Studies of *trans*- and *cis*-xylomollin molecular structures using molecular dynamics simulations

Radia Mahboub

Department of Chemistry, Faculty of Sciences, University of Tlemcen,
B.P. 119, Tlemcen, 13 000, Algeria

E-mail address: radiamahboub@yahoo.com

ABSTRACT

The present work describes the comparative study of the *trans*- and the *cis*-xylomollin structures. We have determined the two bridgehead H₅ and H₉ configurations using simulation calculations for both *trans*- and *cis*- distereoisomers. Molecular Dynamic (MD) simulations of the *trans*- and *cis*- xylomollin were performed with an efficient program. The geometries, interaction energies, bonds, angles, and the Van der Waals (VDW) interactions were carried out in solution and gas phases. This comparative study shows that the *trans*-xylomollin acquires the high configuration energy under the AMBER field using MD method. This molecule reaches its high stable configuration state in solution environment. Our MD simulation results are goods and in agreement with those of literature.

Keywords:

xylomollin; *trans*-fused iridoid; modeling structure; molecular dynamic

1. INTRODUCTION

Heterocyclic molecules are the rings composed of both carbon and one or more heteroatoms. The heteroatom of heterocyclic molecules is generally oxygen, sulfur, or nitrogen, with the latter being particularly common in biochemical systems. Heterocyclic with relatively simple structures are pyrrole (5-membered) and indole (6-membered carbon ring). Rings can fuse with other rings on an edge to give polycyclic compounds. Rings can also fuse on a "corner" such that one atom (always carbon) has two bonds going to one ring and two to another. Such compounds are termed spiro and are important in a number of natural products. They can be unsaturated or aromatic heterocyclic or saturated heterocyclic. Alkaloids, chromenes, coumarins, flavonoids, xanthenes are some examples.

These compounds must be ecologically non-toxic for human. So, the author has classified then into two classes (table 1) [1]. Chemicals derived from the first classes are vital for the preservation and the protection of life processes, and have also theirs toxicological, pharmacological and ecological importance. The second classes are used in modern industry and medicine.

Table 1. Chemical classes of biologically active compounds [1]

First Classes	Second Classes
Aliphatic alcohol, Acids	Gums
Amino acids, Alkaloids	Glues
Carbohydrates, Carotenoids, Hydrocarbons	Alkaloids
Fatty Acids and Polyunsaturated Fatty Acids	Saponins
Lipids, Pheromones, Phorbol esters, Phenolics	
Steroids, Triterpenes, Glucosides	
Tannins	

Iridoids compose a class of natural compounds which have various biological activities such as antiviral [2], anti-allergic, anti-anaphylactic, analgesic [3], antioxidant [4, 5], antimicrobial, antirheumatic, laxative, hypotensive, sedative, antitumor [2,6], immunostimulant [7, 9], antimicrobial [10, 11] and hepatotoxic [13-15]. Others show radical scavenging activity against DPPH, antioxidant activity against β -carotene [12] and significant inhibition of UVB-induced: Activator Protein-1 activity in cell culture [16, 17].

Furthermore, the Xylomollin 1 belongs to the first classes. Our interest of this molecule becomes for its *trans*-fused iridoid terpene structure which has multiply applications in pharmacological.

In the present work, we describe and characterize the molecular structure of *trans*- and *cis*- xylomollin by MD simulation using AMBER as the force fields. We discuss the computational chemistry results of the two diastereoisomer compounds then we compare their two calculations results with the coupling constant effects.

In section 2, we report the stereochemistry for each compound (*trans*- and *cis*-) using the coupling constants.

In section 3, we describe the materials and methods used in this study. In the next section, we have detailed the molecular calculations: i) geometry optimization, and ii) dynamic simulations. In the second part, we discuss the evolution of: i) energies, ii) dihedral angles, and iii) geometry optimization in both vacuum and water environments. We present the calculation results obtained for the two compounds by Molecular dynamics, and then we compare all these results. In the last section, we give the conclusion.

2. STRUCTURE OF XYLOMOLLIN

Xylomollin 1 is the first natural heterocyclic compound of a *trans*-fused secoiridoid hemiacetal acetal. From natural source, its can be isolated in only small amounts and it represents one example of the naturally occurring cyclopentanomoterpenes.

Xylomollin 1 is the (1R, 4a α , 8a β)-hexahydro-1 α -hydroxy-3 β -methoxy-8 β -methyl-6-oxo-1H,3H-pyrano[3,4-c]pyran-4 α -carboxylic acid methyl ester.

(1*R*,3*R*,4*R*,4*aS*,8*S*,8*aR*)-methyl 1-hydroxy-3-methoxy-8-methyl-6-oxo-octahydropyrano[3,4-*c*]pyran-4-carboxylate

Fig. 1. Structure of Xylomollin.

The authors were fascinated by the structure of xylomollin. For its synthesis, they have suggested that a *cis*-bicyclo[3.3.0]octane can be transformed into the *trans*-fused natural product. The transformation occurs by the inversion of stereochemistry at one of the bridgehead carbon [18]. Previously, Nakane and all have partially synthesized the xylomollin and have found that the stereochemical control is realized by an inversion of configuration for one length chain [19].

In literature, we found some structures related to known iridoids [20]. Compound 2 has the 8 (*S*)-secoiridoids unlike kingside 3 and sarracenin 4 have the 8 (*R*) configuration. The two synthetics (-)-1-OMe-2 and its C-3 epimer 5 were prepared from (-)-loganin 6, and have *cis*- configurations (figure 2). The (-)-1-OMe-2 complies with a *gauche* relationship of the bridgehead hydrogens in a *cis*-decalin system ($^3J_{H5H9}$ values: 4.8 Hz and 9.4 Hz) [21]. So, with the $^3J_{H5H9}$ value of 10 Hz found in 1 is too large for a *cis*-fused decalin system; the xylomollin's structure is related to: 5(*S*),8(*S*),9(*R*)-secoiridoid. The all *trans*- diaxial orientation of the methine hydrogens is more regular with the reported 1H NMR coupling constant data [22].

Fig. 2. Structures of secoiridoids.

In the xylomollin 1, the two bridgeheads H₅ and H₉ have *trans*-configuration [23]. The H₅ was shown to possess a β -orientation with a large coupling constant ($\delta_{\text{H}} = 3$ ppm, dddd, 1H, $J = 10$ Hz, H₅) [24], the second one was also proposed to have an α -orientation ($\delta_{\text{H}} = 1.75$ ppm, ddd, 1H, $J = 10$ Hz, H₉) when compared to the literature report [25, 26]. In the *cis*-fused iridoid, authors have shown that the NOE cross peaks between H₅ and H₉ provided the same orientation for the two protons with a small coupling constant in the ¹H-NMR spectrum (4 Hz) [27]. Therefore, the two iridoids have distinct configurations. So, this involve that the compound 1 has *trans*-configuration.

In order to confirm the stereochemistry of compound 1, we have studied the two molecules: *trans*- and *cis*- xylomollin by comparing their calculation data. For this, we have using the Molecular Dynamic simulations at constant temperature both in vacuum and in water.

3. MATERIALS AND METHODS

Molecular Dynamics (MD) is a talented method used to model a simulation of macroscopic systems involving a few molecules. Today, MD tends to become an alternative to experiments in order to provide complements geometry, structural and thermodynamic characteristics. Molecular simulation provides an intermediate method between experiments and classical models. It gives useful predictions to understand the relation property-chemical structure.

All MD simulations were performed with AMBER force field using conjugate gradient algorithm. In vacuum, the system was simulated using Molecular Dynamics with 0.0001 ps step. Temperature was kept constant at 300 K. The starting temperature was taken at 100 K and the step at 20 K. In water, simulations, the system was placed in a box (20 x 20 x 20 Å) containing one molecule of Xylomollin (*trans*- or *cis*-) and 246 water molecules and cut-off 4 Å. Optimization of the molecule was realized in periodic boundary conditions (PBC). The compounds were solvated by added water molecules. The systems were first energy minimized steps with the conjugate gradient algorithm. Then, the position-restrained MD simulation was run 0.5ps.

Fig. 3. Geometry structure of *trans*-xylomollin: (a) in vacuum, (b) in water.

4. RESULTS AND DISCUSSIONS

4. 1. Geometry optimization

Trans- (gauche) conformation of the *trans*-xylomollin structure is confirmed by the dihedral angle ($H_{37}-C_5-C_9-H_{29}$) and the distance bonds. In the isolated and the solvated compound, this torsion angle does not change. So, we have obtained an anhedral angle value of -164.135° (table 2a). This situation is due of the strong Van der Waals collisions in *trans*-xylomollin or between *trans*-xylomollin and water under AMBER.

For the *cis*-xylomollin, the conformation is verified by the same parameters. In that case, we have found that the dihedral angle ($H_{37}-C_5-C_9-H_{29}$) is an anhedral one and its value fluctuate around -40° (table 2b).

Table 2a. Structural properties of *trans*-xylomollin with AMBER.

<i>trans</i> -xylomollin															
Isolated								Solvated							
Bond	D (Å)	Angle	Θ (°)	Dihedral	Φ (°)	Δ	Δ (Å)	Bond	D (Å)	Angle	Θ (°)	Dihedral	Φ (°)	Δ	Δ (Å)
1-2	1.424	4-5-9	107.551	4-5-9-1	68.867	37-29	3.014	1-2	1.424	4-5-9	107.552	4-5-9-1	68.865	37-29	3.014
2-3	1.420	6-5-9	110.206	6-5-9-8	-39.914	37-31	2.464	2-3	1.420	6-5-9	110.206	6-5-9-8	-39.943	37-31	2.464
3-4	1.548	6-5-37	107.707	37-5-9-29	-164.435	37-30	3.044	3-4	1.548	6-5-37	107.707	37-5-9-29	-164.437	37-30	3.044
4-5	1.536	8-9-5	113.742			37-36	3.644	4-5	1.536	8-9-5	113.742			37-36	3.644
5-6	1.529	1-9-5	106.76			37-33	3.026	5-6	1.529	1-9-5	106.76			37-33	3.026
6-7	1.524	8-9-29	106.726			37-34	3.574	6-7	1.524	8-9-29	106.726			37-34	3.574
7-11	1.354					29-30	2.688	7-11	1.354					29-30	2.688
11-8	1.418					29-36	2.332	11-8	1.418					29-36	2.332
8-9	1.547					29-32	2.815	8-9	1.547					29-32	2.815
9-1	1.540					29-31	3.821	9-1	1.540					29-31	3.821
8-10	1.534					29-34	3.797	8-10	1.534					29-34	3.797
3-12	1.422					29-33	2.507	3-12	1.422					29-33	2.507
12-13	1.418							12-13	1.418						
4-14	1.528							4-14	1.528						
14-15	1.230							14-15	1.230						
14-16	1.355							14-16	1.355						
16-17	1.414							16-17	1.414						
1-18	1.412							1-18	1.412						
7-19	1.229							7-19	1.229						

Table 2b. Structural properties of *cis*-xylomollin with AMBER.

<i>cis</i> -xylomollin															
Isolated								Solvated							
Bond	D (Å)	Angle	Θ (°)	Dihedral	Φ (°)	Δ	Δ (Å)	Bond	D (Å)	Angle	Θ (°)	Dihedral	Φ (°)	Δ	Δ (Å)
1-2	1.418	4-5-9	112.998	4-5-9-1	-41.190	37-29	2.300	1-2	1.418	4-5-9	113.037	4-5-9-1	-41.058	37-29	2.299
2-3	1.417	6-5-9	109.897	6-5-9-8	-41.985	37-31	2.466	2-3	1.417	6-5-9	109.966	6-5-9-8	-41.781	37-31	2.465
3-4	1.537	6-5-37	105.678	37-5-9-29	-42.679	37-30	3.031	3-4	1.536	6-5-37	105.628	37-5-9-29	-42.524	37-30	3.030
4-5	1.537	8-9-5	111.714			37-36	3.945	4-5	1.537	8-9-5	111.707			37-36	3.945
5-6	1.531	1-9-5	111.698			37-33	2.526	5-6	1.531	1-9-5	111.724			37-33	2.528
6-7	1.522	8-9-29	106.346			37-34	4.228	6-7	1.522	8-9-29	106.349			37-34	4.228
7-11	1.353					29-30	3.783	7-11	1.353					29-30	3.785
11-8	1.418					29-36	3.043	11-8	1.418					29-36	3.043
8-9	1.556					29-32	2.212	8-9	1.556					29-32	2.213
9-1	1.542					29-31	4.230	9-1	1.542					29-31	4.229
8-10	1.537					29-34	4.172	8-10	1.537					29-34	4.173
3-12	1.422					29-33	3.861	3-12	1.422					29-33	3.864
12-13	1.422							12-13	1.418						
4-14	1.533							4-14	1.533						
14-15	1.230							14-15	1.230						
14-16	1.356							14-16	1.356						
16-17	1.424							16-17	1.414						
1-18	1.411							1-18	1.411						
7-19	1.229							7-19	1.229						

Δ : Non-Bonded Distances

The geometry optimizations of *trans*- and *cis*- xylomollin are realized in vacuum and in water (in PBC with a 246 TIP3P water molecules); using AMBER as the force field. The results are given in tables 3. As consequence, the *trans*- molecule has higher energies (dihedral, Van der Waals (VDW) and total) than the *cis*- structure in both situations (vacuum and solution). The *cis*- molecule acquires inverse situation for the bond and the angle energies.

Table 3a. Geometry optimization properties of *trans*-xylomollin at 300 K with AMBER before MD simulations.

State	Geometry								
	Bond (Kcal/mol)	Angle (Kcal/mol)	Dihedral (Kcal/mol)	VDW (Kcal/mol)	Stretch-bend (Kcal/mol)	H-bond (Kcal/mol)	Electrostatic (Kcal/mol)	Energy (Kcal/mol)	Gradient (Kcal/Å.mol)
Isolated	0.6607	3.5038	9.7106	3.4996	-	-0.0002	0	17.3746	0.0869
Solvated	0.6605	3.5034	9.7104	3.5079	-	-0.0002	0	17.3820	0.0870

Table 3b. Geometry optimization properties of *cis*-xylomollin at 300 K with AMBER before MD simulations.

State	Geometry								
	Bond (Kcal/mol)	Angle (Kcal/mol)	Dihedral (Kcal/mol)	VDW (Kcal/mol)	Stretch-bend (Kcal/mol)	H-bond (Kcal/mol)	Electrostatic (Kcal/mol)	Energy (Kcal/mol)	Gradient (Kcal/Å.mol)
Isolated	0.7699	5.0700	6.0819	3.0612	-	-0.0003	0	14.9831	0.0966
Solvated	0.7691	5.0558	6.0836	3.0681	-	-0.0002	0	14.9767	0.0927

In both environment, the minimum potential energies $E_{MD,min}$ calculated for the geometry optimization using AMBER are higher in *trans*- structure than in *cis*- (respectively 14.8 Kcal/mol, and 10.47 Kcal/mol). We note that the dihedral factor in potential energy is big in the *trans*- molecule than in the *cis*- one when using AMBER field (tables 4a, 4b). For the last situation, we think that the system considers the friction coefficient which affects the structure geometry of molecule in the *cis* form.

Variations are visible for angle, and dihedral energies. The much important difference is happened in the angle energies. The big value (angle energies) in the *cis*- structure is due to strong interactions between atoms and the values of angles (*trans*:- C₄-C₅-C₉ 107.5°, C₁-C₉-C₅ 106.7°; *cis*:- C₄-C₅-C₉ 113°, C₁-C₉-C₅ 112°).

Table 4a. Geometry optimization properties of *trans*-xylomollin at 300 K with AMBER after MD simulations (in vacuum and in water).

State	Bond (Kcal/mol)	Angle (Kcal/mol)	Dihedral (Kcal/mol)	VDW (Kcal/mol)	Stretch-bend (Kcal/mol)	H-bond (Kcal/mol)	Electrostatic (Kcal/mol)	Energy (Kcal/mol)	Gradient (Kcal/Å.mol)
Isolated	0.5663	2.7598	8.5234	2.9413	-	-0.0002	0	14.7907	0.0897
Solvated	0.5899	2.84978	8.4689	2.9680	-	-0.0002	0	14.8764	0.0996

Table 4b. Geometry optimization properties of *cis*-xylomollin at 300 K with AMBER after MD simulations (in vacuum and in water).

State	Bond (Kcal/mol)	Angle (Kcal/mol)	Dihedral (Kcal/mol)	VDW (Kcal/mol)	Stretch-bend (Kcal/mol)	H-bond (Kcal/mol)	Electrostatic (Kcal/mol)	Energy (Kcal/mol)	Gradient (Kcal/Å.mol)
Isolated	0.5235	4.1015	3.9900	1.8550	-	-0.0005	0	10.4707	0.0908
Solvated	0.5795	4.3416	3.9390	2.4038	-	-0.0001	0	11.2638	0.0954

4. 2. Molecular Dynamic simulations

Dynamic simulations were accomplished at constant temperature, using AMBER force field. The conjugate gradient (Polak-Ribiere) algorithm was preferred because for the constant dielectric. We have calculated the xylomollin (*trans*- and *cis*-) dynamic properties in both gas and water. We have employed the TIP3P water molecules model, and chosen the bath relaxation time equals to 0.1 ps. The simulation temperature was fixed to 300 K and the step at 20 K. The run time was 0.5 ps, the step size was 0.0001 ps and the heat time was 0.1 ps. Here, we study the evolution of: i) energies and ii) dihedrals angles in both vacuum and water environments for the two systems: *trans*- and *cis*- xylomollin.

At first, we have calculated all energies for the two systems then represented them in figures 4 and 5. The average energies are in good agreement with the simulation accuracy. The sampling results of step-size of MD method in vacuum and water are presented in figures (a) and (b), respectively.

For the *trans*-xylomollin, the total energies (ETOT) are the same in MD simulations in vacuum and water environments. After equilibration, the MD simulation becomes more stable (respectively 73.58; 75.48 Kcal/mol). The potential energy (EPOT) and the kinetic energy (EKIN) illustrate fluctuations in both environment and vary around (40.66, 47.34) Kcal/mol, and (32.92, 28.14) Kcal/mol (figures 4). In simulation, we observe that the trajectory of potential energy has attained a minimum around 0.4 ps (30 Kcal/mol). In the same time, the kinetic energy provides an opposite situation i.e. its reach a maximum (43 Kcal/mol).

(a)

(b)

Fig.4. Evolution of energies for *trans*-xyломollin with AMBER: (a) in vacuum, (b) in water.

(a)

(b)

Fig. 5. Evolution of energies for *cis*-xyломоллин with AMBER: (a) in vacuum, (b) in water.

For the *cis*-xyломоллин, the positions of all energies stay unaltered (vacuum: ETOT 71.53, EKIN 34.65, EPOT 36.88 Kcal/mol; water: ETOT 72.72, EKIN 30.50, EPOT 42.21, Kcal/mol). All energies are very stable at time greater than 0.1 ps for both environments. Here, the fluctuations disappear in spite of weak interactions between molecules. We note the same situation around 0.4 ps i.e. the potential energy has attained a minimum at 30 Kcal/mol, and in the same time, the kinetic energy provides the opposite situation and reaches a maximum at 43 Kcal/mol.

Comparing the results for the two systems, we note that the total and potential energies obtained for the *trans*- structure are lightly up to those obtained for the *cis*- form. The situation is opposite for kinetic energies. So, here the potential energies correspond to the configuration energies for each diastereoisomer.

Both configuration isomers (*trans*- and *cis*-) are influenced by the same effects: stereo-electronic and anomeric. The *trans*- molecule forbids the tilting of conformation and the structure adopts the stiff form. So, the steric hindrance effect between the two protons H₃₇ and H₂₉ is less and their non-bonded distance is high (3.0 Å). For the *cis*-molecule, the junction (C₅-C₉) of the two cycles allows the possibility to tilt between two chair forms. So, this structure is flexible. As consequence, the two protons H₃₇ and H₂₉ are carried by the concave face and their non-bonded distance (2.3 Å) is less than VDW radius (2.4 Å). We conclude that our Molecular Dynamics is suitable for the two systems, our MD results are in harmony with the coupling constant found in literature (*trans*:- $^3J_{H_{37}H_{29}} = 10$ Hz), and converge to the much stable compound: the *trans*-xyломоллин ($E_{\text{config } trans-} > E_{\text{config } cis-}$).

After analyze the evolution of values of dihedrals angles H₃₇-C₅-C₉-H₂₉ for *trans*-xyломоллин, we have observed that the angle is instable in MD simulation in gas. During this simulation run, the torsion angle oscillates between multiple states. The deviation has two

states of stability: the first until 0.07 ps. The second state has about fully duration of the simulation run in MD calculations (figure 6).

(a)

(b)

Fig. 6. Evolution of dihedral angles for *trans*-xyломollin with AMBER: (a) in vacuum, (b) in water.

In water, the dihedral angle and its deviation present two phases of stability (first at 0.34 ps, second at 0.39 ps) which are separate by short periods in which torsion angle turns quickly into an anhedral angle (two states) (figure 6). Finally, the angle becomes stable and gets a value of dihedral angle which correspond a *trans*- configuration. So, we confirm that our *trans*-system is more stable in water than in gas.

For the *cis*-xylomollin, the dihedral curves are similar in the two environments. The dihedral angles are most anhedral, present instability, and oscillate between multiple states. So, this molecule undergoes tilting of conformation because of the *cis*- cycle junction (figure7). From these results, we confirm that the molecule carries an intermediary conformer structures imposed by the concave face of C₅-C₉ bond.

(a)

(b)

Fig. 7. Evolution of dihedral angles for *cis*-xylomollin with AMBER: (a) in vacuum, (b) in water.

In the actual conditions, the torsion angles seem into anhedral angle for all simulations. Consequently, deviations have an important state of stability while the angle has been affected by the collision. The effect is well represented under AMBER field. So, the structure has been disturbed while the calculation runs. At this transition period, the *cis*-configuration geometry is conserved.

The differences will appear for both configurations (*trans*-, *cis*-) and under the same field (AMBER) where the angle changes in these simulations due to the interaction molecule structures. As consequence, we conclude that our *trans*-system is more stable than the *cis* one principally in water.

5. CONCLUSIONS

We have essentially studied the two bridgehead H₅ and H₉ configurations (*trans* and *cis*) using simulation calculations in order to establish the much stable configuration in the bicyclic structure. So, we have studied the evolution of the geometry optimized properties for the *trans*- and *cis*- xylomollins. We have chosen the MD method to predict much better the characteristics. The geometries, interaction energies, bonds, angles, dihedrals and the VDW interactions were carried out in solution and in gas phase. We have calculated the thermodynamic and structural properties for the two configurations with AMBER force field. Then, we have compared these results with those of literature in order to confirm the stable structure.

The energies are stable in MD simulations under the AMBER field. In water, we note that the total and potential energies obtained for the *trans*- structure are lightly up to those obtained for the *cis*- form. In gas, the situation is similar. We conclude that our Molecular Dynamic method is suitable for the xylomollin systems, and gives good results for the *trans*-molecule.

At a short period, the torsion angle changes due to the interaction of water molecules and turns quickly into dihedral angle. During the simulation time, the *trans*- molecule conserve its configuration geometry, adopts the stiff form, and forbids the steric hindrance effect between the two protons H₃₇ and H₂₉.

We conclude that our simulation under AMBER field gives best results; the *trans*-xylomollin acquires the configuration energy. So, the *trans*-molecule reaches its high stable configuration state in solution environment under AMBER field. Our MD simulation results are good and in agreement with those of NMR data literature.

REFERENCES

- [1] Bandaranayake W. M., *Wetlands Ecology. Manag.* 10(6) (2002) 421-452.
- [2] Akcos Y., Ezer N., Ozcelik B., Abbasoglu U., *Farm. Bilimler Derg.* 23 (1998) 99-103.
- [3] Baruah, C. C., Gupta P. P., Amar Nath, Patnaik Late G. K., Dhawan B. N., *Pharmacol. Res.* 38(6) (1998) 487-492.
- [4] Owen R. W., Mier W., Giacosa A., Hull W. E., Spiegelhalder B., Bartsch H., *Food. Chem. Toxicol.* 38(8) (2000) 647-659.
- [5] Cuendet M., Potterat O., Hostettmann K., *Pharma. Biol.* 37(4) (1999) 318-320.
- [6] Bowers M. D., Puttick G. M., *J. Chem. Ecol.* 14 (1988) 319-334.

-
- [7] Zeyheri H., Baghdikian B., Lanhers M.C., Fleurentin J., Ollivier E., Millard C., Balansard G., Mortier F., *Planta Med.* 63(2) (1997) 171-176.
- [8] Mathad V. T., Raj K., Badhuri A. P., Sahai R., Puri A., Tripathi L. M., Strivastava V. L. M., *Bioorg. Med. Chem.* 6(5) (1998) 605-611.
- [9] Mandal S., Jain R., Mukhopadhyay S., *Indian J. Pharm. Sci.* 60(3) (1998) 123-127.
- [10] Fleming H. P., Walter W. M., Etchells J. L., *Appl. Microbiol.* 26(5) (1973) 777-782.
- [11] Kubo I., Matsumoto A., Takase I., *J. Chem. Ecol.* 11(2) (1985) 251-263.
- [12] Yamano T., Tsujimoto Y., Noda T., Shimizu M., Ohmori M., Morita S., Yamada A., *Food. Chem. Toxicol.* 28(7) (1990) 515-519.
- [13] Cervellati R., Speroni E., Govoni P., Guerra M. C., Costa S., Arnold U. W., Stuppner, H., *Z. Naturforsch C.* 59(3-4) (2004) 255-562.
- [14] Jeong E. J., Lee K. Y., Kim S.H., Sung S. H., Kim Y. C., *Eur. J. Pharmacol.* 588(1) (2008) 78-84.
- [15] Bhandari P., Kumar N., Singh B., Ahuja P. S., *Indian J. Exp. Biol.* 48(3) (2010) 323-328.
- [16] Sang S., He K., Liu G., Zhu N., Cheng X., Wang M., Zheng Q., Dong Z., Ghai G., Rosen R. T., Ho C., *Org. Lett.* 3 (2001) 1307
- [17] Bouyssi D., Monteiro N., Balme G., *Tetrahedron Lett.* 40 (1999) 1297-1300.
- [18] Whitesell J.K., Wang P. K. S., Aguilar D. A., *J. Org. Chem.* 48 (1983) 2511-2515.
- [19] Nakane M., Hutchinson C. R., VanEngen D., Clardy J., *J. Am. Chem. Soc.* 100 (1978) 7079-7080.
- [20] Plouvier V., Favre-Bonvin J., *Phytochem.* 10(8) (1971) 1697-1722.
- [21] Eliel E., *Stereochemistry of Carbon Compounds*, McGraw-Hill., New York, (1962) P.279.
- [22] Whitesell J. K., Wang P. K. S., Aguilar D. A., *175th National Meeting of the American Chemical Society*, Anaheim, Calif, March13-14, 1978.
- [23] Hassam S. B., Hutchinson C.R., *Tetrahedron Lett.* 21(13) (1980) 1209-1212.
- [24] Kubo I., Miura I., Nakanishi K., *J. Am. Chem. Soc.* 98(21) (1976) 6704-6705.
- [25] Krull R. E., Stermitz F. R., Franzyk H., Rosendal Jensen S., *Phytochem.* 49(6) (1998) 1605-1608.
- [26] Foderaro T. A., Stermitz F. R., Hope H., *Tetrahedron Lett.* 33 (1992) 2953-2954.
- [27] Karioti A., Chatzopoulou A., Bilia A. R., Liakopoulos G., Stavrianakou S., Skaltsa H., *Biosci. Biotechnol. Biochem.* 70(8) (2006) 1898-1903.