluble carbohydrates, total nitrogen, total protein, total phenolics and total flavonoids were increased in flower. Water content, organic matter, total and insoluble carbohydrates, total lipids, total tannins, total saponins and alkaloids were increased in leaf, while total ash, acid soluble and insoluble ash, water soluble and insoluble ash and crude fibre were increased in stem. Preliminary phytochemical screening in aerial parts of *Pulicaria* revealed the presence of alkaloids, saponines, flavonoids, tannins and cardiac glycosides. Sterols were absent in stem. Antimicrobial studies by used 7 bacterial strains and 5 fungal strains showed that the plant extract greatly inhibited the growth of most of tested microbial strain. Moreover, anti tumor studies showed that the total extract of the plant produced high effect on the specific carcinoma cell.

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Key words: Chemical composition; Phytochemical; *Pulicaria incisa* subsp. *Incisa*; antitumor; antimicrobial

1. Introduction

The Egyptian deserts are rich in medicinal plants belonging to many families (Boulos, 1995). *Pulicaria incisa* belongs to family Asteraceae is a woolly tomentose annual or short lived perennial with erect stems that are branched mainly from the base. Cauline leaves are oblong, strongly undulate and dentate or incised, clasping and auriculate at the base, while radical leaves are narrowed at the base (Boulos, 2009). Asteraceae (Compositae or Sunflower family) is the largest family of flowering plants in the world. The family includes over 1,600 genera and 23,000 individual species. Many members of the Asteraceae family are important for medicinal, ornamental, and economic purposes (Mueller et al., 2000). *Pulicaria incisa* is used by the natives of some upper Egyptian areas in the form of a decoction, sweetened with sugar as substitute for tea (Amer *et al*., 2007). The plant also used as a traditional medicine for treating heart diseases by Bedouins (Mansour *et al*., 1990; Nabiel, 2003).

So it is of interest to choose *Pulicaria incisa* subsp. *Incisa* herbal plant belongs to this family. The aim of the study was evaluated some metabolomics parameter also biologically evaluation activity of the methanol extracts (70%) of *Pulicaria incisa* subsp. *Incisa*.

2. Materials and Methods

2.1. Plant material

The aerial parts of *Pulicaria incisa* subsp. *Incisa* were collected in April, 2013 from Saint- Katherine area, south Sinai in Egypt. The taxonomic idendification of plant materials was confirmed by Saint Katherine protectorate team.

2.2. Methods

1. Eco-physiological study including determination of the percentage of plant water content of *Pulicaria incisa* subsp. *Incisa* (Rowell, 1994). Determination of certain pharmacopoeial constants of plant material, including inorganic (ash) and organic matter (Brower and Zar, 1984), acid-soluble and acid-insoluble ash, water-soluble and water-insoluble ash (Askar and Treptow, 1993) and crude fibers (British pharmacopoeia, 1980).
2. Investigation of metabolic products including determination of total carbohydrates, soluble and insoluble carbohydrates (Chaplin & Kennedy, 1994), Free and combined sugars were investigated using HPLC for determination free and combind sugers.
3. Total nitrogen and protein content determined by using Kjeldahl method (James, 1995).Free amino acids and protein-amino acids were accomplished according to Pellet and Young (1980) using Amino Acid Analyzer (Beakman system 7300 High Performance analyzer).
4. Total lipids content according to British Pharmacopoeia (1993). Phytochemical study including preliminary phytochemical screening, including steam distillation of volatile oils (Balbaa *et al.,* 1981), Test for Alkaloids (Woo *et al.,* 1977), Test for Glycosides (Treare & Evan, 1985), Test for Cardiac Glycosides (Treare & Evan, 1985), Test for Saponins (Kokate *et al.,* 2001) & (Kokate, 1994), Test for phenols (Ahmad *et al.,* 2005), Test for Phytosterols (Brieskorn *et al.,* 1961) & (Fieser and Fieser, 1959), Test for Tannins (Treare & Evan,

1985), Test for Flavonoids (Geissmann, 1962) & (Khandeal, 2008).

5. Total phenolics were determined with the Folin Ciocalteu as described by (Maurya and Singh, 2010), Total flavonoids by (Samatha *et al.,* 2012), total tannins by (Ali *et al.,* 1991), total saponins according to (Obadoni & Ochuko, 2001) and total alkaloids as described by (Woo *et al.,* 1977).
6. Antimicrobial activity was carried out using the paper disc technique (Duguid *et al.,* 1978), anti-tumor Activity according to Skehen & Storage (1990).

3. Results and Discussion

Metabolomics parameter of *Pulicaria incisa* subsp. *Incisa* flower, leaf and stem contents are summarized in Table (1). Results indicated that, Leaf of *Pulicaria incisa* contained the highest water content and total carbohydrates return to photosynthetic process which performed inside leaves (plastids) needs water and CO2 uptake. Fiber is the amount of cellulose and lignin present in the plant. Stem is the most woodier in plant aerial parts, so it contain the highest crude fiber and ash content to support and transfer water to leaves. The stem of most plant species have greater fiber levels compared to the leaves (Buxon and Redfearn, 1997). Flower and leaf of *P. incisa* contain higher soluble carbohydrates as compared to stem. This result agreed with that observed in *Nerium indicum* by (Vijayvergia and Kumar, 2007). The study also showed that the plant contains a large quantity of total flavonoids and total phenolics especially flower which support its use as herbal tea. Flavonoids have been shown to exhibit their actions on membrane permeability and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase (Li *et al*., 2003). Phenols have antibacterial and anti-inflammatory activities (Vijayvergia and Kumar, 2007), also responsible for antioxidant and free radical scavenging effect of plant materials (Hasanuzzamn *et al*., 2013 & Seladji *et al*., 2014). The studied plant have intermediate amount of total tannins, alkaloids and saponins which concentrate more in leaf aerial part. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993).

Table 1. Metabolomics parameter of *Pulicaria incisa* subsp. *Incisa* aerial parts

Item (%)	*Puliaria incisa* subsp. *Incisa*		
	Flower	Leaf	Stem
Water content	69.48±0.60	76.39±0.43	74.12±0.48
Total ash	16.6±0.25	14.5±0.19	18.3±0.26
Organic matter	83.4±0.25	84.7±0.73	81.7±0.26
Acid soluble ash	11.28±0.17	10.5±0.07	11.5±0.16
Acid insoluble ash	5.3±0.08	4.21±0.05	6.7±0.09
Water soluble ash	11.9±0.18	10.9±0.14	12.07±0.17
Water insoluble ash	4.6±0.07	3.6±0.04	6.2±0.08
Crude fibers	20.5±0.2	14.4±0.17	23.4±0.2
Total carbohydrate	30.5±0.16	33.4±0.19	26.3±0.13
Soluble carbohydrate	13.7±0.07	13.4±0.07	11.3±0.05
Insoluble carbohydrates	16.8±0.08	20±0.08	15±0.07
Total nitrogen	2.91±0.04	2.4±0.06	1.7±0.02
Total protein	18.2±0.29	15±0.38	10.6±0.16
Total lipids	1.2±0.06	1.5±0.03	0.8±0.09

Total nitrogen, total protein reached maximum values in flower and minimum values in stem for plant under investigation. Total lipid reached maximum values in leaf and minimum values in stem and this maybe the ability of leaf to storage water to completed metabolic process. The percentage of inorganic matter (ash), acid soluble ash, acid insoluble ash, water soluble ash, water insoluble ash and crude fibers reached maximum values in stem and minimum values in leaf for plant under investigation. This may be due to the increase of total ion accumulation as a result of increasing soil moisture stress and soil salinity, which agreed with the results obtained by Larcher (1995) & Alli Smith (2009).

In the present study (Table 2) the percentage of Free and Combined Sugars showed that arabinose, sucrose and raffinose were undetected in flower, leaf and stem and the concentration of glucose was the highest one of the separated free sugars in stem. also the concentration of galactose was the highest one of the separated combined sugars in leaf . on the another hand the concentration of fructose was the lower values of the separated free sugars in flower, leaf and stem, also the concentration of ribose, rhamnose, were the lower values one of the separated combined sugars in flower, stem and leaf respectively.

Free amino acids of *Pulicaria incisa* subsp. *Incisa*

The separation of free amino acids of the different parts (flower, leaf, and stem) of the *Pulicaria incisa* was achieved using amino acid analyzer, and each component. The obtained results were calculated and tabulated in Table (3) and Fig. 1, where fifteen free amino acids were presented in flower, leaf and stem. It was obvious that glutamic and Isoleucine was the highest amino acid of the separated free amino acids at flower and stem respectively; proline was the highest amino acid of the separated free amino acids at leaf. On another hand Isoleucine was the lowers amino acid concentration of the separated free amino acids at flower and leaf. Aspartic was the lowers amino acid concentration of the separated free amino acids at stem.

Table 2. Free and combined sugars of *Pulicaria incisa* subsp. *Incisa* aerial parts

Sugar name	RT	*Pulicaria incisa* subsp. *Incisa*					
		Flower		Leaf		Stem	
		F	C	F	C	F	C
Rhamnose	2.35	11.2	9.3	12.6	8.7	-ve	-ve
Arabinose	2.65	-ve	-ve	-ve	-ve	-ve	-ve
Unknown	2.70	2.3	1.4	1.6	1.5	1.1	1.6
Ribose	2.75	1.7	0.5	9.6	14.2	-ve	0.7
Fructose	3.4	1.3	4.3	0.7	-ve	2.3	1.7
Glucose	3.65	12.3	13.4	11.2	14.6	19.4	13.1
Galactose	3.7	13.3	11.5	17.2	20.1	8.9	15.3
Unknown	4.4	12.1	11.7	-ve	-ve	-ve	0.8
Unknown	5.3	15.4	13.2	20.2	18.4	-ve	-ve
Sucrose	6.9	-ve	-ve	-ve	-ve	-ve	-ve
Raffinose	10.3	-ve	-ve	-ve	-ve	-ve	-ve

Table 3. free amino acids of *Pulicaria incisa* subsp. *Incisa* aerial parts

Name of amino acids	*Pulicaria incisa* subsp. *Incisa*		
	Flower	Leaf	Stem
Aspartic	1.486	0.678	0.076
Theronine	0.941	0.682	0.063
Serine	0.710	0.451	0.125
Glutamic	1.873	0.532	0.740
Proline	1.230	0.847	0.517
Glycine	0.698	0.446	0.344
Alanine	0.876	0.355	0.486
Valine	0.816	0.431	0.120
Isoleucine	0.114	0.089	1.0051
Leucine	0.602	0.141	0.452
Tyrosine	0.740	0.414	0.545
Phenyl alanine	0.571	0.223	0.345
Histidine	0.914	0.276	0.240
Lysine	0.342	0.124	0.111
Arginine	0.596	0.134	0.146

Table 4. Preliminary phytochemical screening in aerial parts (flower, leaf and stem) of *Pulicaria incisa* subsp. *Incisa*

Groups	Tests	Methanol Extracts		
		Flower	Leaf	Stem
Alkaloids	Wagner's reagent	-ve	+ve	+ve
	Dragendorrf's reagent	-ve	+ve	+ve
Glycosides	Glycosides test	+ve	+ve	+ve
	Modified borntrager's	+ve	+ve	+ve
Cardiac glycosides	Legal's test	+ve	+ve	+ve
Saponins	Foam test	+ve	+ve	+ve
	Haemolysis tests	+ve	+ve	+ve
Phenols	Ferric chloride test	+ve	+ve	+ve
Phytosterols	Liberman burchard's test	+ve	+ve	-ve
	Salkwaski reaction	+ve	+ve	-ve
Tannins	Gelatin test	+ve	+ve	+ve
	Lead acetate test	+ve	+ve	+ve
Flavonoids	Schinodar's test	+ve	+ve	+ve
	NaOH test	+ve	+ve	+ve
Amino Acids	Xanthoproteic test	+ve	+ve	+ve
	Ninhydrin test	+ve	+ve	+ve
Volatile oil's	Steam distillation	+ve	+ve	+ve

The preliminary phytochemical screening of *Pulicaria incisa* subsp. *Incisa* plant of different parts (flower, leaf and stem) to investigated alkaloids, glycosides, cardiac glycosides, saponins, phenol, sterol, tannins, flavonoids, amino acid and volatile oil present in plant under investigation. Table (4) showed that sterols in stem were absent.

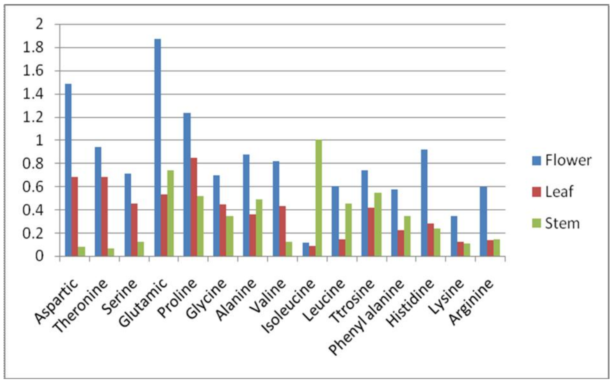

Figure 1. Free amino acids (mg/ml) in aerial parts of *Pulicaria incisa* subsp. *Incisa* during April 2013

Table (5) shows The percentages of total flavonoids and total phenolic acid has increased value in flower and decreased value in stem , The percentages of total tannins, total sponins and total alkaloid has increased value in leaf and decreased value in stem.

Tables (6&7) shows antimicrobial activity of *Pulicaria incisa* subsp. *Incisa*, smallest amount of antimicrobial compounds necessary to inhibit growth of the tested organisms. MIC of total aerial part of *Pulicaria incisa* subsp. *Incisa* extract of the tested medicinal plant was studied against bacterial strains (*S .aureus, Bacillus subtilis, Enterococcus faecalis, K. pneumonia, Escherichia coli* and *Pseudomonas aeruginosa*) and fungail strains (*F.oxysporum, R.solani, A. niger, A.flavus and C.albican*). The results in bacterial strains showed that *Enterococcus faecalis* was the most sensitive test organism with MIC concentration 3.125 mg/ml with inhibition zone 6 mm on another hand the results in fungal strains showed that *F. oxysproum* was the most sensitive test organism with MIC concentration 12.5mg/ml with inhibition zone 8.7 mm. From the above results of antimicrobial effect showed that *Enterococcus faecalis, F.oxysproum* the most sensitive test organism with (6 and 8.7) mm inhibition zone respectively. In addition, gram negative bacteria more sensitive than gram positive bacteria. Figure (2) showed that "inhibitory activity against hepatocellular carcinoma cells was detect under these experiment condition with IC 50 = 2.5 μg" in *Pulicaria incisa* subsp. *Incisa* total aerial parts and according to that we can used *Pulicaria incisa* subsp. *Incisa* total aerial part as antitumor agent.

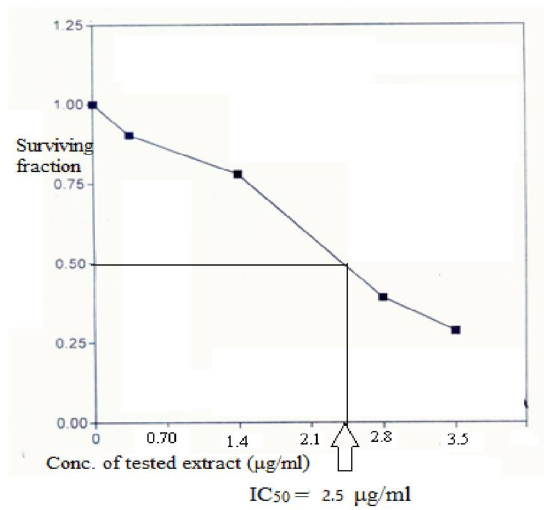

Figure 2. Anti-tumor activity of *Pulicaria incisa subsp incisa* extract against hepatic cell line (HEPG2) & IC_{50} =2. 5μg

Table 5. Total active materials in aerial parts of *Pulicaria incisa* subsp. *Incisa*

Plant parts	Item	Total active materials in different parts		
		Flower	Leaf	Stem
Pulicaria incisa subsp. *Incisa*	Total flavonoids (mg/gm rutin)	208.8±0.72	192.5±0.86	172.8±0.88
	Total phenolic acids (mg/gm gallic acid)	239.5±0.57	153.1±0.44	125.1±0.6
	Percentage of Total Tannins (%)	2.7±0.02	3.75±0.08	2.2±0.03
	Percentage of total Saponins (%)	0.9±0.01	1.3±0.2	0.72±0.03
	Percentage of total Alkaloids (%)	2.5±0.02	3.2±0.05	1.4±0.07

Table 6. Antibacterial activity with *Pulicaria incisa* subsp. *Incisa* during April 2013

Plants	Conc. Per mg/ml	*S. aureus*	*B. subtilis*	*Entero. faecalis*	*K. pneumonia*	*E. coli*	*P. aeruginosa*
Pulicaria incisa subsp. *Incisa*	50	25	28	35	34	37	31
	25	13	18	22	19	30	21
	12.5	08	11	15	12.6	18	16
	6.25	00	05	11	8.1	07	09
	3.125	00	00	06	00	00	00
	MIC	12.5	6.25	3.125	6.25	6.25	6.25

Table 7. Anti-fungal activity with *Pulicaria incisa* subsp. *Incisa* during April 2013

Plants	Conc. Per mg/ml	*F. oxysporum*	*R. solani*	*A. niger*	*A. flavus*	*C. albican*
Pulicaria incisa subsp. *Incisa*	100	32	14	14	8	00
	50	21	09	07	00	00
	25	17	00	00	00	00
	12.5	8.7	00	00	00	00
	6.26	00	00	00	00	00
	MIC	12.5	50	50	100	> 100

Correspondence to:
Prof. Dr. Emad Eldein Abass Ewais
Head of Botany and Microbiology Department
Faculty of Science
Al-Azhar University, Nasr city, Cairo, Egypt
Emails: Ewais-E@yahoo.com

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