J. Mater. Environ. Sci. 7 (8) (2016) 2938-2946
ISSN : 2028-2508
CODEN: JMESC

Phytochemical Screening, Antioxidant and Antibacterial activity of *Lepidium sativum* seeds from Morocco

K. Chatoui[1], A. Talbaoui[2], M. Aneb[2], Y. Bakri[2], H. Harhar[3]* and M. Tabyaoui[1]*

[1] *Laboratory of Materials, Nanoparticles and Environment, department of Chemistry, Faculty of Science, Mohammed V University. Rabat, Morocco.*

[2]*Laboratory of Biochemistry and Immunology, Faculty of Science, Rabat, Mohammed V University, Morocco.*

[3] *Laboratoire de Chimie des Plantes et de Synthèse Organique et Bioorganique, Faculté des Sciences, Université Mohammed V, Rabat, Morocco.*

Received 26 Mar 2016, Revised 24 May 2016, Accepted 02 Jun 2016
**Corresponding author. E-mail: hichamoo79@yahoo.fr / tabyaouihamid@gmail.com; Phone: +212668799942*

Abstract
Methanol and ethyl acetate extract of the seeds of *Lepidium sativum* was preliminary screened with the aim of assessing the availability of some biologically active compounds. The prepared extract was used for the phytochemical screening study which was carried out using standard methods. The phytochemicals screened from the Methanol extract of *Lepidium sativum* showed positive result for flavonoids, Saponoside, tannins, Alkaloid, Stereol and Polyterpene compounds. But ethyl acetate extract showed positive result only for Saponoside and Alkaloid. The evaluation of the antimicrobial activity showed that the methanol and ethyl acetate extracts are most active on the whole of the bacteria tested and revealed a significant antibacterial activity against *Rhodococcus equi*. With regards to IC50 values (50% inhibitory concentration) of scavenging abilities of the DPPH radical, methanol extract exhibited important antioxidant activities with IC50 of 925.22±0.02 ppm. These results can be considered very promising and justify further research, amongst others on the identification of antioxidant and antibacterial components in the active extracts.

Keywords: *Lepidium sativum*; Seeds; Methanol extract; Antioxidant; Antibacterial.

1. Introduction
Medicinal plants have been used from ancient time for their medicinal values. Nowadays, the crude extracts samples from medicinal plants have been shown interest for the development and preparation of alternative traditional medicine [1, 2]. Plants are the best sources for chemical ingredients or phytochemical agents for cure of different diseases. Medicinal plants are an inexhaustible source of molecules with very different biological and pharmacological activities. For this reason, we chose *Lepidium sativum* from medicinal plants because it has not been studied in Morocco, although relatively abundant and widely used in traditional medicine.

Cress (*Lepidium sativum L.*), otherwise known as garden cress, garden cress pepper weed or garden pepperwort, in Morocco is called hab rchad. It's a fast growing annual herb belonging to the Brassicaceae family that is native to Egypt and west Asia but is widely cultivated in temperate climates throughout the world for various culinary and medicinal uses [3].

Seeds contain 27% of protein, 14-26% of lipids, 35-54% of carbohydrates and 8% of crude fiber [4]. The carbohydrates of the *L. sativum* seeds comprise of 90% non-starch polysaccharides and 10% of starch. Cress Seed can be used as a rich source of dietary fiber [3]. *L. sativum* seed contains 20-25% of oil and the main fatty acid is linolenic acid (32-35%) It also contains natural antioxidants (tocopherols and carotenoids) which protect the oil from rancidity. Imidazole alkaloids, lepidine, monomeric alkaloids, sinapic acid and sinapin were reported in

seeds of *L. sativum* [5]. α-tocopherol and β-sitosterol were reported in the unsaponifiable matter of the Cress seeds [6]. It have been reported that phytochemicals which are considered as secondary metabolites components are directly responsible for activity such as antioxidant, antimicrobial, antifungal, anticancer, anti-inflammatory among others [7]. Therefore, screening of chemical constituents in medicinal plants in order to assess for their availability may provide new useful information to the scientific community and in claiming for their therapeutic efficacies. Antibiotic resistance by pathogenic microorganisms (bacteria, viruses, fungi) has become a serious problem because these microbial contaminations still affect public health. [8] Hence the interest in antioxidants (non-toxic) and natural antimicrobials, especially of plant origin, has increased significantly in recent years.
This study therefore aimed at screening chemical constituents of *Lepidium sativum L.*, seeds sample from south west of Morocco in order to provide vital information on their availability. The current study also focuses on the possibility of using *Lepidium sativum* seed as source of low-cost natural antioxidant and antibacterial.

2. **Experimental**

2.1 *Plant material and chemicals*

Cultivated *Lepidium sativum* seeds were collected from Tafraout city, in the souss Massa region (South west of Morocco) (29°44'16.4"N 8°58'16.9"W), Seeds were harvested in June 2014. After harvest, the seeds were sundried and stored at 4 °C until processed.
All the reagents and chemicals were of analytical or HPLC grade from different mark (Loba chimie ; VWR chemicals ; Sigma-Aldrich ; Panreac ; Riedel-de Haen ; Sharlau ; Fluka ; Merck ; SDFCL. They were purchased from Professional Labo (Casablanca, Morocco).

2.2 *Preparation of the crude extract*

The Methanolic, Ethyl acetate and Petroleum ether extracts are prepared following the process described [9]; 150 g of cress seeds were collected, dried in the oven at 70 °C for 4 h and reduced to powder. It was separately macerated with the solvents and allowed to stand for 72 hrs and then filtered. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw capped bottles at 5°C in the refrigerator, until when required for use.

2.3 *Phytochemical screening of Lepidium sativum Linn*

Chemical tests for the screening and identification of bioactive chemical constituents in the Cress seeds were carried with extracts prepared using the standard procedures.

2.3.1 *Flavonoids* [10]

0.5 g of various extract was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests: 5 ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H_2SO_4. The appearance of the yellow coloration indicated the presence of flavonoids.

2.3.2 *Sterols, polyterpenes* [11]

Using LIEBERMANN reagent allows identifying these compounds, Blue-green ring between layers indicates the presence of steroids and pink- purple ring indicates the presence of terpenes.

2.3.3 *Polyphenols* [10]

To 1 ml of various solvent extracts of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green color indicated the presence of phenols.

2.3.4 *Tannins* [12]

Search for catechin tannins is made from reagent Stiasny. 5 ml of each extract were evaporated to dryness. After adding 15 ml of reagent Stiasny the residue, the mixture was kept in a water bath at 80°C for 30 min. The observation of a precipitate in large flakes characterized catechin tannins.

For gallic tannins, we filtered the previous solution. The filtrate was collected and saturated with sodium acetate. The addition of Fecl3drops causes the appearance of a blue-black coloration intense, indicating the presence of gallic tannins.

2.3.5 *Alkaloids* [13]
Alkaloids were characterized from Bouchardat reagent (reagent iodo-iodized) and Dragendorff (reagent iodobismuthate of potassium). 6 ml of each solution were evaporated to dryness. The residue is taken up in 6 ml alcohol at 60 °. The addition of 2 drops of reagent Dragendorff on the alcoholic solution caused a precipitate or orange color. Adding 2 drops of Bouchardat reagent on the alcoholic solution caused a color precipitate reddish brown and indicated a positive reaction.

2.3.6 Saponosides[14]
To find saponins, we contributed in a test tube, 10 ml aqueous total extract. The tube was shaken for 15 s and allowed to stand for 15 min. A height of persistent foam greater than 1 cm indicated the presence of saponins.

2.4 Determination of the antioxidant activity
Trapping free radical DPPH assay was conducted to evaluate the antioxidant activity. This test measures the ability of eliminating free radicals in Methanolic extract. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant that can donate an electron to the DPPH, the purple color, typical free radical DPPH to disintegrate, the mixture was kept in the dark for 30 minutes and (OD) was recorded at 517 nm against a control sample [15].
0.5 ml of Methanolic extract of the test solutions at different concentrations (400, 800, 1200, 1600, 2000 ppm) were mixed with 2.5ml of a methanolic solution of 0.1 mM DPPH. The absorbance is measured by UV / VIS device Spectrometer Lambda 25. The percentage inhibition of the DPPH is calculated by the formula as follows [16].
The standard calibration curve is α-tocopherol (Vitamin E).

$$\% I = (Ac - As) / As$$

Where Ac = absorbance of control and As = absorbance of sample.

2.4 Determination of antimicrobial activity
2.4.1 Microorganism used
The strain used in this work is: *Rhodococcus equi* (GK1: CIP 105335), *R equi* is a bacterium bacillus Gram-positive, can infect humans. Risk groups are immunocompromised individuals such as HIV-AIDS patients or transplant recipients. Infection in these patients resembles the clinical and pathological signs of pulmonary tuberculosis. *R equi* is from the laboratory of Biochemistry and Immunology of the Faculty of Sciences Rabat.

2.4.2 Bacterial culture
From a new culture, we realize a bacterial suspension incubated at 30°C 24 hours at stove. This solution represents the bacterial inoculum which will be used throughout the study.

2.4.3 Realization of antibacterial test by the gel diffusion method
The tests of antibacterial activity, we used the agar diffusion technique Trypto Soybean casein (TSA), Disk of the mark (PRAT DUMAS France), Referenced "A006242-1000 UNITS", 6mm diameter, were placed in screw vials, sterilized in an autoclave at a temperature 121 °C for 20 minutes.
The media are seeded with a few milliliters of the inoculum so as to cover the entire agar surface. The tests were performed according to the method of Vincent aromatogram [17].which consists in depositing impregnated filter paper discs of extracts on the surface of agar in petri dishes previously seeded. The plates are then incubated for 48 hours in the oven hang.

The biological activity manifests itself by the appearance of a microbial growth inhibition halo around the discs containing the test sample. The reading is performed by measuring the diameters of inhibition observed [18, 19].

2.5 Bactericidal activity and cell lysis

The germ (*Rhodococcus equi*) was grown in nutrient broth (TSA) incubated for 24 hours at a temperature of 30 °C. Series of liquid culture medium were prepared, one of them containing 200 µl of viable bacteria inoculated, we added the Ethyl acetate extract of the plant studied and these mixtures were stirred and incubated at temperature of 30 °C, (Ethyl acetate extracts used are diluted in 1% DMSO) the reading OD of each culture to design the bacterial growth, we take 2.5 ml of sample louse measured at a wave length of 600 nm of each point at intervals of eight hours incubation time throughout experience, but to detect genetic press material we centrifuged 1 ml of each tube 1200 g for 5 minutes to remove all traces of bacteria. 250 µl of the supernatant was resuspended with 750 µl PBS to measure the absorption at 260 nm UV spectrophotometer lamspec / visible from each time point. We used untreated as negative bacteria and bacterial control treated with penicillin-streptomycin (PS) (100 µl / ml-100 mg / ml) as a positive control [20, 21, 22].

3. Results and discussion

3.1. *Phytochemical analysis*

According to the percentage of Yield, the highest yield was observed in methanol extract (34.2%) followed by ethyl acetate extract (19.1%) and finally the petroleum ether extract (14.2%).

Phytochemical constituents in the plant samples are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer. [23]. All secondary metabolite components displayed antioxidant and antimicrobial properties through different biological mechanisms. Most of the secondary metabolite components were isolated and identified in the polar plant crude extracts [24]. The main chemical groups identified in petroleum ether, Ethyl acetate and methanolic crude extracts of the *Lepidium sativum* seeds are mentioned in the Table 1.

Table 1: Phytochemical screening of the extract of *Lepidium sativum* seeds

Identified chemicals groups		Extract of the *Lepidium sativum* seeds		
		Methanol	Ethyl acetate	Petroleum ether
Flavonoid		+	+	-
Alkaloid	Mayer	+++	++	++
	Bouchardat	+++	++	++
	Dragendorff	++	++	++
Stereol and polyterpenes		+	-	-
Tannin	Catechic	++	-	-
	Gallic	black Coloring (+)	-	-
Saponiside		+	+	+

Legends: +: presence. - : none detected.

Phytochemical screening of the methanolic, petroleum ether and ethyl acetate extracts of *LS* seed revealed the presence of various medically active constituents. The phytochemical compounds present in the methanolic extract were identified as alkaloids, saponins, sterols, tannins, flavonoids and terpenoids (Table-1). Saponins and alkaloid were also present in ethyl acetate and petroleum ether.

The presence of these chemical constituents in the seeds of *L.S* demonstrates to their antibacterial activity. These phytochemicals are known to show medicinal as well as physiological activity [25]. The occurrence of alkaloids, saponins, sterols, tannins, flavonoids, terpenoids in the methaniloc extracts of *LS* was also reported earlier by George et al., [26].
The detected different bioactive compounds in different extracts of *LS* may be responsible for the antioxidant and antibacterial activities. Several reports are available on flavonoid groups which exhibited high potential biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-allergic reactions [27; 28; 29; 30]. Saponins are also bioactive constituent which involved in plant defense system because of their antimicrobial activity [31]. Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers [31;-35].

3.2. *Antioxidant activity*
The antioxidant activity through free radical scavenging activity (DPPH) method of different extracts of *LS* was determined (Figs. 1 and 2). The principle of antioxidant activity is their interaction to produce oxidative free radicals. The role of DPPH method is that the antioxidants react with the stable free radical. During the free radical reaction, DPPH (a,a-diphenyl-b-picrylhydrazyl) is converted into a,a-diphenyl-b-picrylhydrazine with color change. The rate of color change gradually decreases to indicate the scavenging potentials of the sample antioxidant. The IC50 values of the Methanol extracts of *Lepidium sativum* was 925.22±0.02 ppm whereas the α-tocopherol (standard) were found to be 50.04 ppm. The Methanol extracts of *LS* contain flavonoid, saponins, tannins, alkaloid and Terpenoid. All these bioactive compounds were able to discolor DPPH solution by their hydrogen donating ability [31- 36]. From the results it appears that the Methanol extracts of *LS* possess hydrogen donating capabilities and it will act as an antioxidant.

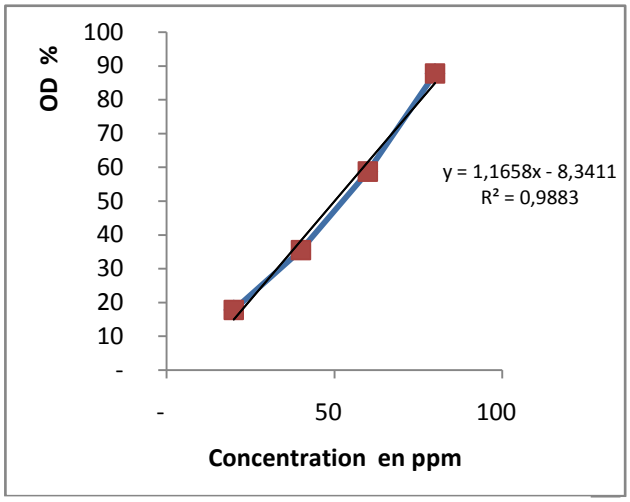

Figure 1: The percentage inhibition of the free radical DPPH by the antioxidant Vitamin E.

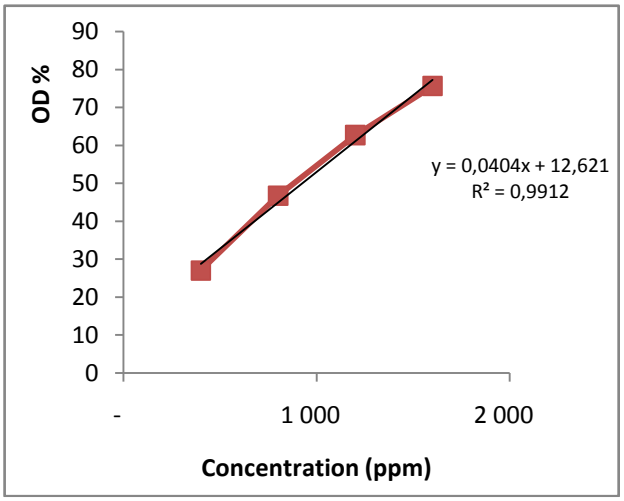

Figure 2: The % inhibition of the free radical DPPH by MeOH extract of *Lepidium sativum* seeds.

The scavenging activity might be due to the presence of total polyphenolic compounds. These polyphenolic compounds include flavonoids, anthraquinones, anthocyanidins, xanthones and tannins [37]. These compounds have been reported to scavenge free radicals, superoxide and hydroxyl radical by single electron transfer [38].
Our result was higher than the finding of Aydemir and Becerik [39] (IC50 value 318.91 ppm) for the Turkish *Lepidium sativum,* In comparison with other Moroccan seeds, the IC50 value of *Opuntia ficus indica* and *Opuntia*

dillenii seed oil (IC50 = 19.79 ± 0.023 and 27.21 ± 0.075 ppm) was lower than our finding for *Lepidium sativum*[40].

3.2. *Biological activity*

3.2.1. *Antibacterial test*

This study of the biological activity of extracts of *Lepidium sativum* seeds has been tested against the *Rhodococcus equi* germ for the evaluation of anti-bacterial activity by the middle agar diffusion method. Allowed us to measure the diameters of the inhibition zones that are located between 7.5-16 mm, in particular for the Ethyl acetate (15.55 mm), (Methanol extracts (13.15 mm) and Petroleum ether extracts have a (10.35 mm)). Similarly reference antibiotics have an active effect on the seed, showing inhibition (30.00 and 34.00 mm), the inhibition is more important for Ethyl acetate extract near the antibiotic doxycycline and inhibition is remarkable for the methanol extract (Figure 3).

Indeed, the plant studied contains antibacterial principles including alkaloids, saponosides and other components which are with a large antibacterial well documented in the literature [41].

On the other hand, the 1% DMSO was applied as negative control test and has no zone of inhibition (Figure 3).

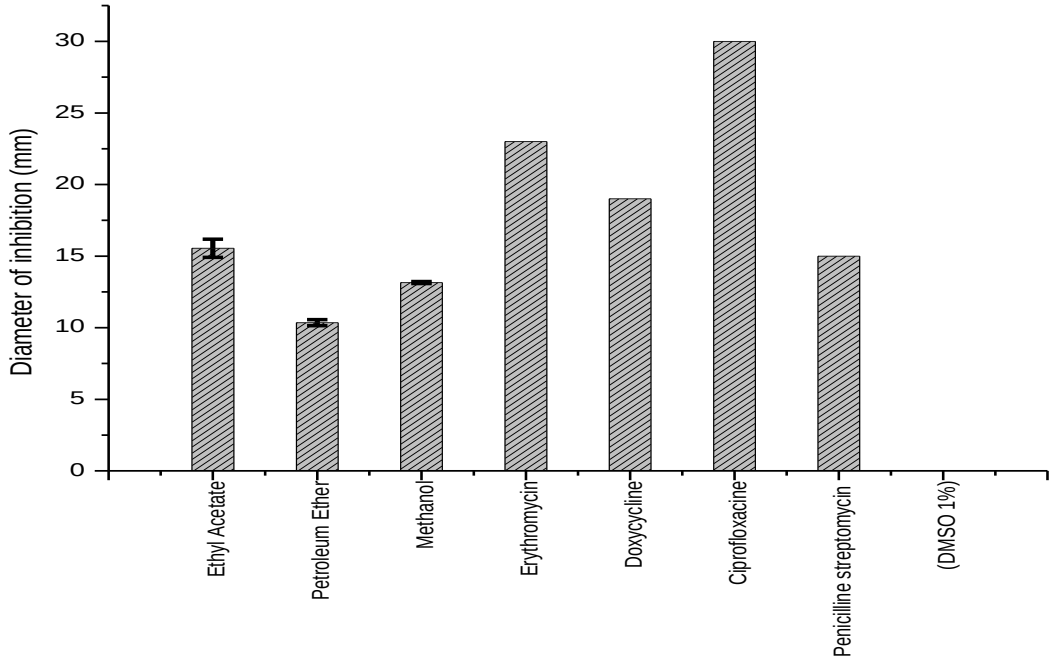

Figure 3: The zones of inhibition (*R eq*) in mm extracts by comparison with reference antibiotics.

Table 2: Zones of inhibition (*R eq*) in mm extracts by comparison with reference antibiotics.

Extract	**inhibition of average diameter in mm**	Antibiotic	**inhibition diameter in mm**
Ethyl Acetate	**15.55 ± 0,6**	Erythromycin	**23.00**
Petroleum Ether	**10.35 ± 0.2**	Ciprofloxacine	**30.00**
Methanol	**13.15 ± 0.07**	Chlorophénicol	**34.00**
		Penicilline streptomycin	**15.00**

Comparisons of the current finding with literature were showed similar result. In vitro disc diffusion method depicted that the methanol extract of seeds of *L. sativium* obtained from Sudan has potent antibacterial activities against *S. aureus*, *E. coli*, *Klebsiella pneumonae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* which was assessed at the concentrations of 2.5, 5 and 10%. For Ethiopian seeds, it revealed that the crude extract of seeds of *L. sativium* have a good inhibition zoon against *S. aureus*, *B. subtillis* and *E. coli*.

3.2.2 *Bactericidal test*

The mode action of the Ethyl acetate extract to the bacterial (*Rhodococcus equi*). The penicillin-streptomycin germ comparison was determined using a 20 µl to concentration of the (EA) extract against 200µl (*R eq*) treated showed growth arrest during the experiment. We read (OD) at 600 nm and observed the curve with the germ (*R.eq*) showed high sensitivity to all of the (EA) extract more than 50% growth inhibition in the presence of the (EA) extract compared to untreated bacteria and it's the same curve positive control by using *PS* (Figure 4).
Bacteriostats limit the growth of bacteria by interacting with the protein synthesis, the DNA replication contributing bacterial multiplication. [42].

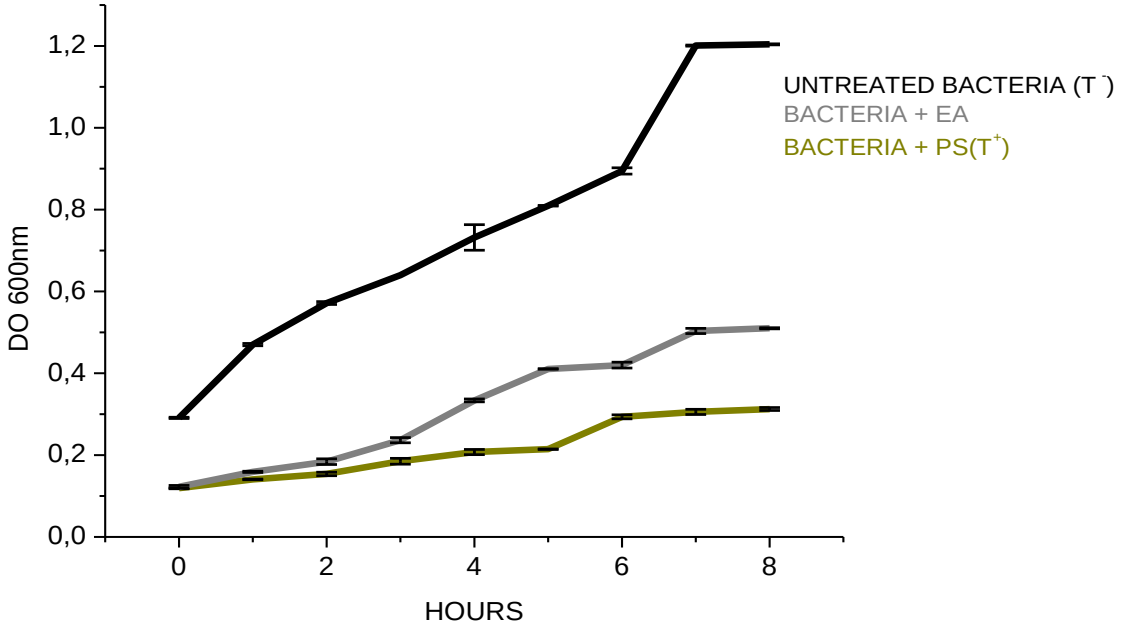

Figure 4: The action of the EA extract and PS on *Rhodococcus.equi* compared to the untreated one.

In addition, a dispersion of the genetic material indicates a deterioration of the bacterial cytoplasmic membrane. Therefore, the integrity of the bacterial cell membrane was examined by analyzing the absorbance at 260 nm (DNA and RNA) by infrared spectrophotometry. The study of reading the cultures treated with the (EA) extract showed a significant leakage compared to untreated bacteria (Figure 5). Similarly with respect to penicillin-streptomycin, to (EA) extract revealed a considerable damage to the bacterial membrane (Figure 5) indicating a violation of the membrane.

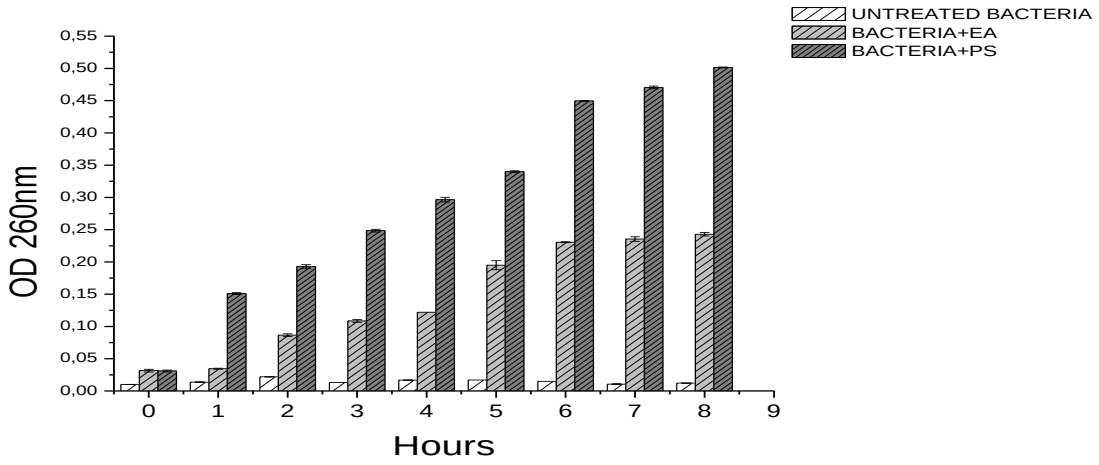

Figure 5: The *Rhodococcus equi* untreated and treated by the EA extract or PS.

Conclusions

In this study, we found that *Lepidium sativum* extracts possess antioxidant and antibacterial activities. The results revealed that Methanol extracts had significant antioxidant. Both extracts (Ethyl acetate and methanol) had antibacterial activity against *Rhodococcus equi*. The antioxidant and antimicrobial activities could be attributed to the flavonoid and tannin identified using Chemical tests. Therefore, *Lepidium sativum* could be an excellent source for natural antioxidant and antibacterial agents for medical and nutraceutical applications. This investigation thus provides a scientific basis for the use of the plant extracts in homemade remedies and their potential use in the treatment of microbial-induced ailments. Further studies may lead to their use as safe alternatives to synthetic antimicrobial drugs.

References

1. H. Baydar, O. Sagdic, G. Ozkan, T. Karadoganatureja. *Food Control*. 15 (2004) 169-172.
2. M.C. Rota, A Herrera, R.M. Martinez, et al. *Food Control*. 19 (2008) 681-687.
3. Gokavi, S. S.; Malleshi, N. G. and Guo, M. *Plant. Food. Hum. Nutr*. 59 (2004)105–111.
4.Mathews et al..*Die Nahrung*. 37(1993)1, 69–71.
5. Maier et al. (2002). Indian Patent No.242/DEL.
6. Lee et al.,*Comp Rev Food Sci Food Safety*, 3 (2004) 1, 21-33.
7. KC. Kakate. 4th ed. Delhi: VallabhPrakashan; Practical pharmacognosy;(1997) 218.
8. Edziri H, Mastouri M, Aouni M, et al. *South Afr J Botany* 80(2012)104–9
9. Williamson, E.M, Okpako, D.T, Evans, F.J,. John Wiley & Sons, Chichester. (1998) 15–23.
10. Ronchetti F. and Russo G. *Phytochem*.; 10(1971) 1385-1388.
11. Harborne JB. Methods of plant analysis. In: Phytochemical Methods (Chapman and Hall, London); 1973.
12. Hegnauer R. Chemotaxonomie der Pflanzen, Bikhäuser Verlag, Basel, Suttgart.6,(1973) 761.
13. Wagner H.Drogenanalyse. Springer Verlag Berlin Heidelberg New York, 1983; 522 pp.
14. Békro Y. A., Békro J. A. M., Boua B. B., Tra B. F. H.and Ehilé E. E.*Rev. Sci. Nat*.4 (2) (2007) 217-225.
15. Marque-Williams W, Cuvelier ME, Berset C. *Food Sci.Technol*. 28(1995) 25-30.
16. Sharififar F, M.H. Moshafi, S.H Mansouri, M.Khodashenas, M, Khoshnoodi, *Food Control*. 18(2007)800–805

17. Cavallo J.D, H. Chardon, C. Chadia, P. Choutet, P.Courvalin, H.Daberrat, H.Drugeon, L.Dubereuil, F.Goldstein, V.Jarvalier, Q.Leclerc, MH.Nicolas-Chamoine, A.Philipon, C.Quentir, B.Rouveix, J. Sirot, C.J. Soussy, *Société française de microbiologie* (2006).
18. Ponce.AG, Frittz.R, Valle D.Del, Roura S.I. *society of food science and technology*. 36(2003)679-684
19. Bidié A.P, Koffi E, N'guessan JD, Djaman A.J.&Guédé-Guina F, *Afr J Trad CAM*. 5 (2008) 294-301.
20. Canillac N, Mourey A. *Aliments Nutr*.13(1995)267-273.
21. Oumzil H, Ghoulami S, Rhajaoui M, Ilidrissi A, Fkih-Tetouani S, Faid M, Benjouad A. *Phytother. Res.* 16 (2002) 723-731.
22. Smith-Palmer A, Stewart J, Fyfe L. *Lett. Food Microbiol*. 26 (1998) 118-122.
23. Hossain, M.A., Nagooru, M.R., *Pharmacognosy Journal* 3 (24)(2011), 25–29.
24. Suresh, S.N., Nagarajan, N. *Journal of Basic & Applied Biology* 3 (1&2),(2009) 59–61
25. Sofowora A. Spectrum Books Ltd., Ibadan, Nigeria, (1993) 191-289.
26. R E George, S K Thomas, M Kunjumon and V Thankamani, *Int J Pharm Bio Sci*. 6(3)(2015) 490 - 497
27. Anyasor, G.N., Ogunwenmo, K.O., Oyelana, O.A., Akpofunure, B.E. *Afri J Biotech* 9 (31), (2010) 4880–4884.
28. Chao, P.D.L., Hsiu, S.L., Hou, Y.C. *Journal of Food Drug Analysis* 10 (4),(2002) 219–228
29. Igbinosa, O.O.,Igbinosa, E.O., Aiyegoro, O.A. *Afri J Pharma and Pharmaco*3 (2),(2009) 58–62.
30.Thitilertdecha, N., Teerawutgulrag, A., Rakariyatham, N., *Food SciTechnol*41, (2008) 2029–2035.
31. Barile, E., Bonanomi, G., Antignani, V., Zolfaghari, B., Ebrahim Sajjadi, S., Scala, F., Lanzotti, V. *Phytochem* 68 (2007) 596–603.
32. Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C., Atangbayila, T.O. *Trop J Pharma Res.*, 7 (3),(2008) 1019–1024
33. Akharaiyi, F.C. *Inter J of Pharma Technol Res* 3 (1),(2011) 478–483.
34. Varahalarao, V., Kaladhar, D.S.V.G.K. *Asian Pacific Journal of Tropical Disease*, (2012) 94–97
35. Sekar, D., Kolanjinathan, K., Saranraj, P., Gajendiran, K. *Inter J Pharma Biol Arch* 3 (5), (2012) 1231– 1235
36. Blois, M.S. *Nature* 4617 (1958) 1199–1200
37. E. Czapecka, A. Mareczek, M. Leja, *Food Chem*. 93 (2005) 223–226.
38. S.V. Jovanovic, S. Steenken, M. Tosic, B. Maryanovic, G. Simic, *J. Am. Chem. Soc*. 116 (1994) 4846–4851.
39. tülin aydemir1 and sedabecerik, *Journal of food biochemistry* 35 (2011) 62–79
40. Z. Ghazi, M. Ramdani, M. Tahri, R. Rmili, H. Elmsellem, B. El Mahi and M.L. Fauconnier, *J. Mater. Environ. Sci*. 6 (8) (2015) 2338-2345
41. Harikrishna D, A. V. N AppaRao, M.C Prabhakar. *Indian J Pharmacol*.36 (4)(2004) 244-250
42. Pankey GA, Sabath LD. *Clin. Infect. Dis*. 38(6) (2004) 864-870