

Anti-inflammatory and Antimicrobial Activity of the Different *Conyza dioscoridis* L. Desf. Organs

Soheir M Zalabani¹, Mona H Hetta¹ and Ahmed S Ismail^{2*}

¹Department of Pharmacognosy, Cairo University, Cairo, Egypt

²Department of Pharmacognosy, Beni-Suef University, Beni-Suef, Egypt

Abstract

Objectives: This study was planned in order to assess the anti-inflammatory and antimicrobial potential of the different organs (leaf, flower and root) of *Conyza dioscoridis* (L.) Desf.

Methods: A preliminary phytochemical and chromatographic screening of the ethanol (70%) extracts was performed prior investigation. Successive extraction of the plant organs was carried out with petroleum ether 60-80°C, chloroform, ethyl acetate and ethanol 90%, and percentage yield of extractives was determined. The anti-inflammatory activity of the total ethanol (70%) extracts was evaluated *in-vivo* by the carrageenan-induced rat paw oedema method, as compared to Indomethacin. The antimicrobial activity of the total ethanol and successive extracts was assessed by the agar dilution method on a set of bacteria and fungi; MICs were determined.

Results: The acute toxicity study of the total ethanol (70%) extracts indicated their safety (LD₅₀ up to 5 g/kg body wt.). The ethanol (70%) extract of the leaf showed the highest percentage of oedema inhibition (76.20%). The *in-vitro* antimicrobial assay revealed that most of the fractions exhibited a significant activity against *Mycobacterium phlei*, as compared to the other tested strains (MICs ranging from 50 to 200 µg/ml); the lowest recorded MICs (50 µg/ml) being those of the successive ethanol extract of the roots and the ethyl acetate extract of the leaves. In addition, the successive ethanol extract of the roots exerted a noticeable effect against *Bacillus subtilis* (MIC, 100 µg/ml).

Conclusion: The data represented in this study demonstrate that the use of *C. dioscoridis* may lower the risk of microbial infections and exert an anti-inflammatory activity, probably due to the presence of phenolics. The use of extracts is recommended to achieve health benefits, rather than pure isolates due to the synergistic and additive effects of their components.

Keywords: *Conyza dioscoridis*; LD₅₀; Anti-inflammatory; Antimicrobial

Introduction

Conyza dioscoridis (L.) Desf. is a popular perennial shrub reputed for its folk medicinal uses. The antimicrobial effect of the ethanol extract of *C. dioscoridis* has been previously assessed against selected Gram-negative and Gram-positive bacteria, and unicellular and filamentous fungi [1]. The antinociceptive effect of the methanol extract of the aerial parts of the plant was evaluated on mice [2]; in addition to its antidiarrheal effect on rabbits [3]. Another study revealed that oral administration of the methanol extract of the leaves (200 mg/kg) reduced the number of fecal discharge produced by castor oil in rabbits exerting a significant antidiarrheal effect [4]; this extract induced a dose-dependent relaxation of rabbit duodenal muscle, the inhibition was attributed to either a calcium-channel, or a possible ganglionic blocking effect. The insecticidal and anti-inflammatory activities of the aerial parts of the plant have been also recorded [5,6].

The present paper aimed to assess the anti-inflammatory and antimicrobial activities of three separate organs of *Conyza dioscoridis* (leaf, flower and root), in order to select the most suitable candidate for further pharmaceutical industrialization.

Materials and Methods

Plant material

Conyza dioscoridis was collected from El-Fayoum, Egypt, 2009. The plant was kindly identified and authenticated by Prof. Dr. Mounir M. Abdel-Ghani, Botany Department, Faculty of Science, Cairo University, Egypt. A voucher specimen is deposited at the Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University.

Preparation of the extractives

The different plant organs, leaf, flower and root (1 Kg, each) of *C. dioscoridis* were air-dried in shade and finely powdered.

A portion (0.5 kg) of each sample was extracted with 70% ethanol till exhaustion, and evaporated under reduced pressure to dryness. The remaining powdered organs were successively extracted with solvents in increasing polarity: petroleum ether (60-80), chloroform, ethyl acetate and ethanol (90%).

Phytochemical and TLC screening of the extractives

The different extractives were subjected to preliminary phytochemical screening [7-9], TLC screening [10], was carried out for detection of individual components.

Determination of LD₅₀: LD₅₀ of the total ethanol extracts of the leaf, flower and root of *Conyza dioscoridis* were estimated according to Spearman and Karber procedure [11]. Thirty male albino mice (25-30 g body weight) were divided into five groups each of six animals.

*Corresponding author: Ahmed S Ismail, Department of Pharmacognosy, Beni-Suef University, Beni-Suef, Egypt, Tel: +201005688322; Fax: +082-2317958; E-mail: ahmed_ph_fut@yahoo.com

Received May 28, 2013; Accepted June 18, 2013; Published June 24, 2013

Citation: Zalabani SM, Hetta MH, Ismail AS (2013) Anti-inflammatory and Antimicrobial Activity of the Different *Conyza dioscoridis* L. Desf. Organs. Biosafety 2:106. doi:10.4172/2167-0331.1000106

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Preliminary experiments were carried out to determine the minimal dose that kills all animals (LD_{100}), and the maximal dose that failed to kill any animal. Several doses at equal logarithmic intervals were selected in between these two doses. Each dose was injected in a group of six animals by subcutaneous injection. All groups of animals were observed for 24 hours, and symptoms of toxicity and mortality rates in each group were recorded, and the LD_{50} calculated.

In vivo anti-inflammatory activity: The anti-inflammatory activity of the total ethanol 70% extracts of the different organs was determined, *in-vivo*, by adopting the carrageenan-induced oedema in the hind paws of rats [12]. Thirty male albino rats, weighing 130-150 g, were divided into 5 groups (each of 6), and orally treated one hour before induction of oedema. Group 1 receiving saline and served as negative control. Groups 2, 3 and 4 were administered the total ethanol extracts of the three organs leaf, flower and root, respectively, at a dose of 50 mg/kg b.wt., each. Group 5 received Indomethacin [13], as standard anti-inflammatory drug (30 mg/kg b.wt). Induction of oedema was performed by sub-planter injection of 0.1 ml of 1% Carrageenan [14], in saline into the pad of experimental animal right paw and 0.1 ml saline in its left hind paw. Four hours after drugs administration, the rats were sacrificed. Both hind paws were excised and weighed separately; the difference in weight between both represents the weight of the oedema.

Antimicrobial activity: The antimicrobial potential of the total ethanol and successive extractives of the three organs of *C. dioscoridis* (leaf, flower and root) was evaluated against selected bacterial and fungal strains by applying the agar dilution method, adopting the method of Clinical Laboratory Standards Institute (CLSI) [15]. Nine organisms were used: *Bacillus subtilis*, *Escherichia coli*, *Mycobacterium phlei*, *Listeria innocua* "LMG 2710", *Enterococcus faecalis*, *Staphylococcus aureus* "Non-pathogenic LMG 3242", *Staphylococcus aureus* "Pathogenic LMG 3240", *Staphylococcus aureus* "Lab. Strain" and *Candida albicans*. The tested organisms were grown on Sabouraud dextrose agar (SAB Agar) and *Candida* identification agar (FLUKA) at 35°C for 24 hrs. Three colonies were suspended in 5 ml saline, then standardized at 530 nm, and the suspension was adjusted to 0.5 McFarland standard and then diluted 10-fold with saline to give organism suspension of (1×10^6 to 5×10^6 CFU/ml). This suspension was then further diluted by putting 1 ml suspension+9 ml saline to give a final suspension volume of 1×10^5 to 5×10^5 CFU/ml. A multiple inoculator was used to inoculate the prepared agar-antifungal plates. A 100 μ l (i.e. 10^4 CFU) of the prepared inoculums were put in the well of multi-inoculator, where each inoculation time by multi-inoculator gave about 10 μ l of prepared inoculums to the plate (i.e. 10^3 CFU).

DMSO was used as negative control plate. Each experiment was performed in duplicates. All plates were incubated at 35°C for 48 hrs. Results were recorded in terms of MIC, which is the lowest concentration of antimicrobial/antifungal agent causing almost complete inhibition of growth or giving no visible growth.

Statistical analysis: Analysis of the data was performed by one way Analysis of Variance (ANOVA), and subsequent analysis was performed using Tukey test. The *p*-values < 0.05 were selected to indicate statistical significance between the groups. Statistical analysis of results was done using analytical software named SPSS statistics 17.0, release (Aug. 23, 2008), Chicago, USA.

Ethics: All animal procedures were performed upon approval from the Ethics Committee of Beni-Suef University, and in accordance with the recommendations of the proper care and use of laboratory animals.

Results

Yield, phytochemical and screening of the extractives

The percentage yields of solvent-free total ethanol (70%) extracts and successive extractives were recorded in Table 1.

Preliminary phytochemical screening of the fractions of the three organs revealed the presence of phenolic compounds, sterols, and/or triterpenes, carbohydrates, and/or glycosides in the three organs. TLC screening showed that terpenes and sterols are detected in petroleum ether and chloroform extracts of all the samples; and flavonoids in the ethyl acetate and ethanol extracts, being much more prominent in the ethanol extract of the root.

In vivo anti-inflammatory activity

The three alcoholic extracts exhibited a significant inhibition of inflammation in the following order (76.20%, 72.61% and 66.67% for the leaf, flower and roots, respectively), Table 2.

Antimicrobial activity

Results of the antimicrobial activity are presented in Table 3. The ethyl acetate extract of the leaf and ethanol extract of the root exhibited the highest growth inhibitory activity (MIC, 50 μ g/ml) against *Mycobacterium phlei*. Concerning *Bacillus subtilis*, its susceptibility was chiefly to the ethanol extract of the root (MIC, 100 μ g/ml). The chloroform extractives of the flower showed, on the other hand, a significant activity against *Listeria innocua* (MIC, 200 μ g/ml). The chloroform extract of both the flowers and roots moderately inhibited the growth of *Staphylococcus aureus* "Non-pathogenic LMG 3242" (MIC, 200 μ g/ml), while *Candida albicans* was mainly susceptible to the ethyl acetate and ethanol extracts of the root (MIC, 200 μ g/ml).

Discussion

The present study was conducted to investigate the bioactivities of a plant used in folk medicine, in order to evaluate the scientific

Extractives	Percentage yield (g/100 g)		
	Leaf	Flower	Root
Total ethanol (70%)	4.97	3.66	3.11
Petroleum ether	1.33	2.99	1.17
Chloroform	10.6	0.71	0.24
Ethyl acetate	0.6	0.97	0.74
Ethanol	3.65	2.56	1.09

Table 1: Percentage yield of successive extractives of the different organs of *Conyza dioscoridis* (L.) Desf.

Groups	Dose mg/kg b.wt.	Weight of rat hind paw \pm S.E.			% of inhibition of inflammation
		Right	Left	Difference	
Gp 1: -ve control	1ml saline	1.44 \pm 0.22	0.60 \pm 0.15	0.84	--
Gp2: Leaf	50	1.14 \pm 0.07	0.94 \pm .14	0.20*	76.20%*
Gp3: Flower	50	1.18 \pm 0.19	0.95 \pm 0.12	0.23*	72.61%
Gp4: Root	50	1.11 \pm 0.16	0.83 \pm 0.20	0.28*	66.67%
Gp5: Indomethacin	30	1.16 \pm 0.09	0.98 \pm 0.07	0.18*	78.57%

\pm S.E.: Mean standard error.

*: Statistically significant compared to the normal control group at *P*<0.05.

Table 2: Acute anti-inflammatory activity of total alcoholic extracts of leaves, flowers and roots of *C. dioscoridis* (L.) Desf. in male albino rats (n=6) using carrageenan-induced rat hind paw oedema.

Microorganism	MIC (µg/ml)											
	Petroleum Ether			Chloroform			Ethyl acetate			Ethanol		
	L	F	R	L	F	R	L	F	R	L	F	R
<i>Bacillus subtilis</i>	400	400	400	400	200	200<	200	400	200	800	800<	100
<i>Escherichia coli</i>	800<	800<	800<	800<	800	800	800<	800<	800	800<	800<	800
<i>Mycobacterium phlei</i>	400	100	200	400	200	100<	50 ≤	100	100	400	800<	50≤
<i>Listeria innocua</i> "LMG 2710"	800<	800	800	800	200	400	800<	800<	400	800<	800	400
<i>Enterococcus faecalis</i>	800<	800<	800	800<	800	>800	800<	800<	800	800<	800<	800
<i>Staphylococcus aureus</i> "Non-pathogenic LMG 3242"	800<	800<	800<	800<	200	200<	800<	800<	800	800<	800<	800
<i>Staphylococcus aureus</i> "Pathogenic LMG 3240"	800<	800<	800<	800<	800<	800	800<	800<	800	800<	800<	800
<i>Staphylococcus aureus</i> "Lab. Strain"	800<	800<	800<	800<	800	800	800<	800<	800	800<	800<	800
<i>Candida albicans</i>	800	400	800	800	400	400	800<	800<	200	800<	800<	200

Table 3: Antimicrobial activity of the successive extractives of the Leaf (L), flower (F) and root (R) of *Conyza dioscoridis* (expressed as MIC µg/ml).

basis of its activity in rheumatic pain and diarrhea. It was reported the antimicrobial, antioxidant and anti-inflammatory activities are related to the presence of phenolic compounds [16]. Inhibition of leukocyte chemotaxis may be involved in the anti-inflammatory action of phenolic compounds, and that one of the anti-inflammatory actions of phenolic compounds is the prevention of the production of oxygen free-radicals by leukocytes [17,18]. Also, related the antimicrobial activity with the presence of phenolic compounds. Among literature, the chloroform extract is effective as antibacterial [19], which is in agree with our finding as most of the chloroform fraction of the three organs are active as antibacterial with MIC ranging from 50-200 µg/ml. Investigation of the activity of plant against non-tuberculous mycobacteria; *Mycobacterium phlei* were reported previously [20], using ciprofloxacin and doxycycline as standard,s and by comparing these results with of *Conyza dioscoridis* fractions; *Conyza* has more activity than other extract and less than the standards, It is, however, important that further studies on isolated pure compounds of *Conyza dioscoridis* should be carried out.

Conclusion

The data represented in this study demonstrate that the use of *C. dioscoridis* may lower the risk of microbial infections and exert an anti-inflammatory activity, probably due to the presence of phenolics. The use of extracts is recommended to achieve health benefits rather than pure isolates due to the synergistic and additive effects of their components.

Acknowledgments

The authors appreciate the help of Dr. Amira Abo Youssef, Pharmacology Department, Beni-Suef University, Egypt, for carrying out the anti-inflammatory assay, and Dr Ahmed O. El Guindy, lecturer of microbiology, Faculty of Pharmacy, Beni-Suef University, in testing the antimicrobial activity of the extractives.

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