Antibacterial Activity of Aqueous Extracts of *Anacyclus Pyrethrum* (L) Link and *Corrigiola Telephiifolia* Pourr. From the Middle Atlas Region-Morocco

Daoudi Amine
Bammou Mohamed
Ibijbijen Jamal
Nassiri Laila
Laboratory of Soil Microbiology and environment, Department of Biology, Faculty of Sciences 11201 Meknes, Morocco

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Abstract

Aqueous extracts of *Corrigiola telephiifolia* and *Anacyclus pyrethrum* were studied in vitro for their antimicrobial properties. These plants were collected from the Central Middle Atlas region. *Corrigiola telephiifolia* Pourr. and *Anacyclus pyrethrum* (L.) Link are widely used as a natural drug for the treatment of various infectious diseases.

Phytochemical screening of the plants showed the presence of flavonoids, saponins, tannins, steroids and alkaloids.

The determination of the polyphenols in the aqueous extract of the two plants revealed the presence of significant amounts of polyphenols (58.5 ± 0.76 mg equivalent of gallic acid (GEA) per gram of extract for *C. telephiifolia,* and 97.57 mg equivalent of gallic acid (GEA) per gram of extract of *A. pyrethrum*).

The antimicrobial activity of the aqueous extracts against three bacterial strains was evaluated on the basis of the inhibition zone using the disk diffusion assay. The aqueous extract of *Corrigiola telephiifolia* is slightly active on *E. coli*. At a concentration of extract 100 µg mL-1, the size of the inhibition zone equal to 9 ± 0.06 mm. Whereas *S. aureus, E. coli* and *K. pneumoniae* showed a sensitivity to the macerate of *Anacyclus pyrethrum* at the concentration 100 µg mL-1 of extract with an inhibition zone 16.55 ± 0.6 mm, 14.95 ± 1.25 mm and 10.83 ± 0.96 respectively.

Keywords: Antimicrobial activity, aqueous extract, Aromatic and medicinal plants, Central Middle Atlas, Morocco

Introduction

The plants have always been the source of medicines, following the presence of many active ingredients that result from therapeutic properties (Ibn Makhlouf, 2008). The plants should be used with great caution, despite their inoffensive reputations (Jean, 2009).

The plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which derive from antimicrobial properties (Muanda, 2010). However, it was not until the early 20th century that scientists became concerned about it (Yano *et al.*, 2006).

People have explored the nature especially plants in search of new drugs (Dabai *et al*., 2012). Medicinal plants are a rich source of antimicrobial agents and are widely used in human therapy, veterinary, agriculture, scientific research and countless other fields (Hussain *et al*., 2012).

Although many plant species have been tested for antimicrobial properties, a large majority have not been adequately assessed (Balandrin *et al*., 1985). In this context, the antibacterial activity of aqueous extracts of two plant species from the Central Middle Atlas region, namely *Corrigiola telephiifolia* Pourr. (سرغينة - Serguina) from the family of Caryophylaceae, and *Anacyclus pyrethrum* (L.) Link (عقير قرحة - Akir karha) from the Asteraceae. These two plants are widely used in Moroccan traditional medicine for the treatment of several diseases: gastroenteritis, spasms and colic, arthritis and rheumatoid, gum diseases and toothache, pathologies of the respiratory system (Bammou *et al*., 2015; Nassiri *et al*., 2016)

The main objective of this work was to screened for the presence of phytochemicals constituents and to determine the antimicrobial capacities of aqueous extracts against two gram negative bacillus strains (*Escherichia coli* and *Klebsiella pneumonia*) and a gram positive cocci strain *Staphylococcus aureus*, respectively responsible for pulmonary, digestive and skin infections.

Material and methods

Monograph of studied plants

Anacyclus pyrethrum (L.) Link (Figure 1: a, b)

Botanical situation

- Kingdom **:** Plantae
- Class : Magnoliopsida
- Order : Asterales
- Family : Asteraceae
- Genre : *Anacyclus* L.
- Species: *Anacyclus pyrethrum* (L) Link
- French name: Pyrèthre d'Afrique
- Arabic name: عقير قرحة - Akir karha

Fig. 1 (a,b): *Anacyclus pyrethrum* (L.) Link (by DAOUDI Amine)

Botanical description: the diameter of the involucre is between 7 to 22 mm; Bracts on three rows, narrowly triangular, green below, blades above, dark-brown margins, fine, eroded. Hemicryptophyte (spring and summer).
Bioclimate: we can find this plant in the semi-arid, subhumid and humid bioclimate.
Distribution: this species is regularly encountered in High mountains- Atlas Saharan, Anti atlas, High atlas, Middle atlas Moroccan, North atlantic plateaux of eastern Morocco (Jerada) Rif (Chaouene, Jbel Assilenh, valley of Tizi-n-lel) (Fennane *et al.*, 2014).
Traditional use: *Anacyclus pyrethrum* is widely used for the treatment of some rheumatism; root powder is recommended for internal use against liver disease, decoction is used to treat cases of bronchitis and enuresis (Sijelmassi, 1996; Daoudi *et al.*, 2014; Bammou *et al.*, 2015), and is therefore recommended for treating oral diseases (Bellakhdar, 1997; Daoudi *et al*., 2014). *A. pyrethrum* is used in the manufacture of dentifrices (Sijelmassi, 1996).
Toxicity: this plant that cannot be devoid of a toxic part. Indeed, according to a study carried out by Daoudi *et al.* (2014), several accidents have been reported. It causes respiratory problems, gastroenteritis, colic, skin irritation, mucous membranes, buzzing of the ears and even loss of consciousness (Bellakhdar, 1997; Hmamouchi, 1999).
Plant chemistry: unsaturated amides, inulin, tannins and resin are identified in the root, also alkaloids and yellow dyes are present (Bellakhdar, 1997; Hmamouchi, 1999).
***Corrigiola telephiifolia* Pourr (Figure 2: a,b)**
Botanical situation
- Kingdom: Plantae
- Class: Magnoliopsida

- Order: Caryphyllales
- Family: Caryophylaceae
- Genre : Corrigiola L.
- Species : *Corrigiola telephiifolia* Pourr.
- French name : Corrigiole
- Arabic name : - سرغينة – Serguina

Fig. 2 (a,b): *Corrigiola telephiifolia* Pourr (by DAOUDI Amine)

Botanical description: *Corrigiola telephiifolia* is an herbaceous plant. The stem leaves oblong-lanceolate, spatulate or oval, somewhat thick, odorous roots. Inflorescence in axillary and terminal glomeruli. Perianth white or a little pinkish, sensibly as long as the sepals. Five stamens shorter than the petals. Whitish anthers. Three stigmata. Akene trigone, crustacean, included in persistent perianth. Hemicryptophyte (spring and summer).
Distribution: the species is distributed throughout the stoney and sandy plains and mountains: Anti atlas (Siroua), High atlas, Middle atlas, Middle Atlantic, North Atlantic Morocco and Rif (Fennane and Ibn-Tattou, 1999).
Bioclimate: this species is located on the semi-arid soft, semi-arid cold, sub humid bioclimate.
Traditional use: this plant is used to treat, respiratory, gastric, dermatological, urogenital, neurological and typhoid diseases (Daoudi *et al*., 2015). Also in aromatic; the roots of serghin are widely used in fumigations, they are the reputation of keeping evil geniuses at a distance (Bellakhdar, 1997) this plant is also used against allergies (Juven *et al.*, 1994; El Hafian *et al.*, 2014).
Toxicity: there is no risk of toxicity during administration of this plant, but the dichloromethane extract of this plant showed potent cytotoxicity (Doudach *et al.*, 2012,b).
Plant chemistry: this plant contains many types of secondary metabolites, including terpenes, phenolic compounds, quinones, alkaloids, saponins and

tannins. The nature and concentration of these compounds depend on the degree of maturity of the plant and the environmental conditions that may influence the capacity of the plant tissue to store and / or synthesis the molecules (Doudach *et al*., 2012,b).

Preparation of plant material

Preparation of aqueous extracts

In this study, we used three modes of traditional extraction: maceration at room temperature, infusion and decoction, the ratio used is 1/10 (w/v) and the condition was as follows:

The Infusion was prepared by adding 100 mL of boiling distilled water to the sample powder (10g) and were left to stand at room temperature for 15 min, and then filtered through Whatman paper.

The decoction was performed by adding to 100 mL of distilled water to the powder (10g), heated and boiled for 15 min. The mixture was left to stand for 5 min and then filtered. The infusions and decoctions were evacuated at 40°C.

The maceration was performed by stirring the plant material powder (10g) with 100 mL of distilled water at room temperature and 150 rpm for 48 h and filtered through Whatman paper. The combined extracts were then evaporated at 40°C.

Quantification of active ingredients

Preliminary phytochemical screening

The freshly prepared extracts were subjected to standard phytochemical analysis to ensure the presence of following phytoconstituents (Harborn, 1998; Adebayo and Ishola, 2009).

Tests for Alkaloids

Mayer's Test: Take 5 ml of extract, few drops of Mayer's reagent is added by the side of the test tube. A white creamy precipitate indicates the test as positive.

Dragendorff's Test: Take 5 ml of extract, 1 or 2 ml of Dragendorff's reagent was added. A prominent yellow/orange precipitate indicates the test as positive.

Test for Saponins

For detection of Saponins the plant extract was subjected for frothing test, 5ml of the extract is vigorously shaken with 8 ml of distilled water in a test tube for 30 sec and was left undisturbed for 20 min. Persistent froth indicating the presence of saponins.

Detection of Tannins

For detection of tannins 3 ml of extract was added to 1% of lead acetate, formation of yellow precipitate indicates the presence of tannins and in the same way 3 ml of extract was treated with 3 ml of FeCl3 appearance of green color indicates the presence of condensed tannins.

Detection of Anthocyanins

For detection of Anthocyanins 3 ml of extract was treated with 3 ml of 2N HCl & NH_3 resulting in to the appearance of pink red colour which turns in to blue violet indicating the presence of Anthocyanins.

Detection of Steroids

For detection of steroids 0.5 ml of extract was dissolved in 5 ml of chloroform to this mixture concentrated sulphuric acid was added from the side of the test tubes uppe layer at surface appeared red and acidic layer showed yellow with green fluorescence indicating the presence of steroids.

Detection of Flavonoids

For detection of flavonoids, alkaline reagent test was performed. The extract was treated with 10% of NaOH solution resulting in to the formation of deep yellow color indicated the presence of flavonoids

Determination of total polyphenols

The polyphenol content was measured using the Folin-Ciocalteu method: 1 mL of Folin reagent (diluted 10 times) was added to 200 µl of aqueous extract and 2 mL of distilled water. The mixture was then in incubation for 4 min before adding 0.8 mL of sodium carbonate (7.5%). The total polyphenols contents were determined after 2 h of incubation and the absorbance was measured at λ_{max} = 765 nanometres. The results were obtained as milligram of gallic acid equivalent (GEA) per gram of extract (Ghedadba *et al*., 2015).

Antibacterial activity by the disk method

Bacterial culture

In order to test the antibacterial potential of aqueous extracts of: *Corrigiola telephiifolia* Pourr., and *Anacyclus pyrethrum* (L) Link. Two Gram-negative bacillus strains (*Escherichia Coli* and *Klebsiella pneumonia*) and a gram-positive cocci strain *Staphylococcus aureus*, respectively responsible for pulmonary, digestive and cutaneous infections, were used in this test. These strains were isolated and identified by the bacteriology department, Mohamed V Meknes hospital centre. One to two colonies were isolated from each fresh culture strain and transferred to tubes containing

sterile distilled water in order to have an initial cell density or turbidity close to that of McFarland 0, 5 (10^6 CFU mL^{-1}).

Preparation of the antibiogram

In the present test, the diffusion technique on agar media was used. Dishes containing Mueller Hinton medium were seeded with 100 µl of each pre-prepared bacterial culture. In each dish corresponding to a strain tested, sterile disks of Whatman paper with a diameter of 6 mm were deposited aseptically and impregnated with concentrations ranging from 25 µg mL^{-1} to 125 µg mL^{-1} of each aqueous extract. In addition, sterile distilled water was used as a negative control.

Finally, gentamicin (30 µg) was used as a positive control for *Escherichia coli* and *Klebsiella pneumonia* whereas Amikacin (10 µg) was the positive control for *Staphylococcus aureus*. The dishes were made in triplicate and incubated at 37 °C. for 24 hours. The objective of the antibiogram is to predict the susceptibility of a microorganism to one or more antibiotics. This sensitivity is expressed by the appearance of a zone of inhibition around the disks. The results of the antimicrobial evaluation of our extracts are reported in millimeters of the zones or diameters of inhibitions, indicating that the microorganisms are inhibited by the extracts tested.

Reading the results

The antibacterial activity is manifested by the appearance of a halo of inhibition of the bacterial growth around the disks impregnated with the aqueous extract. The result of this activity is expressed by the diameter of the inhibition zone (mm) (Djenane *et al.*, 2012).

- Ø <8 mm: non-sensitive bacteria;
- 9 <Ø <14 mm: sensitive bacteria;
- 15 <Ø <19 mm: very sensitive bacteria;
- Ø> 20 mm: extremely sensitive bacteria.

Results and discussion

Determination of polyphenols

Corrigiola telephiifolia Pourr.

Phytochemical screening of the plants revealed some differences in the constituents of the two plants tested. As indicated in Table 1, the powder of *Corrigiola telephiifolia* Pourr. showed the presence of alkaloids and flavonoids. In addition to the relatively average amounts of tannin and steroids. The demonstration of saponin was remarkable, so the height of the foam was clearly visible. Our results corroborate those found by (Timite, 2012), which showed that species belonging to the family Caryophyllaceae

Table 1: Phytochemical constituents of Corrigiola telephiifolia and Anacyclus pyrethrum		
Test	Corrigiola telephiifolia	Anacyclus pyrethrum
Tannins	++	+
Flavonoids	+	+
Alkaloids	+	+++
Saponins	+++	++
Steroids	++	-
Anthocyanins	-	-
+++ = appreciable amount; ++ = moderate amount; + = trace amount; - = completely absent.		

are often rich in saponin and mainly triterpenic saponin. Finally, *Corrigiola telephiifolia* Pourr. is very rich in sterols and triterpenes.

The aqueous extracts of the plant are obtained via the three traditional extraction methods; infusion, decoction and maceration. However, the total polyphenols are assayed via the Folin-ciocalteu method. This method is widely studied because it is standardized, simple, reproducible and the interference with the sample matrix which is often colored is minimized at the long absorption wavelength (765 nm) used (Boukri, 2014). The results showed that the highest concentrations are observed in the macerate of *Corrigiola telephiifolia* with 58.5 mg of gallic acid / g of extract (Figure 3).

In general, the results of phytochemical screening and colorimetric determination revealed the richness of *Corrigiola telephiifolia* Pourr. In total polyphenols this oriented us to evaluate the antimicrobial effect of the plant.

Anacyclus pyrethrum (L.) Link

The phytochemical examination carried out on the ground root extracts of *Anacyclus pyrethrum* (L) Link**.** revealed the presence of alkaloids, flavonoids, tannins and saponin, these results being consistent with that of (Elazzouzi *et al*., 2014). The bibliography indicates the presence in the roots of polyacetylenes compounds and lignans. The roots mainly accumulate alkamides, especially pellitorin, the main constituent of which is found in the roots of *A. pyrethrum*. These nitrogenous and unsaturated substances are associated with the long-known sialagogue effect (Hamimed, 2009). The determination of the polyphenols in the various aqueous extracts (maceration, decoction and infusion) of this plant revealed that the maceration had the equivalent amount of gallic acid in mg per 1 g of the largest extract relative to the other extracts (97, 57 Qt eq Ag mg / 1 g extract) (Figure 3).

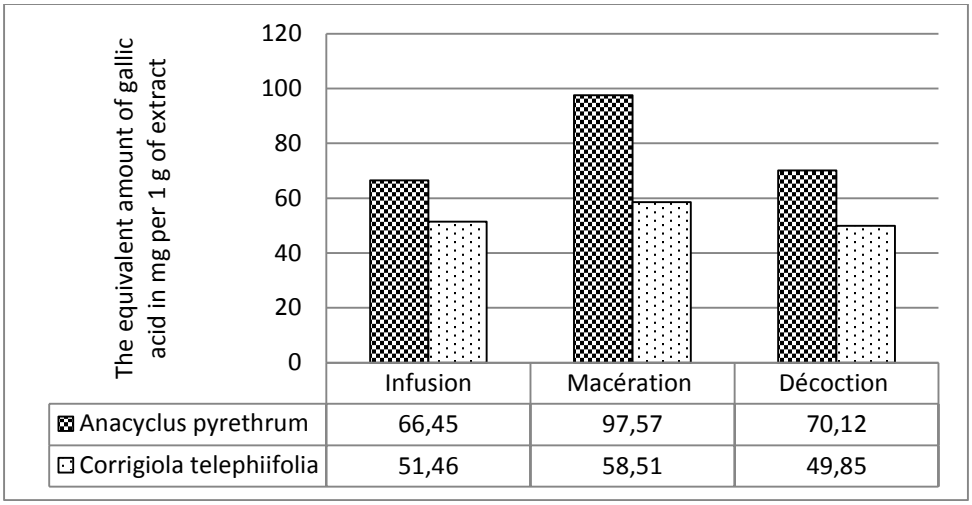

Fig. 3: Determination of polyphenols from aqueous plant extracts

Antibacterial activity of aqueous extracts

The antibacterial activity of aqueous extracts of *Corrigiola telephiifolia* Pourr. is tested against three bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*), the results revealed that the macerate of the plant is only slightly active against *E. coli* with an inhibition zone of 9 ± 0.06 mm at an extract concentration of 100 µg / ml (Table 2).

Table 2: Antibacterial activity of aqueous extract of *Corrigiola telephiifolia* Pourr.

Extract (100 ug/ml)				
Germs	ATB	Decoction	Infusion	Maceration
S. aureus	AK (S)=19	-	-	-
E. coli	GN(S)=20	-	-	9.00 ±0.06
K. pneumoniae	GN(S)=20	-	-	-

(–): No zone of inhibition observed: germ insensitive to the extract.
Average of the inhibition diameters in millimeters

This inhibiting power may be explained by the abundance of saponin. Thus, it is reported in other studies (Saihi, 2011; Doudach *et al*., 2013c) that the extract of *C. telephiifolia* is active against *E. coli*. These findings are also confirmed by the results of our ethnobotanical investigation, which showed that the roots of *C. telephiifolia* are recommended to treat digestive infections.

The relatively high values of polyphenols led us to carry out tests on bacterial strains to unveil the antibacterial activity of this plant. *S. aureus*, *E. coli* and *K. pneumoniae* showed sensitivity to macerate of *Anacyclus pyrethrum* at a concentration of 100 µg mL^{-1} with an area of inhibition of 16.55 ± 0.60 mm, 14.95 ± 1.25 mm and 10.83 ± 0.96 mm respectively (Table

3). These results were consistent with those published by (Doudach *et al*., 2012a ; Elazzouzi *et al*., 2014).

The aqueous extract of *Anacyclus pyrethrum* produces antibacterial effects on other bacterial strains, namely *Listeria monocytogenes* and *Candida albicans* (Selles *et al*., 2012). These results prove that this plant produces an important antibiotic potential, which seems to correlate well with the total phenolic values found. Therefore, these results provide a good basis for further examination of the possible application of *A. pyrethrum* for medicinal purposes, as well as in the pharmaceutical industry.

Table 3: Antibacterial activity of aqueous extract of *Anacyclus pyrethrum* (L.) Link

Extract (100 µg/ml)				
Bacteria	ATB	Decoction	Infusion	Maceration
S. aureus	AK (S)=19	10.60 ± 0.35	6.86 ± 0.77	16.55 ± 0.60
E. coli	GN(S)=20	9.33 ± 0.96	-	14.95 ± 1.25
K. pneumoniae	GN(S)=20	7.20 ± 1.00	-	10.83 ± 0.96

(–) : No zone of inhibition observed: germ insensitive to the extract.
Average of the inhibition diameters in millimeters

Conclusion

This work made it possible to demonstrate the antibacterial properties of the aqueous extracts of *Corrigiola telephiifolia* Pourr. (سرغينة – Serguina) and *Anacyclus pyrethrum* (L.) Link (عقير قرحة - Akir karha), significant inhibition diameters on pathogens and sometimes resistant to antibiotics.

The results of this work could justify certain traditional uses of these plants in certain pathologies such as respiratory, gastric and dermatological, urogenital, neurological affections. However, this work must continue in order to isolate separately the active ingredients responsible for the antibacterial activity.

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