Antibacterial and antifungal screening of four medicinal plants

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1. Introduction

Infectious diseases are so far responsible for 43% of deaths in the poor country, even if they have lost ground in developed societies (1% of all deaths) due to hygiene and urban sanitation, anti–infectives and vaccines[1].

In Algeria, as in other developing countries, infectious diseases remain until this day a public health problem because of their frequency and severity. The situation is further more concern because of the emergence of new strains and the emergence of uncomon infections that are resistant to conventional treatment[2–3].

Given these challenges posed by the use of antibacterial agents available, it is essential to find effective new antimicrobial substances and broad spectrum of action. One of the strategies of this research is to explore the plants used in traditional medicine. Plant extracts have been traditionally used in folk medicine against various diseases[4].

Indeed, 80% of the African population still uses traditional medicine for which the majority of therapy involves the use of active ingredients of medicinal plants. These plant species also important to the health of populations and should be studied scientifically to better use[5].

However, although the flora of Algeria is rich and varied, it is still little exploited scientifically especially in the field of the fight against infectious diseases[6].

The goal is to find more plants with antimicrobial...
properties as well as to rationalize the use of medicinal plants. This work focuses on four plants used effectively in various traditional treatments of various diseases in Algeria and elsewhere. The in vitro antimicrobial activity of hydroalcoholic and chloroformic extracts of the plants were studied against some bacteria involved in infectious diseases.

2. Materials and methods

2.1. Plant material

The proposed material for the study (Table 1) was harvested in different area at different periods and were stored in the dark at ambient temperature in our laboratory. The plants were identified and authenticated by Dr. Nadjib Rahmoun, Département de Biologie, Section of Plant Biology, Tlemcen University.

2.2. Ethnopharmacological study

In order to know the way in which the selected plants were traditionally used, it should be started initially by ethnopharmacological investigation of these plants. This study was performed by conducting a questionnaire that contains the photo, vernacular name, traditional use, used parts and extraction method of the plant. This questionnaire was given to herbalists, nurserymen, botanists, pharmacists and traditional users.

2.3. Preparation of extracts

Plant crude extracts were prepared according to the method of Sharma (1990) that was fully described in the previous paper[7]. Extraction was carried out from the crushed dry leaves. Briefly, 25 g of powdered plant material was soaked in 100 mL of solvent. Various extractions were carried out using hydroalcoholic and chloroform solvents. Each mixture was stirred for 24 h. At the end of each extraction the extract was passed through a Whatman No. 1 filter paper (Whatman, UK). The volatile filtrates were concentrated under vacuum on a rotary evaporator at low temperature 30 °C. The extracts were stored at 4 °C until further use.

The extraction of Aloe vera (A. vera) was performed by the aspiration of mucilage sheets with a syringe.

The yield is calculated by the following formula:

\[ \text{Rendement (g)} = \frac{m_0}{m_1} \times 100 \]

Where \( m_0 \) is evaporated extract mass, and \( m_1 \) is initial vegetal mater mass.

2.4. In vitro biological assay

2.4.1. Evaluation of the antibacterial activity

The in vitro antimicrobial activity of the examined extracts was assessed by the Kirby–Bauer disk diffusion and the solid agar dilution methods, according to the recommendations of the National Committee for Clinical Laboratory Standards[8,9].

A panel of 12 well–documented pathogenic bacteria was used in the study. The following bacteria, obtained from the laboratory. Antibiotiques Antifongiques: Physico–Chimie, Synthèse et Activité Biologique, Département de Biologie, Tlemcen University, were used: Pseudomonas aeruginosa ATCC 27853 (P. aeruginosa), Escherichia coli ATCC 25922 (E. coli), Salmonella typhimurium ATCC 13311 (S. typhimurium), Acinetobacter baumannii ATCC 19606 (A. baumannii), Klebsiella pneumonia ATCC 700603 (K. pneumonia), Enterobacter cloacae ATCC 13047 (E. cloacae), Citrobacter freundii ATCC 8090, Proteus mirabilis ATCC 35659 (P. mirabilis) (Gram–negative bacteria), Staphylococcus aureus ATCC 25923 (S. aureus), Bacillus cereus ATCC 10876 (B. cereus), Enterococcus faecalis ATCC 49452 (E. faecalis), Lysteria monocytogenes ATCC 15313(Gram–positive bacteria), and the yeasts: Candida albicans ATCC IP 444 (C. albicans), C. albicans ATCC 10231, C. albicans ATCC 26790. These are Gram–positive and Gram–negative bacteria known to be the cause of many infectious diseases such as skin diseases, respiratory, digestive and urinary systems.

2.4.2. The disk diffusion method

The disk diffusion method against bacteria and yeasts was fully described in the previous paper[10]. Positive controls were made with gentamycin and ciprofloxacin for bacteria. Amphotericin B was used as positive control for yeasts. An additional negative control disk without any sample but impregnated with the equivalent amount of dimethyl sulfoxide solvent was also used in the assay. The antimicrobial activity was considered beyond a diameter of 9 mm or more. The data were the mean of three replicates.

2.4.3. Solid agar dilution method

Mueller–Hinton agar medium was prepared in the flasks and sterilized. To 19 mL this medium, 1 mL of extract was added in order to get the fold serial dilution desirable. The Petri dish was thoroughly mixed by stirring. A negative control was also prepared in the same way using solvent dimethyl sulfoxide and water. The Petri dish was inoculated with 1 µL of bacterial suspension adjusted and diluted to

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**Table 1**

Parts, harvest periods and regions of the used plants.

| Local name | Family/Latin binomial | Voucher specimen N° | Tested part | Harvest period | Harvest area       |
|------------|----------------------|---------------------|-------------|----------------|--------------------|
| Carob      | Leguminosae/C. siliqua | LPBpec.C.S. 15.02   | Fruits and seeds | April 2013     | Nédroma (Algeria)  |
| Sahar      | Aloaceae/A. vera      | 010102              | Leaves      | May 2013       | Marsa El Kbir (Algeria) |
| Siwak      | Salvadoraceae/S. persica | 2215                | Roots       | April 2013     | Timimoune (Algeria) |
| Kaff maryam| Cruciferae/A. hierochuntica | 14562               | Whole plants| May 2013       | Mecca (Saudi Arabia) |
the concentration of $10^7$ CFU (0.5 McFarland standard diluted to 10%), and the final inoculum required is $10^5$ CFU per spot. Gentamycin and ciprofloxacin were used as positive controls for bacteria. The minimum inhibitory concentration (MIC) of *C. albicans* was performed as for bacteria but the culture medium used was supplemented with Mueller–Hinton, 2% glucose and 0.5 mcg/mL methylene blue with pH of 7.4. The fungal suspension was set at 0.12–0.15 ($\lambda=530$ nm) at final concentration of $1\times10^{-5}$–$5\times10^{-7}$ CFU. Amphotericin B was used as positive control.

All the plates were inoculated at 37 °C for 24 h. The MIC (of bacteria and yeasts) was considered as the weakest concentration for which there was no visual growth. The data were the mean of three replicates.

### 3. Results

#### 3.1. Ethnopharmacological study

This study was carried out by performing a questionnaire which was placed at the disposal of users of various professions. The results obtained are shown in Table 2. It can be noted that the fruits are the common part of the four plants. All the plants were used traditionally in different treatment and with different extraction methods.

### Table 2

Ethnopharmacological uses of the medicinal plants tested.

| Plants       | Traditional uses       | Parts used              | Modes of uses         |
|--------------|------------------------|-------------------------|-----------------------|
| *A. vera*    | Beauty products         | Roots, leaves           | Dry powder            |
| *A. hierochuntica* | Hypoglycemic,          | Roots, leaves and      | Infusion, maceration  |
| *C. siliqua* | Stomach problems,      | Seeds and fruits        | and dry powder        |
| *S. persica* | Tooth brushing, against| Roots and fruits        | Infusion, maceration  |
|              | Github, Moisturizing   |                         | and dry powder        |
|              |                        |                         |                       |

### Table 3

Extraction yields of the studied plants.

| Plant                | Part   | Solvents          | Concentration (g/mL) | Yield (%) |
|----------------------|--------|-------------------|----------------------|-----------|
| *C. siliqua*         | Seeds  | Hydroalcoholic    | 7.00                 | 10.00     |
|                      |        | Chloroform        | 0.09                 | 1.40      |
| *S. persica*         | Roots  | Hydroalcoholic    | 7.00                 | 2.00      |
|                      |        | Chloroform        | 0.06                 | 1.12      |
| *A. hierochuntica*   | Whole  | Hydroalcoholic    | 7.00                 | 1.40      |
|                      | plant  | Chloroform        | 0.09                 | 2.00      |
| *A. vera*            | Mucilage | Hydroalcoholic | 5.50                 | 22.00     |

#### 3.2. Yield

The preparation of extracts from different parts of selected plants was performed using two solvents of different polarities. The yields obtained by different extracts are shown in Table 3. All hydroalcoholic extracts showed the best yield compared with the results of the chloroform extracts. *A. vera* showed a yield of about 22.00%.

### Table 3

Antimicrobial activity of the tested extracts.

| Bacteria          | *C. siliqua* | *S. persica* | *A. hierochuntica* | *A. vera* | Gentamycin | Ciprofloxacin | Amphotericin B |
|-------------------|--------------|--------------|--------------------|-----------|------------|---------------|----------------|
| Gram–negative     |              |              |                    |           |            |               |                |
| *A. baumannii*    | 26           | 15           | 8                  | 20        | 18         | 7             | 8              | 14.50         | 24.5          | –             |
| *C. freundii*     | 7            | 9            | 6                  | 10        | 6          | 6             | 6              | 26.00         | 32.5          | –             |
| *E. cloaca*       | 10           | 6            | 7                  | 8         | 6          | 6             | 10             | 18.00         | 20.0          | –             |
| *E. coli*         | 16           | 18           | 7                  | 6         | 7          | 8             | 6              | 21.50         | 36.0          | –             |
| *K. pneumoniae*   | 7            | 24           | 7                  | 8         | 6          | 8             | 6              | 14.00         | 24.0          | –             |
| *P. aeruginosa*   | 30           | 24           | 15                 | 13        | 25         | 19            | 10             | 21.33         | 33.0          | –             |
| *P. mirabilis*    | 6            | 7            | 6                  | 6         | 6          | 6             | 7              | 23.00         | 33.3          | –             |
| *S. typhimurium*  | 24           | 22           | 7                  | 6         | 9          | 8             | 10             | 25.00         | 35.5          | –             |
| Gram–positive     |              |              |                    |           |            |               |                |                |                |                |
| *B. cereus*       | 30           | 24           | 20                 | 23        | 10         | 9             | 6              | 23.00         | 33.5          | –             |
| *E. faecalis*     | 22           | 16           | 7                  | 8         | 7          | 7             | 7              | 26.00         | 32.5          | –             |
| *L. monocytogenes*| 23           | 18           | 8                  | 6         | 6          | 7             | 6              | 18.00         | 20.0          | –             |
| *S. aureus*       | 8            | 26           | 7                  | 16        | 6          | 10            | 6              | 21.33         | 33.0          | –             |
| Yeasts            |              |              |                    |           |            |               |                |                |                |                |
| *C. albicans* ATCC 444 | 30   | 11           | 17                 | 11        | 25         | 21            | 18             | 21.00         | 21.0          | –             |
| *C. albicans* ATCC 10231 | 10   | 11           | 18                 | 10        | 9          | 12            | 7              | 20.00         | 20.0          | –             |
| *C. albicans* ATCC 2690 | 9    | 9            | 13                 | 8         | 8          | 17            | 11             | 21.00         | 21.0          | –             |

–: no MIC observed.

### 3.3. Antibacterial activity

Evaluation of the antibacterial activity of hydroalcoholic and chloroform extracts of the studied plants was determined initially by the disk diffusion method against different bacteria. These bacterial strains are Gram–negative species frequently encountered in infectious diseases.

The results of the diameters of inhibition zones are shown in the Table 4. It can be noted that the most interesting plant is *Ceratonia siliqua* (*C. siliqua*) which showed antibacterial activity against five Gram–negative bacteria. The diameters of the zones of inhibition were between 15 mm and 30 mm against *A. baumannii, E. coli, K. pneumoniae, P. aeruginosa* and *S. typhimurium*. The hydroalcoholic and chloroform
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between 13 and 25 mm. The
was very sensitive to the whole
showed no interesting activity against
all Gram–negative bacteria. This can be explained by the
inadequate method used in the extraction. P. aeruginosa
has been the most sensitive against the whole plants extracts of
and A. hierochuntica.
The strain B. cereus was very sensitive to the whole extracts except that of A. vera. All Gram–positive strains
were susceptible to both extracts C. siligua, except S. aureus
that was resistant to chloroform extract. The diameters of the
zones of inhibition were between 16 mm and 30 mm. S. persica
has shown inhibition zones whose diameter were
between 16 mm and 23 mm against A. baumanii and P. aeruginosa.
A. vera and A. hierochuntica showed no interesting
activity against all positive bacteria grams.
The results of MICs of the Gram–negative and Gram-
positive bacteria are shown in Table 5. It can be noted that
the best results were obtained with chloroform extract of all
plants. The lowest MICs obtained by C. siligua were 0.13 mg/mL
against P. aeruginosa, E. coli and E. cloacae. MICs
against C. freundi and A. baumanii were 0.200 and 0.250 mg/
ml respectively. In addition, the lowest MICs obtained by
S. persica were 0.05 mg/mL against K. pneumoniae and P.
aeruginosa. MICs against K. pneumoniae and C. freundi were
0.35 mg/mL. Other results were between 0.35 and 43.75 mg/
ml. The plant A. hierochuntica showed MICs of 0.07 mg/mL
against C. freundi and P. aeruginosa. Contrary to what was
expected, the lowest MIC of A. vera was 4.29 mg/mL against
E. cloacae. The lowest MIC of C. siligua was 0.130 mg/mL
against E. faecalis for the chloroform extract and against
S. aureus for the hydroalcohol extract. However, S. persica
showed a rather high MIC (43.75 mg/mL) against B. cereus
and S. aureus.
Against C. albicans, chloroform extracts was more active
than hydroalcohol extracts of the all plants (Table 5). The
lowest MIC was recorded for A. hierochuntica (0.07 mg/mL)
and C. siligua (0.125 mg/mL).

4. Discussion
The antimicrobial properties and the use of four plants
to fight against infectious diseases have been reported in
several studies. Methanolic extracts of A. hierochuntica, C.
siligua have antioxidant and antimicrobial properties[12-14].
Miswak acts as antibacterial agent. While studying the effect
of miswak pieces on bacteria in periodontitis and dental
caries, Chaurasia et al. concluded that the antibacterial
effect was most pronounced on Porphyromonas gingivalis,
Actinobacillus actinomycetemcomitans, and Haemophilus
influenzae, less than on Streptococcus mutans, and least
on Lactobacillus acidophilus[15]. They also reported that
the antibacterial effect of miswak suggests the presence of
volatile active antibacterial compounds. Whereas, in our
investigation, A. vera extract does not show any interesting
activity. These findings have also been mentioned in
literatures, especially that this plant is well known for its
poly saccharides and anthraquinone derivatives[16].
Finally, antimicrobial extracts from tested plants can be
assumed to be useful to the producing plant in warding off
infectious diseases and there is therefore a compelling
reason to suppose that anti–infective agents could be active
against human pathogens. The screening assays justify the
use of the investigated plants in the Algerian ethnomedicine.
The results of these screening investigations confirm the
great potential of plants of the Algerian ethnomedicine
for production of bioactive compounds and are useful for
rationalizing the use of medicinal plants in primary health
care. The results complement a major research endeavor
in establishing a relationship between the use of plants by
scientific communities and especially clinical knowledge of
the plant. The phytochemical characterization of the extracts,
the identification of responsible bioactive compounds and
quality standards are necessary.

Table 5
MICS (mg/mL) of the tested extracts against bacteria.

| Bacteria | C. siligua | S. persica | A. hierochuntica | A. vera |
|----------|------------|------------|------------------|--------|
|          | Chloroform | Hydroalcohol | Chloroform | Hydroalcohol | Chloroform | Hydroalcohol | Chloroform | Hydroalcohol |
| Gram– | A. baumanii | 0.250 | – | – | 43.75 | 11.25 | – | – | 8.00 | – |
| negative | C. freundi | 2.000 | 21.87 | 0.75 | 0.35 | 0.07 | 5.47 | – | 0.50 | 0.16 |
| E. cloacae | 0.130 | – | 1.50 | – | – | – | – | – | 4.29 | 0.50 |
| E. coli | 0.130 | 5.46 | – | 43.75 | – | – | – | – | 0.50 | 0.08 |
| K. pneumoniae | – | – | 0.05 | 0.35 | – | – | – | – | 34.38 | 8.00 |
| P. aeruginosa | 0.130 | 175.00 | 0.05 | 5.47 | – | 87.50 | 34.38 | 0.50 | 0.25 |
| Proteus mirabilis | – | – | – | – | – | – | – | – | 0.50 | 0.08 |
| S. typhimurium | – | – | – | – | – | – | – | – | 8.00 | – |
| Gram– | B. cereus | 1.000 | 0.70 | 43.75 | 43.75 | – | – | – | 0.50 | 0.64 |
| positive | E. faecalis | 0.130 | 5.46 | – | – | – | – | – | 16.00 | 0.25 |
| L. monocytogen | 5.000 | 87.50 | – | – | – | – | – | – | 8.00 | 0.25 |
| S. aureus | – | – | 0.13 | 43.75 | – | – | – | – | 0.50 | 0.25 |
| Yeasts | C. albicans ATCC 444 | 0.125 | 175.00 | 0.47 | 175.00 | 2.25 | 43.75 | – | – | – |
| C. albicans ATCC 10231 | 0.125 | 43.75 | – | 87.50 | – | – | – | – | 34.38 | – |
| C. albicans ATCC 20700 | 2.000 | 43.75 | – | 87.50 | 0.07 | 5.47 | – | – | – | 8 |

–: no MIC observed.
Conflict of interest statement

We declare that we have no conflict of interest.

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