

Full Length Research Paper

Anatomical, phytochemical and pharmacological studies of roots of *Cnicus benedictus* L.

Chabane Djamila¹, Assani Akym², Mouhoub Faiza¹, Bourakba Chahinez¹, Nazeli Nacer-bey³.

¹Research Laboratory on Arid Zones (L.R.Z.A), Faculty of Biology, University of Sciences and Technology (USTHB), Bab Ezzouar, Algiers 16111, Algeria.

²Cargill Guelph Department of Quality Control 165 Dunlop Dr, Guelph, ON N1L 1P4.

³Research and Development Center CRD Saïdal. Algiers, Algeria.

Accepted 25 April, 2013

Cnicus benedictus L., named Blessed thistle, is a medicinal endemic plant in Algeria, well known by locals as the "march grass" for its effectiveness against burns and wounds. This study aims to provide information on the anatomical properties of the roots, their capacity of chemical compounds in relationship with reduction of burned area. Anatomical studies include cross sections of the roots. Phytochemical screening methods were applied to identify the major chemical compounds in this species. In addition, pharmacological tests were performed on the rats by measuring the diameter of wound healing skin after application of the root extract in comparison with commercial healing ointment "Baneocin".

Key words: *Cnicus benedictus* L., anatomical study, phytochemical screening, pharmacological analysis, wound healing.

INTRODUCTION

The east regions of the country possess rich floristic wealth and diversified genetic resources of medicinal plants. Ethnobotanical research carried out underlined the characterizing traditional knowledge with the local community of *Cnicus benedictus* L. (Chabane et al., 2008). As known the ethnobotanical information and the traditional uses of plants, helps ecologists, pharmacologists, and botanists in their efforts (Abebe and Hagos 1991, Abebe and Aheyu 1993, Ibrar et al., 2007). The knowledge of the histological structure of the various organs of the plant containing the active principle is also very important. Anatomical characters constitute a help in the identification in morphological features of many plants (David et al., 2008). Botanical knowledge and phytochemical analyses of possible active compounds must to be done simultaneously (Jantan, 2004), in order to underline the holistic nature of traditional medicine and works towards its integration into a modern health care system. The present study focuses to find a relationship between anatomic structures containing natural substances in different tissues of plant roots, besides phytochemical screening methods were applied for identifying the major chemical compounds groups of these species.

In addition, pharmacological study was performed to reveal the efficiency of natural root extracts on the skin.

MATERIALS AND METHODS

The plants were collected, and the species was identified with the help of Flora of Bonnier (1999).

Harvesting plant material.

All parts of plants were collected; roots were separated and were dried at 25°C in the dark to avoid the photo-oxidation of phenolic compounds, and in a well ventilated and stored for the preparation of root's powder by using an electric mill and stored in sealed boxes.

Anatomical study

The fresh plants were gently collected from natural areas; roots were separated and fixed in alcohol 70% before cutting with razor blade. For the anatomical description the material was sectioned free-hand, in transverse directions. Sections were emptied in bleach (sodium hypochlorite 12 °C) for 1H and then rinsed with distilled water and then treated with 1% acetic acid

*Corresponding author. E-mail: chabanedj@yahoo.fr.

for 1mn. The sections were stained by double staining with methyl green and Congo red for 20mn each one in agreement with usual techniques in plant anatomy (Langeron, 1949).

Chemical screening

To know the composition of plant secondary metabolism compounds, the plant samples were dried in total darkness, sifted powdered and dissolved in various solvents ranging from methanol, chloroform, diethyl ether etc. After filtration, the residue was obtained and analysed by colorimetric reactions or precipitation (Bruneton, 1999).

Pharmacological study

This pharmacological activity consisted to test in the laboratory the extracts of roots, which aim the identification of a possible healing effect. Plant powder is obtained by grinding the dried roots of Blessed thistle. In a glass mortar, add 10g of the plant powder 50g vegetable jelly "vaseline", until a homogeneous mixture. This mixture produces a thick cream. Upload the petrolatum which has a carrier (fats) facilitates the dissolution of the powder and its distribution on the skin during application. This cream was applied on the skin of rats. In parallel, the Baneocin marketed ointment (compound of the association between the two pharmacological agents (neomycin - bacitracin) was tested as reference product. Before the beginning of the wound-healing experiments, the dorsal skin of rats was shaved, a wound was made in the dorsal area of the rats, noted that two batches of each rats are used in this experimentation. Healing protocol used is the application of the test product on wounds caused previously. These sores its rounded surface of 1cm², total removal of the skin of the thoracolumbar region thereby excision. Applications will be scheduled daily until complete epithelialisation of the wound and it takes about 15 days. Every day, the wounds were treated with the different preparations until complete wound healing. The wounds were visually observed. The first step is the application of the cream from the roots mixed with Vaseline, a second batch of rats was treated with marketed ointment "Baneocin" on wounds caused previously. The applications will be on a daily basis until complete healing of the wound (15 days). In this part, two criteria of evaluation were considered: healing time and the diameter of healing the surface of wounds.

It is noted that each sample fingerprint, the possible emergence of crust is removed with gauze soaked in saline 0.9%. This facilitates the removal of the surface of the wound. The imprints of wounds area are taken from a transparent sheet, and surfaces are calculated by means of architect's Autocad. The statistical evaluation of the data was made by the Test of Student. Data of experiments represented by three replicates from each experiment.

RESULTS AND DISCUSSION

Medicinal plants have always been considered a healthy source of people's lives. Today, human health is becoming more sensitive towards the disease and so the use of medicinal plants could be the best solution. The therapeutic properties of natural medicinal plants are very useful in curing various diseases (Bellakhdar 2006, Teklehaymanot and Giday 2007). The method used for this study seems to give an idea about the natural product existed in organs of medicinal plants by anatomic study, its chemical composition and its pharmacological activities to confirm or not the healing effect on the skin widely known by the indigenous population.

Anatomic study

Concerning anatomic study, all cut root sections 4-5 mm in thickness and circular shape in outline with fissures shows anatomic primary marked feature of development (figures 1 and 2). The root surrounded by the phellogen of subepidermic origin with suber and phelloderm containing a secondary structure strongly developed with the cambium already installed in parenchyma elements. In details, the outermost zone consists of radial bands of rectangular, tangentially elongated, thin-walled cork cells in 8 to 12 rows named "suber". Around the parenchyma exists 1 to 2 rows of bands of phelloderm followed by many layers of parenchyma cells with calcium oxalate scattered in the parenchyma. The main characteristic element of parenchyma is the presence of secretary canals especially in the cortical region, as well in the bast as in the wood. Noted, that the secretary canals are anatomic structures often filled with essential oils, each channel consists of two secretary layers of cells around a light called light channel, secretary cells and protective cells. Secretary canals fit forming concentric circles at the cortical parenchyma as shown in (figures 1 and 2). The innermost zone consists to the endodermis, last layer of the cortex, formed from elongated very thin-walled cells, pericycle followed by the typically alternating radial vascular bundles (15 – 17) arranged in a circle while the root is young, this cell organization is developed in common pattern of the secondary vascular cylinder with time, the cambium induced the formation of secondary xylem and secondary phloem in the parenchymatous pith with few parenchyma cells in the center. With regard to the root, the characteristics observed for the species under study are in line with the general root organization in the Asteraceae, peripheral phellogen, cambium in the inner region, Fritz and Saukel (2011) have noted the discrimination of most genera and species of Asteraceae was possibly based on the anatomy of underground parts of plants. Calcium oxalate crystals appear in the cortex as clusters, this anatomic apparition and their location was been useful in taxonomic classification (Kuster, 1956). Similar observations were previously reported several plants by



Figures. 1.2. Anatomic structure of outer part containing a circle of secretor canals in transverse sections of root of *Cnicus benedictus* L. Bar = 20 μ m. Figs shows (S=Suber; Ph= Phelloderma, P = Parenchyma, SC= Secretary canals. Bars = 80 μ m).

Table 1: Preliminary phytochemical analysis of *Cnicus benedictus* L. with different reagents used.

Compounds	Chemical reactions (Chlorydric acid with or without other product)
Flavonoids	----
Anthocyanes	----
Leuco anthocyanes	----
Tannins	----
Gallic tannins	----
Catechin tannins	----
Quinones free	----
Quinones combined	----
Saponins	+++
Alkaloids	+++
Glucosides	+++
Amidon	----
Coumarines	+++
Sennosides	----

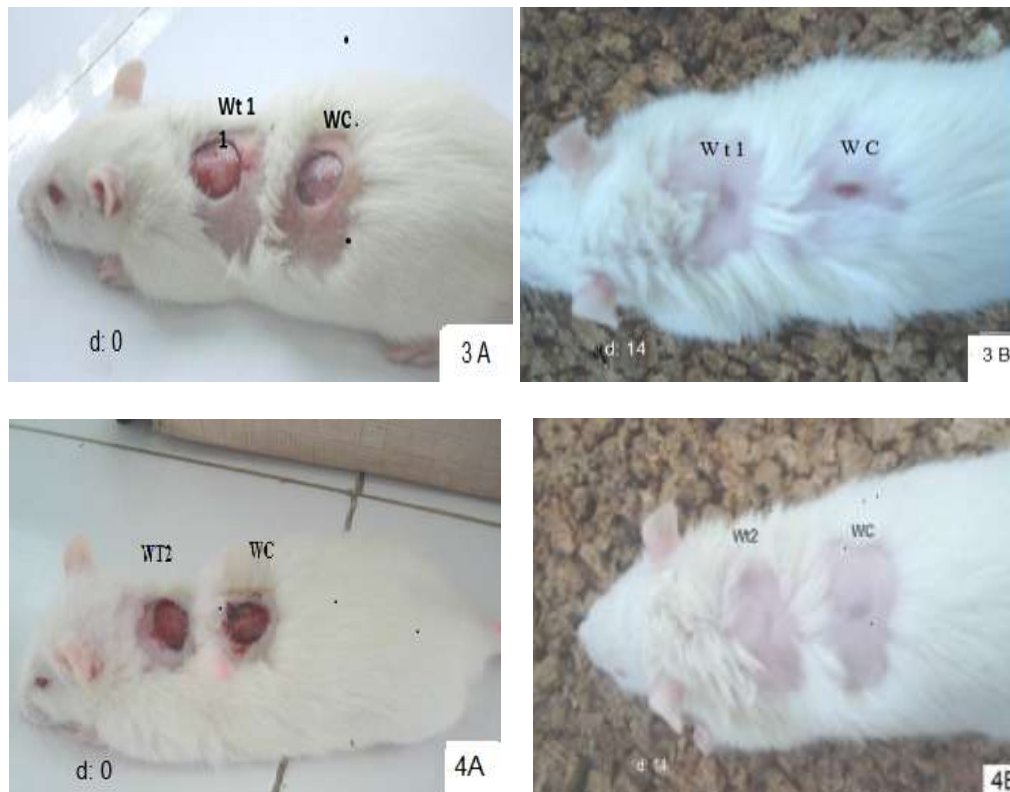
'----'indicates the absence and '+ + +' indicates the high degree of the presence of the compounds respectively.

(Rowe et al., 2004, Williams et al., 2008). Tămaş (2005), underlined the importance of anatomical structure plants. This study could be the main criteria for the establishment of the drugs identity (Hutira and Moldovan 1971, Tămaş 1972, Toma and Rugină 1998). The anatomical characters may be used for identifying some vegetable medical product of medicinal plants. This work led to the morphological description, botany, anatomy to the determination of bioactive substances followed by a pharmacological study to be exploited in the medical field. It is therefore interesting to carry out extensive studies on these plant species during the flowering period, proliferation of roots to obtain a large mass to produce a good amount of cream of Blessed Thistle. The same activity was reported in other plants from the same synantherees family by other scientists. Ghasemi et al. (2009), has showed that natural extracts from *Achillea kellarensis* (family of synantherees) could be very useful in wound healing activity in wistar rats.

Phytochemical analysis

The results of phytochemical screening have showed many phenolic compounds, the root powder contains a

high quantity of Saponins, alkaloids, starch, glycosides and Coumarins (see Table 1). All these secondary metabolites present in other herbs are known to exhibit medicinal properties. However, flavonoids, anthocyanes and all forms of tannins were absent in the root powder. Previous many works have showed that the chemical constituents contained in *Cnicus benedictus* L. are sesquiterpene lactone glycosides such as cnicin (0.2-0.7%), polyacetylen (Umehara et al., 1993); lignans such as trachelogenin, arctigenin (Ulbelen and Berkan, 1977); flavonoids; tannins (8%); and essential/volatile oils (0.3%) such as cinnamaldehyde (Vanhaelen-Fastre, 1973). Lignans such as trachelogenin may contribute to the bitter characteristics of blessed thistle. Cnicin has also been identified as a principal bitter ingredient in blessed thistle (Vanhaelen-Fastre, 1974). Several studies have reported many medicinal effects from the active compounds from *Cnicus benedictus* L. such as anti-inflammatory, antimicrobial, antibacterial, antiviral effects in relationship with the skin healthy. Comparing the results of phytochemical analysis with skin healing laboratory rats under the effect of root powder, indicates the effectiveness of Chemical compounds



Figures 3, 4. Assessment of surface wounds in rats treated by different preparation. Fig 3. Rats treated by cream prepared from root powder of *Cnicus benedictus L.* Fig 4. Rats treated by ointment reference (Baneocin®). Figs shows d: 0 and d: 14. At day 0 (before application) and day 14 (after 14 days of application). WC: Wound control, Wt1: Wound treated by cream prepared from root powder. Wt2: Wound treated by ointment reference (Baneocin®).

Table 2. Healing activity by percentage (%) reduction of wounds: "plant powder» and "Reference ointment" on mice skin

percentage (%) reduction of wounds						
Time variation in days	0	4	7	9	11	14
Root powder mixture	0	6.5	65.68	87.57	94.08	98.81
Baneocin® ointment	0	27.95	65.59	84.94	91.39	95.69

Table 3. Healing activity by average diameter of area of wounds: "plant powder» and "Reference ointment" on mice skin
Statistical analysis averages expressed in days; plant powder and reference ointment.

Healing activity						
Time variation in days	0	4	7	9	11	14
Root powder mixture	1.69	1.58	0.58	0.21	0.1	0.02
Baneocin® ointment	1.86	1.34	0.64	0.28	0.16	0.08

identified namely saponins, which facilitate the penetration of substances to the skin, alkaloids, anesthetics, analgesics and bactericide. Glycosides and coumarins obtained successively have antiseptic and anti-inflammatory activities, in addition to essential oils, which have effects: antiseptic, antibacterial, anti-inflammatory, often applied as an ointment or compress to their properties fungicide, bactericide, antiseptic and healing.

Pharmacological activity

The pharmacological activity of *Cnicus benedictus L.* (Figure 3 and 4), has showed a high level of wound

healing activity, the results obtained indicate that treatment of rats with the test product isolated from root's powder shows a total duration of wound healing after 14 days against more than fourteen compared to the ointment control "Baneocin®". The diameter of the zone of injured skin decreased with time in comparing to those treated with ointment application (see Table 2 and 3).This pharmacological test showed that the polyphenols of *cnicus benedictus L.* roots have a big wound healing activity. Results obtained underline that wounds treated with the crude extract of the plant and ointment (Baneocin®) show a rapid healing of the skin in 14 days. However, the comparison of the activity of the plant powder with the reference product on rats show

that the cream of *Cnicus benedictus L.* has a potent activity with a percentage reduction of wound (98.81 %) at day 14, against those treated by the ointment (95.69%). In addition, statistical analysis (Student test) reveals a little significant difference. Indeed, these results reveal a good healing activity of the plant powder; it has the same effectiveness as the Baneocin®, known for its healing effect, confirming the traditional use widely reported by the population. Concerning untreated wounds of control rats, these show a healing of skin in average in 20th day. Note that the wounds treated with the plant powder have not reported skin infections, and this has been the bactericidal effect of the plant. These observations reveal the importance of the plant extract in skin healing; this way of treatment by use of plants is becoming very useful in human health. The use of this plant to heal skin's disease has been extensively applied by population. By the way, data from the literature mention the great potential of use of *Cnicus* for therapeutic treatment of the skin. According to Lutterodt et al. (1999), the phytochemical constituents such as tannins, flavonoids, alkaloids, cyanogenetic, glycosides, reducing sugar and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores. It appears that secondary metabolites and all other active principles of the plant have been shown to be responsible for the healing reaction. The wound healing of *Cnicus benedictus L.* was reported by other authors by using other parts of the plant as leaves and flower heads (Kataria, 1995; Vogel, 1997; Chevalier, 2000). Many other important effects of different parts of this plant on the skin were determined as antimicrobial activity attributed to cnicin and polyacetylene constituents (Grieve, 1973; Chevalier, 2000; Van Wyk and Wink, 2004). The main product responsible of this healing reaction is cnicin which has antibacterial and antifungal activity. Similarly, cnicin was the most active sesquiterpene against a variety of fungi in another study (Panagouleas et al., 2003). On the other hand, one patent discloses antifungal proteins active against plant pathogenic fungi, isolated from the seed of blessed thistle. Therefore, more studies need to be conducted to search for compounds responsible of active cell proliferation of injured zone.

CONCLUSION

According to the results of this investigation, the anatomical study and phytochemical underlined its medicinal character. During analysis, the extract of roots of *Cnicus benedictus L.* applied to the skin of rats of laboratory depilated allowed to compare the evolution of the diameters of heal produced. This work deserves to be confirmed by histological studies to comparing the effect of healing of skin. Therefore, conducting a subsequent study on another animal species is crucial. This could be rewarding and lead us to the discovery of new active substances with high healing power. The results of this study support the

easy usage of the studied root plant powder and suggest that root powder contains compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

ACKNOWLEDGMENTS

We thank women and men of east region especially from Bordj bou arreridj for the great collaboration during the surveys.

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