World Journal of Environmental Biosciences

Available Online at: **www.environmentaljournals.org**

Volume 8, Issue 1: 54-62

ISSN 2277- 8047

Biocatalytic Preparative Methods of Asymmetric Alcohols using *Pergulariatomentosa* L.; Its antibactrial activity

Bilal Khaled[1,2],Lakhdar Sekhri[1]*, Mohammed Said Nedjimi[2], Abdelaali Atmani[1] Hamza Bensaci[1]

[1]Lab. Dynamic Interactions and Reactivity of Systems, Process Engineering Department, Faculty of Applied Sciences, University KasdiMerbah, Ouargla30000, Algeria.
[2]University EL oued Faculty of Sciences B.P 789, 39000 EL Oued, Algeria.

ABSTRACT

The present work is aimed mainly to investigate the bioreduction of acetophenone as preferred model substrate of simple ketones and aromatic ketones for asymmetric reaction and also aimed to compare the antibacterial activities of four extracts; Ethanol/H_2O, diethyl ether, butanol, and ethyl acetate extracts of Pergulariatomentosa on five bacteria strains: Escherichia coliATCC11303, Bacillus cereus ATCC10987, Bacillus cereus ATCC14579, Pseudomoasaeroginosa ATCC 27853, and Salmonello ATCC700623 using disc diffusion method. The results revealed that all extracts exhibited a certain bioactivity against all tested gram positive and gram negative bacteria at 1000 and 5000 µg/ml. The highest Inhibition rate of Bacillus cereus ATCC10987 is 16 mm at the concentration 5000 µg/ml in ethyl acetate extract. Moreover a moderate inhibition diameter of 6 mm with the lowest concentration of dichloromethane extract (600 µg/ml) was recorded against Pseudomoasaeroginosa ATCC 27853. The bioreduction of acetophenone with Pergulariatomentosa L.could be reduced to chiral 1-phenylethanol with attractive enantioselectivity. The results obtained in the present study suggest that the Pergulariatomentosa L., can be used in treating diseases caused by the tested organisms and provides a new route to produce chiral alcohols, as the platform chemicals for enantiomerically pure pharmaceuticals, through asymmetric reduction of the corresponding prochiral ketones.Further chemical and pharmacological investigations may be carried out to isolate and identify the chemical constituents in the selected plants responsible for the antimicrobial activity.

Keywords:*Bioreduction phytochemical analysis, Pergularietomentosa, salmonella, antibactrial activity*.

Corresponding author: Lakhdar Sekhri
e-mail✉ sekhril @ yahoo.fr
Received: 26 October 2018
Accepted: 29 March 2019

1. INTRODUCTION

Today there is an incessant and imperative necessity to find out new antibacterial compounds with various chemical structures and new mechanisms of action because there has been a shocking increase in the prevalence of new and reemerging contagious syndromes. This is why ethnobotany and ethnopharmacology are working to select of suitable extraction techniques (Tushar et al. 2017; Shivraj et al., 2017; Saha et al., 2017), and identify, all over the world, plants that are known to be active and for which it is up to modern research to specify the properties and validate their use (Ashakkumarand Ramaswamy, 2013), The search for new ones molecules must be undertaken within plant biodiversity using ethnopharmacological data. The medicinal plants have been used for ages as remedies for human diseases. In recent years there has been a flood of papers describing the synthesis and isolation of some natural products and study of their biological antimicrobial activities (Bensaci et al., 2016; Bassam et al., 2005; Sekhri et al., 2008; Babarbiet al., 2015;Thirupathaiah et al., 2008; Singh et al., 2007; Suresh Kumar et al., 2006). Most bacterial infections are treated with antibiotics, but at present time the natural herbal treatments (folk medicine) has spread dramatically without resorting to drugs and synthetic materials. However, due to the appearance of new strains of the bacteria and the weakness of chemotherapeutics and antibiotic resistance exhibited by pathogens has led to the screening of several medicinal plants for their potential antimicrobial activity (El-Hilaly et al., 2003). An increasing number of reports dealing with the evaluation of the antimicrobial effects of different extracts of various medicinal plants are frequently available (Babpour et al. 2009; Pelt et al., 2001; Babamer et al., 2012; Abu Zarga et al., 2013). However, due to the emergence of new strains of bacteria and weak chemotherapeutic agents and antibiotic resistance of pathogens, several medicinal plants have been tested for their potential antimicrobial activity (Ishrak et al., 2000; Benkeblia, 2004).

This has led us to focus more on the study of medicinal plants such as *Pergulariatomentosa L.*

Pergulariatomentosa L., Commonly known as Ghalka in Algeria, is perennial shrub about 50-60 cm high, reaching 1 m in good conditions (A guide (IUCN)., 2005;Biola et al., 1993; Doaigey, 1991). It is poisonous plant that is known to be distributed in the Saharian and Sub-Saharian countries of North Africa

(Shabana et al., 1990) including Algeria, Niger, and Egypt (Al-Farrajand Al-Wabel, 2007).This plant is also common in the Middle-East region including Saudi Arabia (Khalid A. Asiry.2015) and Jordan (Husseine et al., 1999). The plant is known to be as producing corrosive white latex that may severely harm the skin.

In spite of being poisonous, *Pergulariatomentosa*is used extensively in traditional medicine by North Saharian and SubSaharian populations. A decoction of the leaves and stems is used for the treatment of bronchitis and tuberculosis, a medication that should be taken with great care and is forbidden for pregnant women. The plant was reported to have molluscidal activity (Hammiche andMaiza, 2006) and persistent hypoglyceaemic effects (Al-Farrajand Al-Wabel 2007). The roots are used for the treatment of bronchitis, constipation and skin diseases (Arafa et al., 2006). Several reports mentioned this plant which used for the treatment asthma and as antirheumatic agent (Arafa et al., 2006; Piacente et al., 2009).

We recently reported the isolation and characterization of two new triterpenes along with five other known compounds reported for the first time from *Pergulariatomentosa*(Babaamaret al., 2013). Now we wish to report the study and evaluation of the antibacterial of *Pergulariatomentosa L.* against several *Gram-positive* and *Gram-negative* bacterial strains *in vitro*.

2. EXPERIMENTAL

2.1. Bioreduction of acetophenone by using*Pergulariatomentosa L.*

2.1.1. General methods

Acetophenone1a, was purchased from Aldrich. These chemicals were used without further purification. Thin-chromatography (TLC) was performed using precoated plates (Aluminium foil, silica gel 60 F_{254} Merck, 0.25mm). Merck 60 silica gel (230-400 mesh) was used for flash chromatography. Optical rotations were determined on EuromexPolarimeter PM. 5400 (Mitscherlich type polarimeter).

All 300 MHz and 75 MHz ^{13}C NMR spectra were run on a Bruker AC 300 NMR spectrometer. Both ^{1}H NMR and ^{1}C NMR spectra were recorded using $CHCl_3$ as internal standard; Infrared spectra were recorded using a Perkin-Elmer 783 spectrometer equipped with a PE 600 data station.

2.1.2. Biocatalysts

Fresh *Pergulariatomentosa L.* was collected from the Negrine desert-Tebessa-Algeria. To increase the contact of the substrate with the biocatalyst, the external pulp of the plants was removed and the rest was carefully cut into small thin pieces (approximately 1 cm long slice).

2.1.3. Synthesis of the chiral alcohols 1b

The chiral alcohol **1b**was synthesized by the reduction of the corresponding acetophenone 1a using *Pergulariatomentosa L.* as biocatalysts.

2.1.4. General procedures for biotransformations:

Typical reaction mixture of (0.02 mol) appropriate ketone **1a** 2% (W/V) of glucose or i-PrOH (in the case of solid ketones), 20 ml of phosphate buffer (pH = 6.5) was added to 25g of cultured plants suspension in 80 mL deionized water. The reaction mixture was agitated in orbital incubator shaker (150 rpm) at 30°C for 2 days. The progress of the reaction was monitored by TLC. The plants pieces were then removed by filtration, washed with deionized water and the filtrate was extracted with petroleum ether (3x100ml). The petroleum ether fraction was dried over anhydrous ($MgSO_4$) and the solvent was evaporated to get the final product. Then chemical yield and enantioselectivity were determined. Each experiment was parallelly repeated at least three times. Then the average value and standard deviations were given.

The product was identified by comparing their data with those of authentic samples on TLC, by IR, and ^{1}HNMR spectra. The presence of alcoholic group in the final product was chemically confirmed by acetyl chloride test.

2.1.5. Identification of chiral alcohol (1b) by optical properties spectroscopic data:

Phenylethanol (1b):*(R)*-(1b) was obtained in 37% yield, $[\alpha]_D^t$+24 (*c* 5, MeOH); 53% ee. The absolute configuration *(R)*-(1b) was estimated by analogy with {Lit., (Basavaiah et al., 2006), $[\alpha]_D^t$+45 (*c* 5, MeOH) for *R*-isomer}; The IR and ^{1}H and ^{13}C NMR spectra of (1b) were identical to those of authentic samples(Sekhri, 1998; Drew et al., 1997);^{1}H ($CDCl_3$, 300 MHz): δ (ppm): 1.5 (3H, d, $\underline{CH_3}CHOH$-), 4.7 (1H, br.s, $\underline{OH}$), 5.2 (1H, q, -$\underline{CH}OH$), 7.3-7.4 (4H, m, Ar-H) **(Figure 1)**; ^{13}C ($CDCl_3$, 75 MHz): δ (ppm): 22.8 ($\underline{CH_3}CHOH$), 69.9 (-$\underline{CH}OH$),), 127.1 (-$\underline{CH, Ar}$), 127.6 (-$\underline{CH, Ar}$), 128.9 (-$\underline{CH}$, Ar), 146.1 (C, Ar) **(Figure 2)**; ν_{max}(KBr Disk, Cm^{-1}): 3340-3060 (OH) **(Figure 3).**

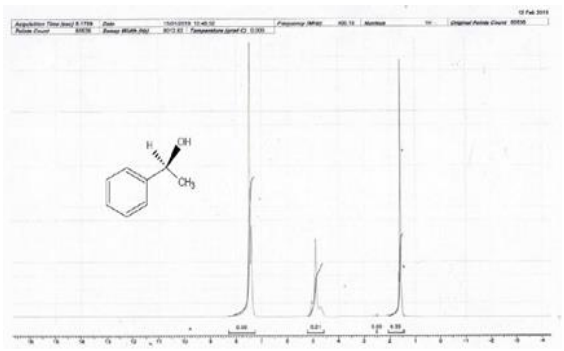

Figure 1: The ^{1}H spectre of *R*-1-phenylethanol **(1b)**

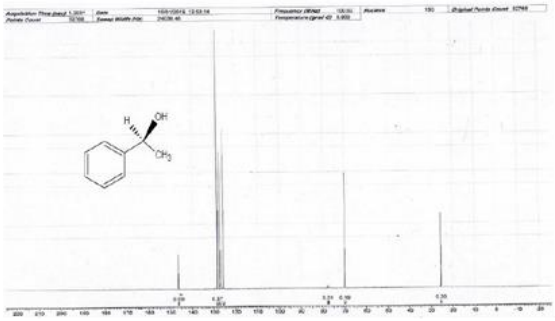

Figure 2: The ^{13}C NMR spectre of *R*-1-phenylethanol **(1b)**

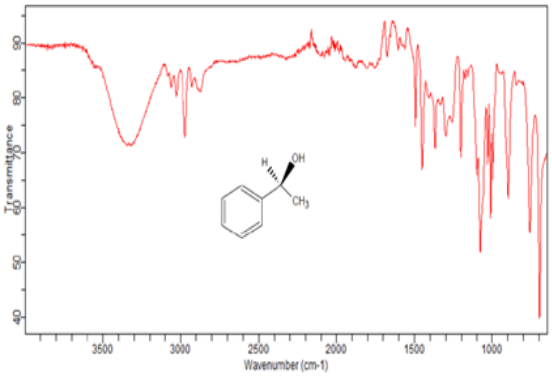

Figure 3: The IR spectre of *R*-1-phenylethanol **(1b)**

2.2.2 Preliminary Phytochemical Analysis

Qualitative Phytochemical analysis of the stalksplant powder was determined as follows:

Resins (200 mg plant material in 10 ml distilled water, filtered); a 10 ml filtrate + 4% HCl, the apearance of turbidity indicated the presence of *Resins*. ***Volatile oils*** (200 mg plant material in 10 ml distilled water, filtered), then a filter paper saturated by the filtrate and exposed to the UV rays, bright rose color indicated the presence of Volatile oils (Mukundam et al. 2012). ***Coumarins*** : In a test tube was placed 1g in 10 ml of distilled water, then covered with filter paper after being soaked in a diluted solutin of NaOH. The test tube was placed in boil water bath for a few minutes and then exposed to UV rays, yellow-green indicated the presence of Coumarins(Geisman, 1962). ***Terpenes*** (Liebermann-Burchardreaction : 200 mg plant material in 10 ml chloroform, filtered) ; a 2 ml acetic anhydride + conc. H_2SO_4. Blue-green ring indicated the presence of Terpenes(Al-Abid, 1985). ***Phenols***(200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml $FeCl_3$, blue-Green precipitate indicated the presence of Phenols (Al-Abid, 1985).***Tannins*** (200 mg plant material in 10 ml distilled water, filtered) ; a 2 ml filtrate + 2 ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins. ***Alkaloids*** (200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayor's reagents/Wagner's reagent/Dragendroff reagent, creamishprcipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids (Harbone, 1973). ***Saponins***(frothing test: 0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated the presence of saponins. ***Cardiac glycosides*** (Keller-Kilani test: a 2 ml filtrate + 1 ml glacial acetic acid + Fe Cl_3 + conc. H_2SO_4); green-blue color indicated the presence of cardiac glycosides. ***Steroids*** (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml acetic anhydride + conc. H_2SO_4. Blue-green ring indicated the presence of steroids. ***Flavonoids*** (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon, pink-tomato red color indicated the presence of flavonoids (Oguyemi, 1979). ***Flavons***: 10 ml of solution of plant powder in ethanol (50%) was added to 10 ml of KOH soltion (50%), and then equal amounts of this solution and extracted plant were mixed, yellow color, indicated the presence of Flavons (Harbone, 1984).

2.2.3 Extraction of plant material

The extracts were prepared by soaking 200 g of the leaves powder in petroleum ether for 24 hours in order to get rid of the fat and chlorophyll. The mixture was then filtered and the residue soaked again in a mixture of EtOH/water (70/30) for 24 hours with shaking from time to time and then filtered. The procedure was repeated three times and the filtrates were combined before being evaporated under reduced pressure. The resulting extracts were diluted with distilled water and left overnight. The filtrates were subjected to extraction by various solvents with increasing polarity (petroleum ether, dichloromethane, ethyl acetate, and butanol). The organic phases were separated and evaporated. The resulting residues were stored at 4°C.

2.2.4 Microorganisms

All bacterial standard strains: *Escherichia coliATCC11303, Bacillus cereus ATCC10987, Bacillus cereus ATCC14579, Pseudomoasaeroginosa ATCC 27853*, and *Salmonello ATCC700623* were obtained and diagnosed in Microbiology Laboratory, *TidjaniHadam Hospital, Bir Elater W. Tebessa. Algeria*.

2.2.5 Preparation of the bacterial culture media

3.7 of muller Hilton agar were mixed with hot distilled water and autoclaved at 121°C and 2 atm. for 15 min. After autoclaving, it was allowed to cool to 45°C in a water bath. Then the medium was poured into sterilized Petri dishes with a uniform depth of approximately 5 mm (Harbone, 1973).

2.2.6 Preparation of plant extract impregnated discs

Whatman N°1 filter paper was used to prepare discs of 6 mm in diameter. They were sterilized by autoclaving and then dried during the autoclaving cycle. The discs were then impregnated with extract of the plants (Oguyemi, 1979).

2.2.7 Disc diffusion method

Disc diffusion method for antimicrobial susceptibility test was carried out according to the standard method by Kirby-Bauer to assess the presence of anti-bacterial activities of plant extracts. A bacterial suspension adjusted to 0.5 McFarland standard ($1.5x10^8$ CFU/ml) was used to inoculate Mueller Hinton agar plates evenly using a sterile swab. The discs impregnated with the plant extracts were placed individually on the Mueller Hinton agar surface. The discs were spaced far enough to avoid both reflection waves from the edges of the petri discs and overlapping rings of inhibition. The plate was then incubated at 37°C for 18 hours in inverted position to look for zones of inhibition. Zones of inhibitions produced by the sensitive organisms were demarcated by a circular area of clearing around the plant extract impregnated discs. The diameter of the zone of inhibition through the center of the disc was measured to the nearest millimeter. The resulting residue of all extracts stored at 4°C were tested at concentrations of 400, 600, 800, 1000 and 5000 µg/ml and were prepared in DMSO.

3. RESULTS AND DISCUSSION

3.1. Bioreduction of acetophenone by using*Pergulariatomentosa L.*

Asymmetric transformations invariably involve the conversion of two dimentional substrate into a three dimentional product. For prochiral ketones such as acetophenone reduction shown in **Figuure 4**, addition to the back face gives 1-phenyl alcohol with *R* configuration, while addition to the back face gives alcohol with *S*configuration.The problem of course is that most common reducing agents, such as sodium borohydride or lithium aluminium hydride, react equally readily with either face. The most obvious solution to this problem is to use a hydride source which itself is enantiomerically pure in principal such as reagent will transfer the hydride to each face of the ketone through diastereoisomerically distinct transition state, which gives at least a fighting chance of an enrgy difference, and preference for addition to one face over the other.

Moreover, plants are potential biocatalysts used as the alternative solution to this problem, since they are easily obtainable from markets and easily manipulated. *Pergulariatomentosa L.*was selected as the biocatalysts.

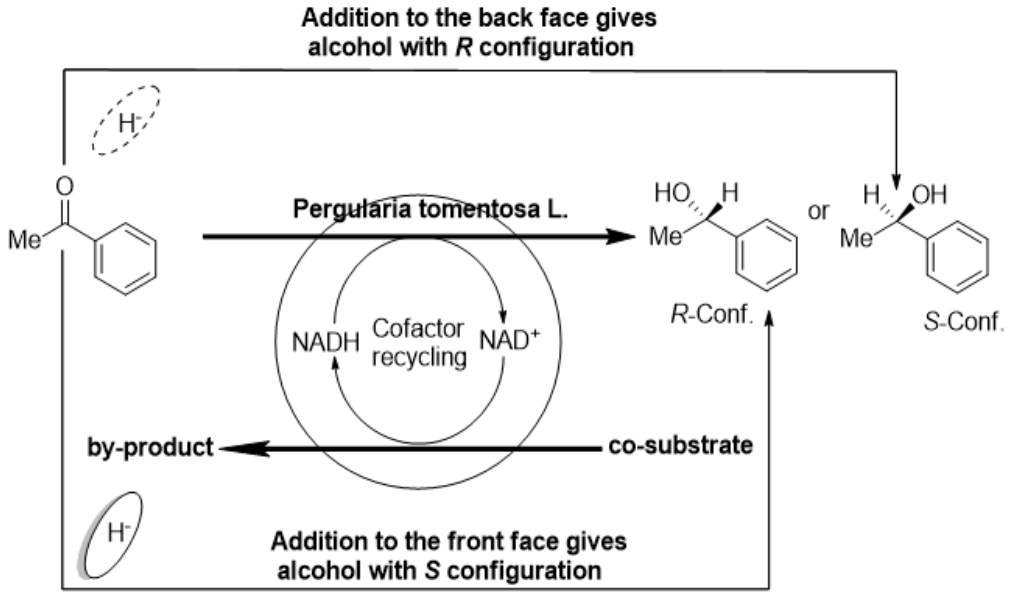

Figure 4: Asymmetric reduction of acetophenones catalyzed by plant tissue

3.1.1. Bioreduction of acetophenone with*Pergulariatomentosa L.*

Acetophenone is a preferred model substrate of simple ketones and aromatic ketones for asymmetric reaction. Acetophenone could be reduced to chiral 1-phenylethanol with attractive enantioselectivity. The yield and ee are 37 and 53.3% respectively and the reaction reached the equilibrium within 2 days (48h). We reported recently, the best results can be obtained by asymmetric reduction of 4'-haloacetophenones (X=Cl) in terms of the yield and ee. The yield and ee are 55.5 and 77% respectively and the reaction reached the equilibrium within 2 days (48h) (Nedjimi, and Sekhri, 2016).

The halogen-containing aromatic ketones such as 4'-chloroacetophenone is more acceptable to plant cells than simple aromatic ketone and the halogen bond on the phenyl group enlarges the difference between the two groups on both sides of the carbonyl, therefore improving the enantioselectivity of this reduction reaction (Nedjimi, and Sekhri, 2016).

3.1.2. Determination of optical activity of chiral products:

Optical properties of the products obtained from the prochiral were studied with the help of polarimeter using the following method. C % solution of the chiral alcohols in a suitable solvent (methanol or chloroform) was prepared and introduced to the polarimeter tube. The optical rotation values were determined individually for the product of each of the prochiral substrates. Specific rotation values are then calculated using the relation (eq. 1).

$$Specific\ rotation = \frac{\alpha}{1 \times c} \qquad (1)$$

Where,

α = angle of rotation

l = length of polarimeter tube (1dm)

c = concentration of the solution (g/ml)

Further enantiomeric excess values of the chiral products are determined by the equation (eq. 2)

$$ee\% = \frac{Observed\ \ Specific\ \ rotation}{Specific\ \ rotation\ of\ \ pure\ enantiomer} \times 100 \qquad (2)$$

These results will be served for the bioreduction of various acetophenones such as 4'-haloacetophenones (X=F, Cl, Br and I) with *Pergulariatomentosa L.*

3.2. Antibacterial activity

The preliminary phytochemical analysis of the crude powder of *Pergularietomentosa* plant collected showed that this plant contains many active ingredients: *Coumarins, tannins, volatile oils, terpenes and alkaloids*, one of the antioxidants of the bacteria responsible for the effect of microbs, also contains flavonoids including glycosides antioxidant and phenols and saponins.

Results for antibacterial activity as obtained with *Pergularietomentosa* plant revealed that the four different extracts tested in vitro by agar disc diffusion against six bacterial species. **Table 1**: summarizes the microbial growth inhibition of tested extracts of this plant that showed significant bacterial activity against all the tested bacteria (*Escherichia coliATCC11303, Bacillus cereus ATCC10987, Bacillus cereus ATCC14579, Pseudomoasaeroginosa ATCC 27853,* and *SalmonelloATCC700623*), where the maximum activity was recorded against *Bacillus cereus ATCC10987* and a maximum inhibition diameter of 16 mm with the Ethyl acetate extract at concentration 5000 µg/ml. Moreover moderate inhibition diameter of 6 mm with the lowest concentration of dichloromethane extract 600 µg/ml was recorded against *Pseudomoasaeroginosa ATCC 27853*. On the other hand the three extracts at concentration 400 (µg/ml)were ineffective againstall the tested bacteria. *Escherichia coliATCC11303, Bacillus cereus ATCC10987, Bacillus cereus ATCC14579, Pseudomoasaeroginosa ATCC 27853,* and*SalmonelloATCC700623.* Moreover the three extracts at concentration 600, 800 (µg/ml) showed no effect against *Bacillus cereus ATCC10987, Bacillus cereus ATCC14579, Pseudomoasaeroginosa ATCC 27853,* and*SalmonelloATCC700623.* However moderate effects against *Escherichia coli* isrecorded with the Ethyl acetate at this concentrations. Moderate inhibition was recorded with all extracts at 1000, 5000 (µg/ml) againstthe tested bacteria.

Table 1: Antibacterial activity of extracts of *PergulariaTomentosa*

		Diameter of inhibition zone (mm)				
Bacteriastrains / **Conc. Of extracts (µg/ml)**		*Salmonella* ATCC700623	Pseudomoasaeroginosa ATCC 27853	Escherichia coliATCC11303	Bacillus cereus ATCC10987	Bacillus cereus ATCC14579
Dichloromethaneextract	400	-	-	-	-	-
	600	-	6	-	-	-
	800	-	7	-	-	-
	1000	6	7	8	7	6
	5000	7	7	9	7	6
Ethylacetateextract	400	-	-	-	-	-
	600	-	-	6	-	-
	800	8	-	7	7	-
	1000	8	6	7	9	8
	5000	8	6	9	16	10
Butanol extract	400	-	-	-	-	-
	600	-	-	-	-	-
	800	-	-	-	7	-
	1000	6	6	8	7	6
	5000	8	6	10	7	6
EtOH/H2O	400	-	-	-	-	-
	600	-	7	-	-	-
	800	-	8	-	-	-
	1000	6	8	7	7	7
	5000	8	8	8	9	7

The results showed the influence of the extract concentration on the growth of the bacteria tested as shown in **Figures-5-9.**

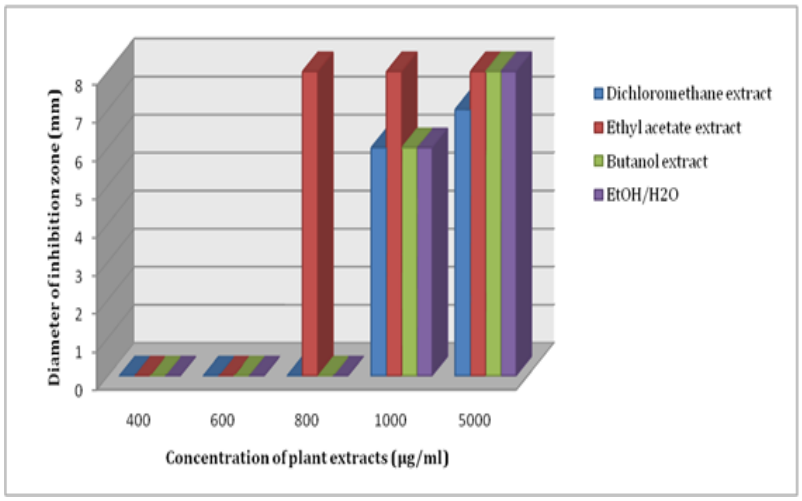

Figure 5: The influence of three extract concentration of *Pergularietomentosa*vs the inhibition diameter on *Salmonello ATCC700623.*

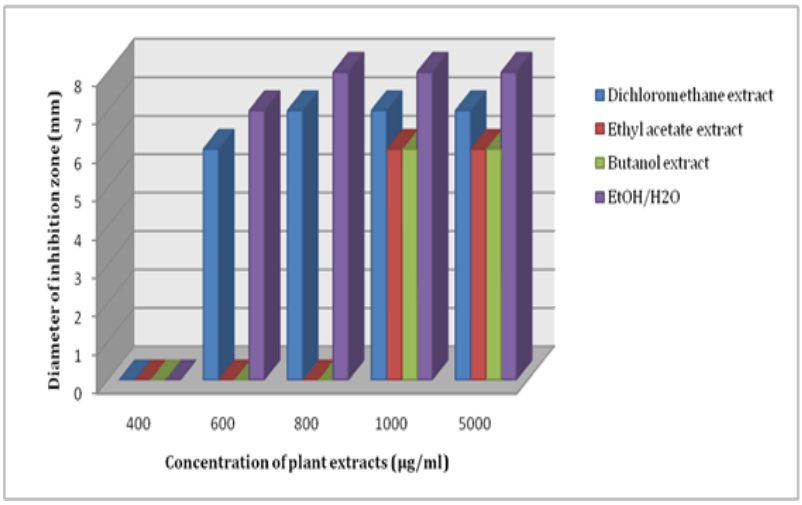

Figure 6: The influence of three extract concentration of *Pergularietomentosa*vs the inhibition diameter on *Pseudomoasaeroginosa ATCC 27853.*

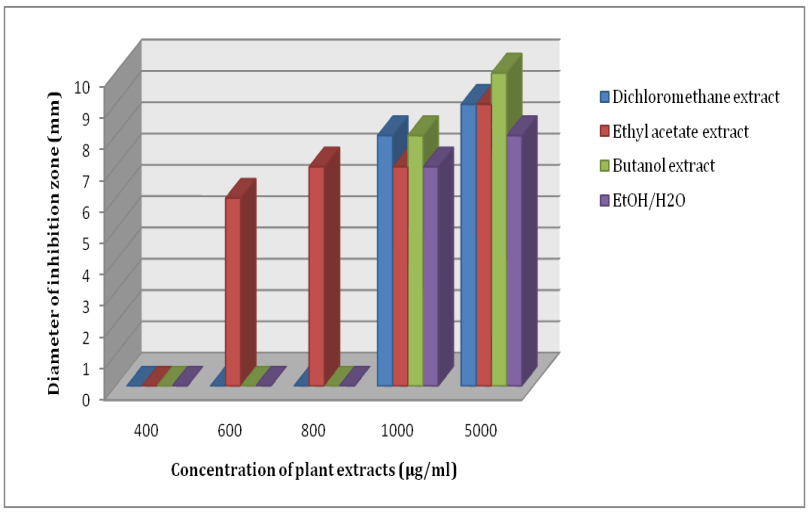

Figure 7: The influence of threeextract concentration of *Pergularietomentosa* vs the inhibition diameter on *Escherichia coli ATCC11303.*

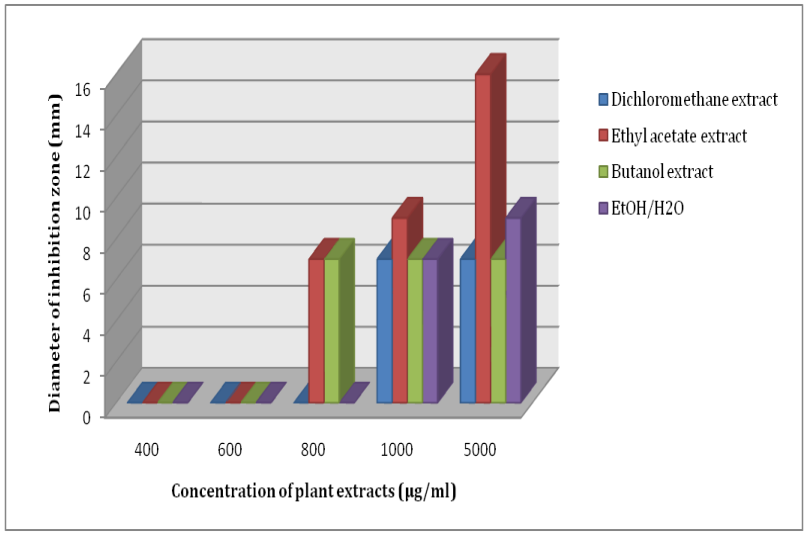

Figure 8: The influence of three extract concentration of *Pergularietomentosa*vs the inhibition diameter on *Bacillus cereus ATCC10987.*

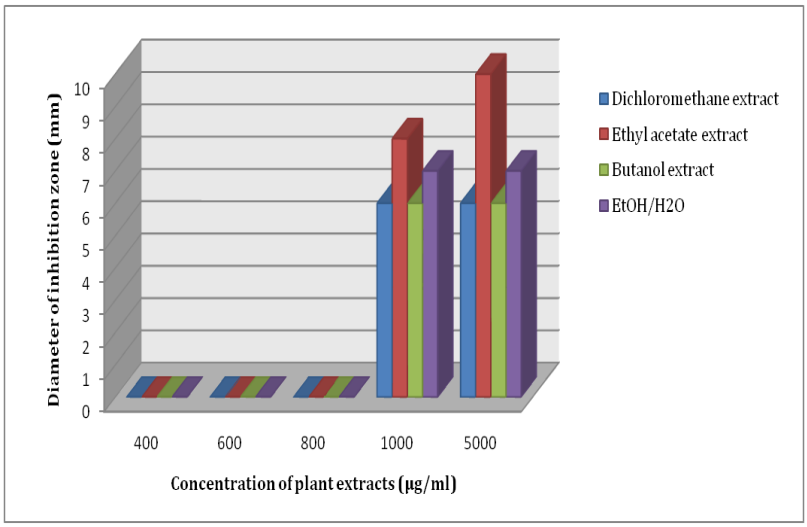

Figure 9: The influence of three extract concentration of *Pergulariatomentosa*vs the inhibition diameter on *Bacillus cereus ATCC14579*.

Further chemical and pharmacological investigations may be carried out to isolate and identify the chemical constituents in the selected plant responsible for the antimicrobial activity.
Generally, the four extracts of this plant are more or less effective towards the tested bacteria and ethyl acetate extracts are more potent compared to butanol, dichloromethane and Diethyl ether extracts.

4. CONCLUSION

The bioreduction of Acetophenone with *Pergulariatomentosa L.*can be effectively reduced to the corresponding chiral alcohol **1b** by the applied plants tissue. Moreover, only *R*- form configuration chiral alcohols could be obtained through these asymmetric reduction reactions. Among various co-substrates, glucose found to be the best for regeneration of co-factors. *P Pergulariatomentosa L.* was chosen as the biocatalysts and the acetophenone derivatives could be reduced by this plant tissue with high enantioselectivity. This provides a new route to produce chiral alcohols, as the platform chemicals for enantiomerically pure pharmaceuticals, through asymmetric reduction of the corresponding prochiral ketones.
This study also underscored the antimicrobial activity of one chenopodiaceae species namely : *Pergulariatomentosa L* using four extracts; Ethanol/H_2O, diethyl ether, butanol, and ethyl acetate extracts with increasing polarity against four bacteria strains: This medicinal plant averred to be effective against *Escherichia coli, Bacillus cereus, Bacillus cereus, Pseudomoasaeroginosa,* and *Salmonella*. The results partially justify the claimed uses of the selected plant in the traditional system of medicine to treat various infectious diseases caused by the microbes.

5. ACKNOWLEDGMENT

The authors wish to express their sincere thanks to Algerian Ministry of Higher Education and Scientific Research (MHESR) for sponsoring the present study and providing the necessary facilities to carry out this research. The authors are also thankful to the staff of Microbiology Laboratory,

TidjaniHadamHospital, Bir Elater W. Tebessa, Algeria, for their assistance and providing the necessary facilities to carry out this work.

REFERENCES

1. Abu Zarga, M. H., Al-Jaber, H. I., Baba Amer, Z. Y., Sakhrib, L., Al-Qudah, M. A., Al-humaidi, J. Y., ... &Afifi, F. U. (2013). Chemical composition, antimicrobial and antitumor activities of essential oil of Ammodaucusleucotrichus growing in Algeria. *Journal of Biologically Active Products from Nature*, *3*(3), 224-231.
2. Al-Abid,M.R.Zurrzusamme mse turungder,Abschla B.(1985) membrane in Phoenix dactylifera. *Wurzburg University. Wuzzburg, FR of Germany. pp*, 153-140.
3. Al-Farraj, A. S., & Al-Wabel, M. L. (2007). Heavy metals accumulation of some plant species grown. *J. App. Sci*, *7*(8), 1170-1175.
4. Arafa .I.Hamed, Plaza, A., Balestrieri, M. L., Mahalel, U. A., Springuel, I. V., Oleszek, W., ...&Piacente, S. (2006). Cardenolide glycosides from Pergulariatomentosa and their proapoptotic activity in Kaposi's sarcoma cells. *Journal of natural products*, *69*(9), 1319-1322.
5. AshakkumarR.,&Ramaswamy M. (2013). Determination of DPPH free radical scavenging Oxidation effects of methanolic leaf extracts of some Indian medicinal plant species. J.*Chem Bio Physci Sec., 3* (2), 1273-1278.
6. Babaamar Z., Sekhri L., AlJaber H., Al-Qudah M. A., & Abu Zarga M. H., (2013). Extraction and identification of triterpenoids from *Pergularia Tomentosa L* Revue Éditée par l'Université Kasdi Merbah Ouargla *Annales des Sciences et Technologie, 5*, 1-5.
7. Babamer Z., Sekhri L., Al-JaberHala I., Al-Qudah M. A., Abu Zarga M. H. (2012). Two new taraxasterol-type triterpenes from Pergularia tomentosa growing wild in Algeria . *Journal of Asian Natural Products Research*. 14(12)1137-1143.
8. Babarbi, E., Haffas, M., Guerri, M., &Sekhri, L. (2015). Synthesis of some fluorinated phosphonium iodide derivatives; study of their biological antibacterial activities and the effect of synergy of these compounds with penicillin-G and penicillin-V. *Biomedical and Pharmacology Journal*, *3*(2), 277-282.
9. Babpour Ebrahim, S. Abdolhamid Angaji , S. Mahdi Angaji (2009) Antimicrobial effects of four medicinal plants on dental plaque. Journal of Medicinal Plants Research Vol. 3(3), pp. 13
10. Basavaiah, D., Rao, K. V., & Reddy, B. S. (2006). (2S)-2-Anilinomethylpyrrolidine: an efficient in situ recyclable chiral catalytic source for the borane-mediated asymmetric reduction of prochiral ketones in refluxing toluene. *Tetrahedron: Asymmetry*, *17*(7), 1041-1044.
11. Bassam Abu-Shanab, Ghaleb Mohammad Adwan, Dahood Abu-Safiya, Naser Jarrar, Kamel Adwan .(2015). Antibacterial Activity of RhusCoriaria. L Extracts Growing in Palestine. *IUG Journal of Natural Studies*, *13*(2), 147-155.
12. Benkeblia, N. (2004). Antimicrobial activity of essential oil extracts of various onions (Allium cepa) and garlic (Allium sativum). *LWT-food science and technology*, *37*(2), 263-268.
13. Bensaci, H., Sekhri, L., Atmani, A., &Tabchouche, A. (2016). A qualitative phytochemical analysis and a comparative study of the antibactrial activity of Retamastalks (raetam). *Orient. J. Chem*, *32*(1): 1-7.
14. Biola F., Alogninouwa T., El Bahri L., Ali M., Kaboret Y., &Fayoumi B., (1993), Etude expérimentale de l'intoxication des caprins par Pergularia tomentosa L Revue d'Elevage et de MedecineVeterinaire des Pays Tropicaux 46, 591-598.
15. Doaigey, A. R. (1991). Occurrence, type, and location of calcium oxalate crystals in leaves and stems of 16 species of poisonous plants. *American Journal of Botany*, *78*(12), 1608-1616.
16. Drew, M. D., Lawrence, N. J., Fontaine, D., Sehkri, L., Bowles, S. A., & Watson, W. (1997). A Convenient Procedure for the Reduction of Esters, Carboxylic Acids, Ketones and Aldehydes using Tetrabutylammonium Fluoride (or Triton® B) and Polymethylhydrosiloxane. *Synlett*, *1997*(08), 989-991.
17. El-Hilaly, J., Hmammouchi, M., &Lyoussi, B. (2003). Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco). *Journal of Ethnopharmacology*, *86*(2-3), 149-158.
18. Geisman T. A., (1962). *Chemistry of Havonoids compounds.* Macmillan Co. New York. 90-101.
19. Hammiche, V., &Maiza, K. (2006). Traditional medicine in Central Sahara: pharmacopoeia of TassiliN'ajjer. *Journal of ethnopharmacology*, *105*(3), 358-367.
20. Harbone J. B., (1984). *Phytochemical methods. A guide to modern techniques of plant analysis*. 2nd ed., Chapman and Hall. London. P: 288.
21. Harbone J. (1973). *Phytochemical methods*. Chapman and Hall. London.
22. Husseine H. I., Alrajhy D., &Hashem S. M., (1999). Molluscicidal activity of Pergularia tomentosa (L.), methomyl and methiocarb, against land snails. Int. J. Pest. Manag. 45, 211-220.
23. Ishrak .k. Khafagi., &Dewedar, A. (2000). The efficiency of random versus ethno-directed research in the evaluation of Sinai medicinal plants for bioactive compounds. *Journal of Ethnopharmacology*, *71*(3), 365-376.
24. Khalid A. Asiry.(2015). The effect of different extracts of Fattaka fruits (Pergularia tomentosa L.) on controlling the Dengue Fever vector (Aedes aegypti) larvae under laboratory conditions. Global Journal of Biology,Agriculture &Health Sciences .4(4)26-29
25. Mukundam B., Shagufa A., &Swarnamoni, D. A., (2012). A comparative study of the antibacterial activity of the ethanolic extracts of Vitex negundo L., Fragaria vesca L., Terminalia arjuna and Citrus maxima *Asian J. Pharm. Bio res., 2* (3): 183-172.
26. Nedjimi, M. S., &Sekhri, L. (2016). A Comparative Study of the Effect of Various Kinetic Parameters on Asymmetric Bioreduction of Prochiral Ketones Catalyzed by Phoenix dactylifera L. *Research Journal of*

Pharmaceutical Biological and Chemical Sciences, *7*(4), 1198-1209.

27. Nedjimi, M. S., &Sekhri, L. (2016). Asymmetric Bioreduction of Prochiral Ketones Catalyzed by Cynarascolumus L, Terfeziasp and Phoenix dactylifera L. *Biomedical & Pharmacology Journal*, *9*(2), 469-476.
28. Oguyemi, A. O. (1979). In Sofowora A. In *Proceedings of a Conference on African Medicinal Plants. Ife-Ife: Univ Ife* (pp. 20-22).
29. Pelt, J. M. (2001). Les nouveaux actifsnaturels. *Ed., Marabout, Paris*.
30. Piacente, S., Masullo, M., De Nève, N., Dewelle, J., Hamed, A., Kiss, R., &Mijatovic, T. (2009). Cardenolides from Pergulariatomentosa display cytotoxic activity resulting from their potent inhibition of Na+/K+-ATPase. *Journal of natural products*, *72*(6), 1087-1091.
31. Saha, S., Barua, B., &Sikdar, D. (2017). Phytochemical screening, phenolic content and antioxidant activity of wild date palm (Phoenix sylvestrisRoxb.) fruit extracted with different solvents. *International Food Research Journal*, *24*(6), 2534-2542.
32. Sekhri L., (1998). PhD thesis, Studies on the asymmetric synthesis of new phosphines and amines and the symmetric reduction of esters ,acides ,ketones and aldehydes Constantine University, Algeria.
33. Sekhri L., Kadri M. L., Chaoula S., &Snigra M., (2008) Synthesis of penicillin derivatives and study of their biological antibacterial activities. *Biomedical & Pharmacology Journal, 1* (2): 257-264.
34. Shabana, M. M., Mirhom, Y. W., Genenah, A. A., Aboutabl, E. A., &Amer, H. A. (1990). Study into wild Egyptian plants of potential medicinal activity. Ninth communication: hypoglycaemic activity of some selected plants in normal fasting and alloxanised rats. *Archiv fur experimentelleVeterinarmedizin*, *44*(3), 389-394.
35. Shivrai Hariram Nile,Arti Shivraj Nile, Young-Soo Keum (2017) Total phenolics ,antioxidant,antitumor,and enzyme inhibitory activity of Indian medicinal and aromatic plants extracted with different extraction methods .3 Biotec ,7(76):1-10
36. Singh K., Gautam J., Jain A. K., & Mishra, P. K., (2007). Synthesis of Benzimidazole Derivatives and Study of Their Antimicrobial and Antifungal Activities *Oriental Journal of Chemistry, 23* (2), 641-650.
37. Suresh Kumar S., Rajesh R., SiddiquiAnnes A. (2006). Pharmacognostical, Phytochemical and Antimicrobiological Studies on Root and Aerial Parts of Ipomoea Obscura (Ker-Gawl). *Oriental Journal of Chemistry, 22* (3): 641-652.
38. Thirupathaiah, A., Rao, G. V., &Takaishi, Y. (2008). A lignan from the roots of Crossandranilotica. *Biomedical & Pharmacology Journal*, *1*(2), 311.
39. Tushar. D, Sonalshah, N.A. Gajbhiye, Satyanshukuma (2017)**.** Effect of extraction metheods on yieled ,phytochomical constituent and antioxidant activity of Withana somnifera ;Arabian journal of chemistry .v10 (1) :1193-1199
40. Union internationale pour la conservation de la nature et de sesressources. (2005). *A guide to medicinal plants in North Africa*. IUCN.