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Chemical Characterization and Antibacterial Activity of Phases Obtained from Extracts of *Artemisia herba alba*, *Marrubium vulgare* and *Pinus pinaster*

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ABSTRACT

The objective of this study is the phytochemical characterization and antibacterial activity of *Artemisia herba alba*, *Marrubium vulgare* and *Pinus pinaster*. The antibacterial potential of different fractions of ethyl acetate, diethyl ether and 1 butanol extracts of the three plants were tested on four strains of bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. The results of phytochemical screening showed a rich and varied composition of secondary metabolites: flavonoids, tannins, terpenes, sterols, coumarins and saponins which have characterized all the crude extracts, while alkaloids particularize *Pinus pinaster*. This result was confirmed by analytical thin layer chromatography on some secondary metabolites such as flavonoids, tannins, terpenes and sterols of the three phases obtained after exhaustion of three plant extracts with organic solvents of increasing polarity. The TLC on polyamide gel revelation by NEU reagent, allowed to assume that the plants tested contain various flavonoids. The chromatographic analysis on silica plate has highlighted several fluorescences with the NEU reagent, reagent of Liebermann-Burchard and FeCl₃ suggesting the presence of flavonoids, terpenes, sterols and tannins. Spectral analysis of methanol extracts advance the predominance of natural flavonoids: Flavonol, Flavone, Flavanone, Flavanol or Anthocyanine. The antibacterial activity of different fractions performed on solid agar medium showed little or no effect; this implies that the antibacterial activity proved with crude extracts of these plants in previous studies is probably due to a synergistic action in chemical ingredients present in the extracts.

Keywords: *Artemisia herba helba*; *Pinus pinaster*; *Marrubium vulgare*; phases; antibacterial activity.

INTRODUCTION

Despite the efforts of chemists in the synthesis of new molecules, more than 25% of prescribed drugs in developed countries derive directly or indirectly from plants¹. However, as drug sources, plants remain underutilized especially in the field of medical microbiology². Certainly most prescribed antibiotics derived from microorganisms, but it is also clear that the plant antimicrobial agents have their place in the arsenal of drugs prescribed by clinicians³. The lifetime of each antibiotic is limited after which microorganisms are developing resistances. The phenomenon of bacterial resistance to antibiotics is due in large part to the massive antibiotic prescribing by doctors and maladministration. Also, the use of antibiotics in agriculture as a promising growth and preventing infection is suspected of contributing to the development of resistant strains not only in animals but also in human populations. These difficulties have aroused our interest in research other antibacterial substances that can be an alternative against antibiotic resistance. The aim of the present study is to evaluate the antibacterial activity and to investigate

chemical composition of three medicinal plants growing wild in Algeria. The work has concerned bark of *Pinus pinaster* (pinaceae), flowering tops of *Marrubium vulgare* (lamiaceae) and aerial parts of *Artemisia herba alba* (Asteraceae).

MATERIALS AND METHODS

Preparation of plant material

Harvested plants (*Artemisia herba Alba*, *Marrubium vulgare*, *Pinus pinaster*) were sprayed and dried just before the extraction, using a knife mill. The extraction of the powders obtained (30g) was made by cold soaking in a hydroalcoholic solution of 70% ethanol (1: 5, v / v), stirred on a magnetic plate (700 r / min for 30 min). The solutions were then clarified by filtration on Whatman paper, concentrated by vacuum evaporation of alcohol and lyophilized to provide dry crude extracts. These extracts were kept in low temperature (-10 ° C). Phytochemical studies and biological activities of various organs (aerial part of AHA, flowering tops of MUV and PPI bark) were performed in the laboratory for the production of therapeutic substances (LOST) of the Faculty of Exact

Sciences, laboratory of micro-molecular biochemistry and phytochemistry of the Faculty of natural Sciences and Life, University of Constantine, the microbiology laboratory of the Constantine CHU, and microbiological engineering and applications laboratory of Constantine University 1.

Preparation and analysis of phytochemical phases

The chemical tests focused on alcoholic extracts of the aerial parts of AHA, luminaries flowered of MUV and bark of PPI. The extraction was made according to the method described by Merghem (1995)⁴. Fractionation of the hydroalcoholic extracts was conducted by successively using organic solvents of increasing polarity: Petroleum ether, diethyl ether, acetate ethyl and 1. butanol to obtain four fractions: diethyl ether (DE), ethyl acetate (EA), the fraction 1. butanol (1.B) and the aqueous fraction (AQ). The extracts were subjected to various phytochemical tests (thin layer chromatography, UV-Visible spectro photometry, chromatography and high performance liquid chromatography) to highlight the secondary metabolites of most biological activities of each plant species. These tests were conducted according to the procedures described⁵.

Determination of antibacterial activity

The antibacterial activity of the obtained phases (ethyl acetate, diethyl ether and 1.butanol) was tested *in vitro* by the agar diffusion method ⁶. Three bacterial clinically isolates were tested: *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*.

RESULTS AND DISCUSSION

Characterization of the main chemical constituents

The result of the phytochemical screening is summarized in table 1 and figures (1,2,3). The "+" indicates the presence of the group of chemical compounds in quantities greater than the detection limit, and the sign "-" means a negative reaction. The present study allowed us to identify different chemical groups present in the bark of PPI, flowering tops of MUV and aerial parts of AHA through characterization of reactions that have been confirmed by thin layer chromatography. Three bioactive groups are identified in all the extracted: sterols and triterpenes, flavonoids and tannins, while *Marrubium vulgare* and *Artemisia herba alba* lack of coumarins and saponins. However, alkaloids are disclosed only in the extract of *Pinus pinaster*.

Chemical studies on species of *Artemisia* indicate that all classes of compounds are present in the genre with particular reference to terpenes and flavonoids according to Wright (2002) ⁷. In our case, we have not detected the presence of saponins and alkaloids. However Sellami et al. ⁸ confirm the presence of alkaloids. The molecules identified in AHA are sesquiterpene lactones, coumarins and acetylenes ⁹. The results of the current study would probably be due to the choice of solvent and the extraction mode. Alkaloids proved only in crude extracts of PPI. Stermitz et al. (1994) ¹⁰ identified their presence in several pine species. A wide variety of terpenes has been identified in the genus *Pinus* ¹¹⁻¹³. The main tannins found in pines, particularly studied from maritime pine (*Pinus maritima* L.) are oligomers of two to seven units of flavan-3-ol. ¹⁴ Phenolic compounds found in the pines are varied

¹⁵. Phytochemical Studies have indicated that many species of *Marrubium* included the presence of flavonoids and phenylethanoidess, diterpenoids, phenolic compounds and essential oil ^{16, 17}. Polyphenols (total phenols, flavonoids, condensed and hydrolysable tannins) were quantified in different organs of *Marrubium vulgare* from Mount Tessala (western Algeria) which relate to our work ¹⁸.

UV spectra of the four phases (figure 3) for the PPI extract conclude to the predominance of natural flavonoids flavonol, flavone, flavanone, Flavanol or Anthocyan. Regarding AHA extract, the results indicate the predominance of Flavone and flavanone type of flavonoids and dihydroflavanol in diethyl ether phase, In Phase Water predominance of flavonoid type flavanone and dihydroflavanol. Other phases of ethyl acetate and 1-butanol can advance the predominance of type flavone flavonoids, and flavanone dihydroflavanol or Isoflavone. The chemical screening on TLC showed relatively few classes of compounds. The revelation iron chloride showed the systematic presence of tannins ¹⁹. The TLC on polyamide gel revelation by NEU reagent, allowed us to assume that the plants (AHA MUV, PPI) contain different flavonoids, as well as acids phenolic. We note in this qualitative analysis that the phases (ED, AE and 1B) of the aerial parts of *Artemisia herba alba* are the richest of total flavonoids, terpenes and sterols and tannins followed by flowered parts of *Marrubium vulgare* and finally the bark of *Pinus pinaster*. Maimoona et al. ²⁰ reported that usually most of flavones and flavonols are obtained in the dichloromethane fractions and ethyl acetate extract of pine because of their polar nature. The presence of flavonoids and phenolic simple as the phenolic acid in various species of pine has been variously reported ²¹⁻²³.

Table 1: Results of the qualitative phytochemical screening of hydroalcoholic extracts of different plant species.

Content	<i>Marrubium vulgare</i>	<i>Pinus pinaster</i>	<i>Artemisia herba alba</i>
Tanins	+	+	+
Sterols & terpenoids	+	+	+
Coumarins	-	+	+
Flavonoids	+	+	+
Alkaloids	-	+	-
Saponins	+	+	-

+ : presence, - : absence.

Antibacterial activity

The results registered in table 2 showed a low activity for most of our samples on most bacterial strains (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* (diameter of inhibition <12 mm)). *Marrubium vulgare* reveals activity on *Escherichia coli* on the ethyl acetate phase and on the diethyl ether and 1 butanol phases against *Pseudomonas aeruginosa*. However, the three phases of the three extracts showed no activity against *Proteus vulgaris*. *Artemisia herba alba* Asso is widely used in the Algerian folk medicine. The phenolic compounds present in the plant could be candidates for some of its biological activities and therefore for its therapeutic use. According to Seddik et al.

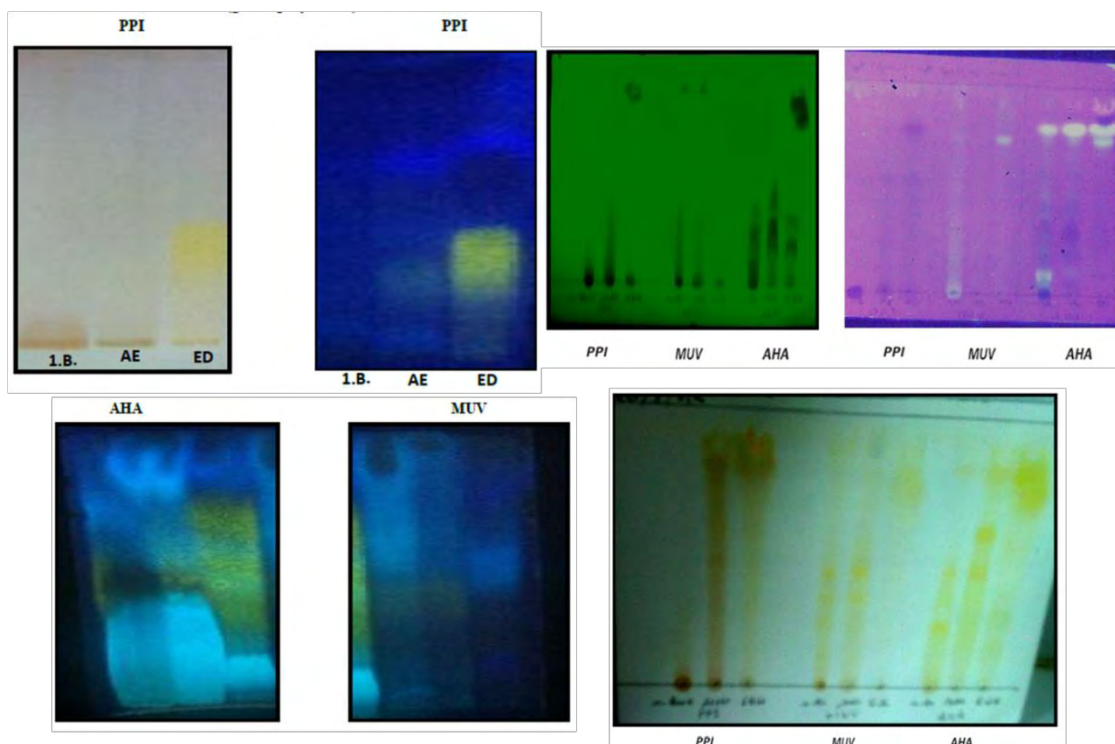


Figure 1 : Analytical TLC of the different plant extracts. AHA: *Artemisia herba alba*, MUV: *Marrubium vulgare*; PPI: *Pinus Pinaster*: DE : diethyl ether; EA : ethyl acetate ; 1B : 1 butanol.

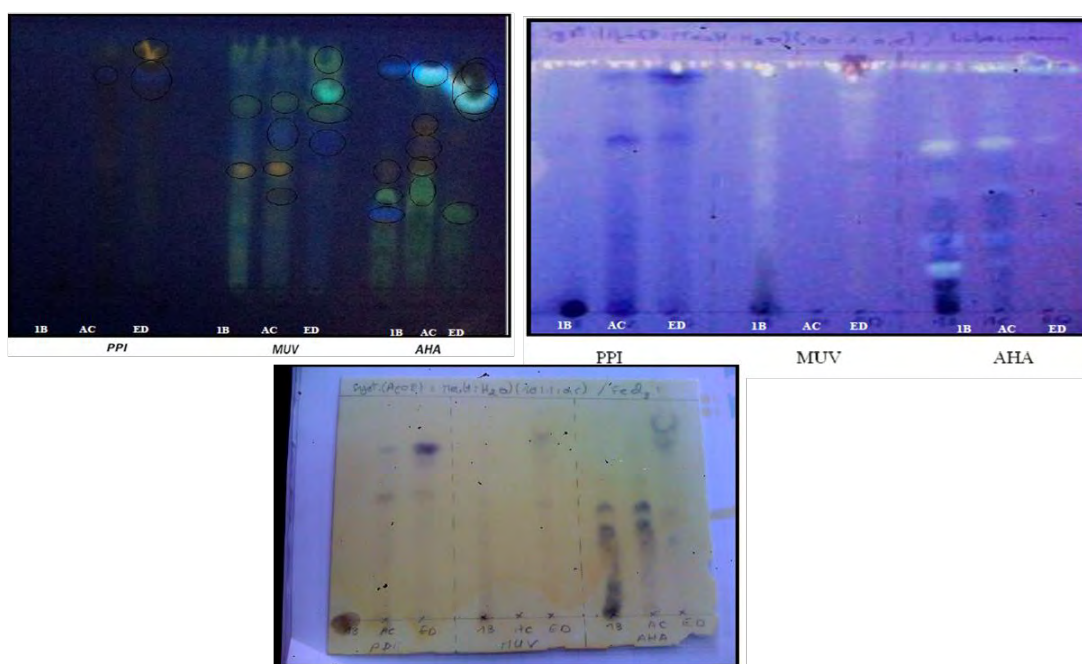


Figure 2: Chromatograms of different phases. AHA: *Artemisia herba alba*, MUV: *Marrubium vulgare*; PPI : *Pinus Pinaster*: DE : diethyl ether; EA : ethyl acetate ;1B : 1 butanol.

²⁴, high levels of flavonoids (apigenin and luteolin) are present in the ethyl acetate phase in addition to the phenolic acids (protocatechic acid, caffeic acid, gallic acid derivatives and ferulic acid). The aqueous phase contains small amounts of phenolic acids and the chloroform phase contains phenolic acids and flavonoids aglycone. The antibacterial activity of extracts of *Artemisia* and phenolic compounds was also evaluated against some bacterial strains.

This extract contains a large amount of phenolic compounds. This is consistent with our results for the phases of ethyl acetate, diethyl ether and 1 butanol of *Artemisia herba alba* against *Pseudomonas aeruginosa* and diethyl ether phase against *staphylococcus aureus*. In the study of Aljebouri ²⁵, the *Artemisia* methanolic phase was most effective against Gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, followed by chloroform extract, acetone and finally the aqueous

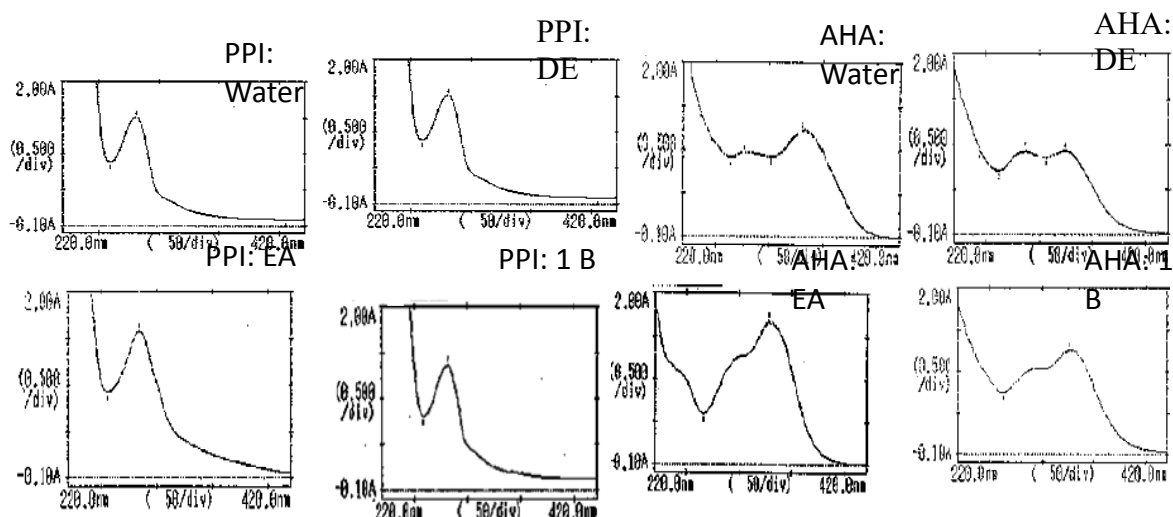


Figure 3: UV absorption specters of the different plant extracts. AHA: *Artemisia herba alba*, MUV: *Marrubium vulgare*; PPI : *Pinus Pinaster*: DE : diethyl ether; EA : ethyl acetate ;1B : 1 butanol.

Table 2: Antibacterial activity of different phases on tested strains.

		DE	EA	1B
PPI	<i>P. aeruginosa</i>	-	+	+
	<i>E. coli</i>	-	-	-
	<i>S. aureus</i>	+	+	+
	<i>P. vulgaris</i>	-	-	-
MUV	<i>P. aeruginosa</i>	+	-	+
	<i>E. coli</i>	-	+	-
	<i>S. aureus</i>	-	-	-
	<i>P. vulgaris</i>	-	-	-
AHA	<i>P. aeruginosa</i>	+	+	+
	<i>E. coli</i>	-	-	-
	<i>S. aureus</i>	-	-	-
	<i>P. vulgaris</i>	-	-	-

AHA: *Artemisia herba alba*, MUV: *Marrubium vulgare*; PPI : *Pinus pinaster*; + : low activity ; - : no activity ; DE : diethyl ether; EA : ethyl acetate ;1B : 1 butanol.

extract. This result is in agreement with Seddik et al.²⁴ which have shown that aqueous extracts of *Artemisia herba alba* had low antibacterial activity against *Escherichia coli*.

Generally, the low or the absence of *in vitro* antibacterial activities of the isolated molecules or fractions suggest a possible synergy between these compounds which were active in the total crude extracts as reported elsewhere²⁶. This is the case of the three tested plants *Pinus pinaster*, *Marrubium vulgare* and *Artemisia herba alba* which showed a significant antibacterial activity when tested with crude total extracts against the same bacterial strains. Several hypotheses can be made to justify the lack of response in the above *in vitro* tests. A) Traditional medicine uses the juice of the leaves, whose chemical composition is impossible to reproduce experimentally with solvents and possible associations and synergies of compounds that can take place naturally. B) The fresh plant is used in traditional medicine, in this study, for conservation reasons, we have chosen to dry the plants.

This preliminary drying step can cause chemical changes of the compounds and cause the loss of the activity. C) The tested concentrations are not sufficient to show activity. D) The non-polar active ingredients are only partially soluble in the liquid test medium; it is not excluded that the concentrations required for the activity of a relatively non-polar compounds have been non-sufficient. This is one of the limits of this test.

Conclusion

The phytochemical study of the three tested plants *Artemisia herba alba*, *Pinus pinaster* and *Marrubium vulgare* revealed a wealth of secondary metabolites such as flavonoids, tannins, terpenes and sterols, coumarins, saponins and alkaloids that distinguishes *P. pinaster*. The antibacterial activity of different fractions showed little or no effect; this implies that the antibacterial activity demonstrated in other studies with crude extracts of these plants is probably due to a synergistic action in chemical ingredients present in the extracts.

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