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Article

Computational and Experimental Studies on Antimicrobial Activity of the Bark of *Annona muricata* against Some Selected Human Pathogenic Bacteria and Fungi

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Abstract: Medicinal plants have traditionally provided a source of hope for novel drug compounds with effectiveness to combat antimicrobial resistance, as plant herbal mixtures have made large contributions to human growth and health. Antimicrobial resistance is presently one of the main threats facing human therefore causing dreadful economic and health impact. The phytochemical analysis of the extracts of *Annona muricata* bark were investigated. The extracts also showed higher minimum antifungal activity when compared to the commercial antibiotic Amphotercin B. The extracts compete favorably with commercial available antibacterial streptomycin in terms of the minimum zones of inhibition and minimal inhibitory concentrations. The prominent antibacterial activity of these extracts on tested bacterial shows that very small amount of the extracts is needed to inhibit the growth of all the tested bacteria except *Staphyloccus aureus* which might be due to the phytochemicals found in them. Also, the docking study observed in this work revealed the

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effectiveness of some active components of *Annona muricat*a against both selected bacteria and fungi. Annonaine inhibited *Candida albican* (PDB ID: 1q42) and *Fusarium oxysperium* (PDB ID: 5od4) and Coreximine Real and Reticuline inhibited *Aspergillus flaws* (PDB ID: 1cf3) and *Penicillium camemeri* (PDB ID: 2mhv) respectively more than other studied compounds. Annonaine inhibited *Clostridium sporogenes, Entrococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphyloccus aureus* cell lines than other studied compounds and Coclaurine inhibited *Bacillus subtilis*. This study provides scientific evidence of traditional use of *Annona muricat*a part for medicinal purpose and indicates its potency for the development of antimicrobial agents.

Keywords: *Annona muricata*, medicinal plants, phytochemicals, antibacterial activity and antifungal activity

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1. Introduction

Anona muricata is also called sour sop, it is an evergreen plant that is mostly distributed in tropical regions of the world and a wide array of ethno medicinal activities is contributed to different parts of *Annona muricata*, and indigenous communities in Africa and South America extensively use this plant in their folk medicine [1]. Researchers have reported that *Annona muricata* possess medicinal property including anticancer, anticonvulsant, anti-arthritic, anti-parasitic, antimalarial, hepatoprotective and anti-diabetic, analgesic hypotensive and anti-inflammatory [1].Researchers have increased their focus towards obtaining advantages from medicinal plants after observing more side effects, high cost of synthetic drugs and development of resistance of microbes to synthetic drugs compared to their benefits [2]. A review on "global crisis of antimicrobial resistance " by Jim O'Neill, estimated that over 700,000 lives are lost yearly due to antimicrobial-resistant infections which might result to reduction in human population between 11 million and 444 million leading to a reduction in global economy by 0.1-3.1% by 2050, if effective antimicrobial agents are not discovered [3,4]. The side effects of antimicrobial resistance are worse in underdeveloped countries, including Nigeria, where the cost of treating resistant infections that are associated with deaths are unaccounted for [5]. The use of phytochemical constituents of plants for pharmaceutical purpose nowadays, has increased rapidly in many countries due to resistance of synthetic drugs to human pathogenic microorganisms and also due to reported of World Health Organization (WHO) that medicinal plants are the best source to obtain a variety of drugs [6].

Moreover, the side effects of indiscriminate use of commercial antimicrobial drugs employed in the treatment of infectious diseases has compelled scientist to search for new antimicrobial substances

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from medicinal plants [6]. The antibacterial and antifungal effects of medicinal plants are attributed to interaction of and bioactive compounds (phytochemicals) found in their parts [7, 8]. Scientific compilation of studies on antimicrobial activity of medicinal plants would help to synthesis drugs with high potential antimicrobial activity from which new antimicrobial drugs with no resistance to human pathogenic microorganisms could possibly be isolated, characterized and elucidated.

The detection of few molecular compounds used for protein – protein crossing point target possess several dares. Docking studies deals with the inter-relationship that exist between ligand (drug-like molecules) and receptor (enzyme). It helps in locating the opposite active site in the enzyme. The docking calculation could be presented as dock score which is the geometric means of calculating the power of the non-covalent interaction that exist between two molecules once the docking is completed. [9-11].

In view of this, ten (10) molecular compounds were selected from the molecules present in the bark of *Annona muricata*. Therefore, the aim of this work is to calculate molecular descriptors which describe the anti-fungi and anti-bacterial activities of the studied compounds using quantum chemical method via density functional theory method as well as the relationship between the selected compounds and the studied enzymes (3ZIH, 4Q0Y, 2N50, 2RQX, 2J5O, 4G8X for bacteria cell lines and 1CF3, 1Q42, 5OD4, 2MHV for Fungi) [11-19] were examined. The calculated compounds are annonaine, anomuricine, anomurine, asimilobine, atherosperminine, coclaurine, coreximine real, nornuciferine, reticuline and stepharine.

2. Methodology

2.1. Sample Collection

The barks of *annona muricata* were collected from Irele town in Ondo state Nigeria and were authenticated in the botany department of Obafemi Awolowo University, Ile-Ife, Osun state. The tested bacteria include: *Bacillus subtili, Clostridium sporogenes, Entrococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa and Staphyloccus aureus* while tested fungi are *Candida albican*, *Fusarium oxysperium, Penicillium camemeri* and *Aspergillus flaws.* These bacteria and fungi were obtained from the microbiology laboratory of the Department of Microbiology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun state.

2.2. Preparation of Plant Extract

About 500g of powdered *annona muricata* bark was extracted with 4 L of methanol for 48 hours with adequate agitation at the intervals of 3 hours. The extracting solvent was decanted and filtered after 48 hours of extraction, partition with n-hexane and ethyl acetate and concentrated on a digital rotary

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evaporator (Heidolph laborata 4010) to obtain the n-hexane and ethyl acetate extracts of the *annona muricata* barks.

2.3. Phytochemical Analysis of Methanol, N-hexane and Ethyl Acetate Extracts of Annona muricata Bark

2.3.1. Determination of hydroxyl anthraquinones

About 10 mL of benzene was added to 5.0 g of each extract, the solution was filtered and 5 mL of 10% NH_3 was added to the filtrate and was vigorously shaken. The occurrence of red colouration in the ammoniacal phase confirmed the presence of hydroxyl anthraquinones.

2.3.2. Determination of saponins

About 0.5 g of each extract was transferred into a test tube, 5 ml of distilled water was added and the mixture was vigorously shaken. Formation of bubbles which persistent for 20 min confirms the presence of saponins [20].

2.3.3. Determination of cardiac glycosides

To 2 ml of each extract, 1 ml glacial acetic acid, few drops of $FeCl_3$ and 1 ml of concentrated H_2SO_4 were added. The appearance of brown ring at the interface confirmed presence of cardiac glycosides [20].

2.3.4. Determination of tannins

10 ml of distilled water was added to 0.3 g of each of the concentrated extracts and filter. Then 5 ml of the filtrate was treated with freshly prepare $FeCl_3$ solution. A greenish dark coloration was observed which confirmed the presence of tannins [20].This procedure was repeated for all the extracts.

2.3.5. Determination of alkaloids

0.5 g of the concentrated extract was stirred with 5 ml of 1% HCl on water bath and filtered; 1 ml of the filtrate was then treated with few drops of Meyer's reagent. Occurrence of turbidity confirmed alkaloid was present [20].This procedure was repeated for all the extracts.

2.3.6. Determination of flavonoids

0.5 g of the concentrated extract was dissolved in 5 ml of $AlCl_3$, 2 ml methanol, 2 ml of concentrated HCl, 2 ml of KOH solution and few drop of magnesium turning was added. Observation of pink coloration confirmed the presence of flavonoid [20].This procedure was repeated for all the extracts.

2.3.7. Determination of phenols

0.4 g of the concentrated extract was treated with $FeCl_3$ solution and intense coloration was

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observed which confirmed the presence of phenol (Sofowora, 1999). This procedure was repeated for all the extracts.

2.3.8. Determination of phlobatannis

The occurrence of a red cloudy colouration observed when the each aqueous extract was boiled with 1% HCl confirmed the presence of Phlobatannis.

2.3.9. Determination of reducing sugar

About 0.5 g of the extract was dissolved in 5 mL distilled water, two drops of Fehling solution was added and the solution was heated. The disappearance of the blue colour of Fehling solution and appearance of red precipitation confirmed the presence of reducing sugar.

2.4. Determination of the Antifungal Activities of Methanol, N-hexane and Ethyl Acetate Extracts of Annona Muricata Bark

Fungal isolates were grown on Sabouraud dextrose agar at 25 ^{0}C till they sporulated. The fungi spores were harvested into the broth and standardized. 200 µL of the standardized broth was used to seed 20 mL of Sabouraud dextrose agar in Maccartney bottles and these transferred into a sterile petri dish after swirling. Wells (6 mm diameter) were bored on the agar with sterile cork borer. 1 mg/L of the each extract was transferred into a well and another well was filled with 1 mg/L of amphotericin B as a positive control. Proper diffusion of compound into the agar was ensured and the plates were incubated at 25 ^{0}C for 96 hours and the zones of inhibition were determined.

2.5. Determination of the Antibacterial Activities of Methanol, N-hexane and Ethyl Acetate Extracts of Annona muricata Bark

The tested bacterial were inoculated into tubes of peptone water and incubated for 18 hours at a temperature of 37 ^{0}C before usage. Each of the cultures was adjusted to standard of 0.5 Mcfarland turbidity. 200 µL of the standardized cell suspensions were used to seed 20 mL sterile molten Mueller Hinton agar in Maccartney bottles and transferred into sterile plates , swirled and allow to coagulate. A (6 mm diameter) sterile cork borer was used to bore wells into which 0.2 mL of 2 mg/L of each extract were transferred. About 1 mg/L of the positive control (Streptomycin) were transferred into the remaining wells. After the pre-diffusion of the cultured plates, they were incubated at 37 ^{0}C for about 24 hours. The diameters of zones of inhibition were measured.

2.6. Determination of Minimum Inhibitory (MIC) of Methanol, N-hexane and Ethyl Acetate Extracts of Annona muricata Bark

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Two folded dilutions of each of the most active extracts 20, 10, 5, 2.5, 0.625 mg/L were prepared and transferred into each well bored into the agar plates. After proper diffusion, they were incubated at 37 ^{0}C for about 24 hours for the bacterial stains. Samples were taking from plates with no visible stains growth of MIC assay, sub cultured on freshly prepared agar plates and incubated at 37 ^{0}C for about 48 hours.

2.7. Computational Method

Quantum chemical method via density functional theory with the use of 6-31G* basis set which comprise of Becke's gradient exchange [21] and Lee, Yang and Parr correlation [22] was use to optimize selected ten (10) molecular compounds (Figure 1). Highest occupied molecular orbital (E_{HOMO}) energy, lowest unoccupied molecular orbital (E_{LUMO}) energy, band gap, chemical potential, chemical hardness, dipole moment, hydrogen bond donor (HBD), hydrogen bond acceptor (HBA) were the molecular descriptors obtained with the use of Spartan '14 software by wavefunction Inc. More so, the relationship between the studied ligands and the studied bacteria and fungi cell lines were observed via docking study. The software used to observe the link between the studied compounds and the enzymes are Discovery studio, AutoDock Tool, AutoDock Vina and Pymol. In this work, different grid size and grid centres as well as 1.000Å (grid point spacing) were used for this docking Study. The exhaustiveness value used for the docking runs was set to be 8 (default value) and nine (9) conformation each were examined together with their individual affinity.

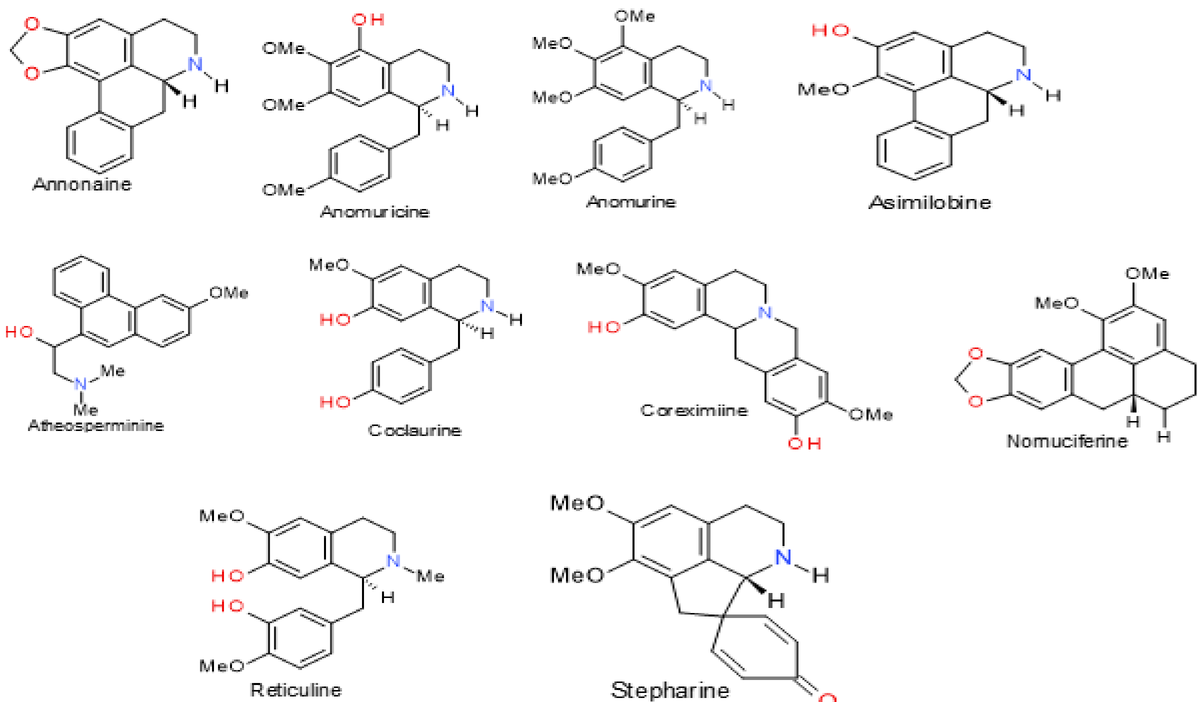

Figure 1: Schematic Structure of the studied compounds [23]

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3. Results and Discussion

3.1. Phytochemical Analysis of Methanol, N-hexane and Ethyl Acetate Extract of Annona muricata Bark

This study revealed that the methanol and ethyl acetate extract contained all secondary metabolites investigated except for alkaloids which was absent in both extract while the n-hexane extract only contained, cardiac glycosides, flavonoids, reducing sugar, saponins, tannins and flavonoids as shown in table 1.0. The presence of these phytochemicals indicate that the bark of *Annona muricata* possess some chemical compounds and medicinal properties that can be used in drug discovery.

Table 1. Result of phytochemical analysis of methanol, n-hexane and ethyl acetate extracts of *Annona muricata* bark

Tests	Methanol	n-hexane	Ethyl acetate
Alkaloids	-	-	-
Flavonoids	+	+	+
Phenols	+	-	+
Tannins	+	+	+
Saponins	+	+	+
Reducing sugar	+	+	+
Hydroxyl anthraquinones	+	-	+
Cardiac glycoside	+	+	+
Phlobatannis	+	-	+

The + (plus sign) for positive results for the tested bioactive compounds while – (minus sign) for the negative results for the tested result.

Antifungal activity of methanol, n-hexane and ethyl acetate extracts of *Annona muricata* bark

The preliminary screening for antifungal activity of *Annona muricata* bark showed, that the methanolic extract exhibited maximum inhibitory zone (27 mm) against *Aspergillus flaws*, the n-hexane extract showed maximum inhibitory zone (38 mm) against *Penicillium camemeri* while the ethyl acetate extract showed maximum inhibitory zone (31 mm) against *Fusarium oxysperium* as shown in table 2. Results obtained from this study, shown that, *Annona muricata* extracts possess higher antifungal activity than the commercially available antibiotics. For instance, Amphotericin B showed lower maximum zones of inhibition compare to all extracts of *Annona muricata* against the tested fungi. This suggests that the use of Annona *muricata* extracts can be of great significance for therapeutic treatment due to the antimicrobial properties it possess. The antifungal investigation in this study corroborates with studies

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of many authors.

Table 2. Result of the antifungal activities of the methanol, n-hexane, DCM, n-butanol and ethyl acetate extract of *annona muricata* leave

Tested Fungi	Inhibition zones (mm)			
	methanol 2 mg/L	n-hexane 2 mg/L	Ethyl ecetate 2 mg/L	Amphotercin B 1 mg/L
Aspergillus flaws	27	25	24	20
Candida albican	26	26	25	22
Fusarium oxysperium	25	28	31	21
Penicillium camemeri	26	38	26	23

Antibaterial activity of methanol, n-hexane and ethyl acetate extracts of *Annona muricata* bark

The inhibition zone diameters of the tested bacteria against the various extracts are shown in table 3. The inhibition zones ranges from 21- 31 mm. The extracts inhibited the growth of all the tested bacteria and compete favorably with Streptomycin use as standard. Highest inhibition zone of the extract was observed in ethyl acetate extract against *Bacillus subtilis* and methanol extract against *Klebsiella pneumonia* (31mm) while the lowest inhibition zone was observed in n-hexane extract against *Staphyloccus aureus* (21mm). The extracts compete favorably with the commercial available antibacterial streptomycin in terms of the minimum zones of inhibition The efficiency of *Annona muricata* as antibacterial for testing microorganisms agrees with those of [24] who demonstrated that Safflower meal has a good antimicrobial activity against a pathogen called (*L. monocytogenes).* The antimicrobial activity of *Annona muricata* against the tested bacteria might be as a result of the phytochemicals present in them which is in line with the report of [25] that the antimicrobial activity of plant extracts are due to the presence of phenolic compounds found in them. [25, 26] reported that the antimicrobial action of *Annona muricata* is likely due to membrane disruption by polyphenols and leakage of cellular constituents. Consequently, membrane-disrupting compounds cause the spillage of cellular contents and interfere with metabolic enzymes, bringing about the inactivation of bacteria [24].

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Table 3. Result of the bacterial activities of the methanol, n-hexane and ethyl acetate extract of *annona muricata* leave

Bacteria isolates	Inhibition zones (mm)			
	methanol 2 mg/L	n-hexane 2 mg/L	Ethyl acetate 2 mg/L	Steptomycin 1 mg/L
Bacillus subtilis	24	25	31	27
Clostridium sporogenes	29	27	27	24
Entrococcus faecalis	27	29	26	26
Klebsiella pneumonia	31	22	28	27
Pseudomonas aeruginosa	26	30	26	25
Staphyloccus aureus	26	21	26	24

Minimal inhibitory concentrations (MIC) of Annona Muricata extracts against bacteria

The minimum inhibitory concentrations (MICs) are the lowest concentrations of an antimicrobial that will prevent the visible growth of a microorganism after overnight incubation, while the lowest concentration of an antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media is called least bactericidal concentrations (MBCs) as [27] MICs are used mainly by diagnostic laboratories to ascertain resistance and a research tool to determine the *in vitro* action of new antimicrobials.

The minimum inhibitory concentration (MIC) test was carried out against *Bacillus subtili, Clostridium sporogenes, Entrococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa and Staphyloccus aureus* as shown in table 4. The result obtained shows that *Annona muricata* extracts has antibacterial activity against all the tested bacterial with MIC values ranging from 0.0156 – 1.0000 mg/ml except for ethyl acetate extract against *Clostridium sporogenes* and the n-hexane extract against *Entrococcus faecalis.* The results also shows that the n- hexane and ethyl acetate extract compete favourably with the standard control streptomycin used against all bacterial species except for the activity of n-hexane extract against (*Entrococcus faecalis* and *Staphyloccus aureus*) and the activity of ethyl acetate extract against (*Clostridium sporogenes* and *Staphyloccus aureus*) respectively. All the extracts also have least MIC values for *Staphyloccus aureus.* The prominent antibacterial activity of these extracts on tested bacterial shows that very small amount of the extracts is needed to inhibit the growth of all the tested bacteria except *Staphyloccus aureus*. The MIC values obtained in this study were lower than that obtained from the study of aqueous and methanol extracts of Safflower against the tested bacteria by [28].

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Table 4. Result of the minimum inhibitory concentration (MIC) of the methanol, n-hexane, and ethyl acetate extract of *annona muricata* bark

Bacteria isolates	Minimum Inhibitory Concentration MIC (mg/ml)			
	methanol	n-hexane	Ethyl ecetate	Streptomycin
	2 mg/ml	2 mg/ml	2 mg/ml	1 mg/ml
Bacillus subtilis	0.1250	0.5000	1.000	0.5000
Clostridium sporogenes	0.0625	0.5000	N.D	0.5000
Entrococcus faecalis	0.5000	N.D	1.000	0.1250
Klebsiella pneumonia	0.5000	1.000	1.000	1.000
Pseudomonas aeruginosa	0.1250	1.000	1.000	0.5000
Staphyloccus aureus	0.0156	0.0156	0.0156	1.000

N.D = Not Detected

Docking Score Result

The obtained result i.e. the binding affinity for the studied ligand-receptor relationship were shown in Table 6 and 7. The calculated binding affinity obtained from the docking of the studied ligand and the bacteria cell lines are shown in Table 5. According to [29] compounds with lower binding affinity value depict the molecular compound with propensity to inhibit. Therefore, the activity of the studied ligand were observed on four different fungi enzymes and it was discovered as shown in Table 5 that Annonaine possess the ability to inhibit *Candida albican* (PDB ID: 1q42) and *Fusarium oxysperium* (PDB ID: 5od4) as well as Corexímine and Reticuline inhibited *Aspergillus flaws* (PDB ID: 1cf3) and *Penicillium camemeri* (PDB ID: 2mhv) respectively.

Table 5: Docking score results for fungi receptors

Compound	*Aspergillus flaws* (kcal/mol)	*Candida albican* (kcal/mol)	*Fusarium oxysperium* (kcal/mol)	*Penicillium camemeri* (kcal/mol)
Annonaine	-9.4	-7.7	-7.0	-6.3
Anomuricine	-8.6	-6.3	-5.9	-6.0
Anomurine	-8.1	-5.9	-6.1	-5.6
Asimilobine	-8.5	-6.8	-6.3	-5.8
Atherosperminine	-7.6	-6.1	-5.6	-4.9
Coclaurine	-9.2	-6.0	-6.4	-6.0
Coreximine	-9.5	-6.5	-6.2	-6.1
Nornuciferine	-9.2	-6.5	-6.7	-6.5
Reticuline	-8.3	-6.3	-5.9	-6.6
Stepharine	-8.9	-6.3	-6.5	-6.0

Moreover, ten selected molecular compounds contained in *Annona Muricata* were also docked against 6 bacteria cell lines (*Bacillus subtilis, Clostridium sporogenes, Entrococcus faecalis, Klebsiella*

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pneumonia, Pseudomonas aeruginosa and *Staphyloccus aureus*). As shown in Table 6, it was shown that Annonaine played a serious role in inhibiting activity of *Annona Muricata*. In this work, Annonaine possesses the ability to inhibit *Clostridium sporogenes, Entrococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphyloccus aureus* cell lines. Coclaurine inhibited *Bacillus subtilis*. The interactions between the studied ligands and the studied fungi and bacteria cell lines were displayed in Figure 2-11.

Table 6: Docking score results for bacteria receptors

Compound	*Bacillus subtilis* (kcal/mol)	*Clostridium sporogenes* (kcal/mol)	*Entrococcus faecalis* (kcal/mol)	*Klebsiella pneumonia* (kcal/mol)	*Pseudomonas aeruginosa* (kcal/mol)	*Staphyloccus aureus* (kcal/mol)
Annonaine	-6.1	-7.6	-6.3	-5.2	-6.9	-6.9
Anomuricine	-5.5	-6.5	-5.4	-5.0	-5.9	-5.7
Anomurine	-5.9	-6.6	-5.1	-5.0	-5.4	-5.8
Asimilobine	-5.7	-7.0	-5.8	-4.9	-6.2	-6.0
Atherosperminine	-5.3	-6.3	-5.3	-4.8	-5.1	-6.2
Coclaurine	-6.2	-7.0	-5.5	-5.1	-5.7	-6.4
Coreximine	-5.7	-7.3	-5.4	-5.0	-6.4	-6.1
Nornuciferine	-5.8	-7.4	-5.7	-4.8	-6.6	-5.9
Reticuline	-5.8	-6.0	-5.7	-5.1	-5.9	-6.2
Stepharine	-5.7	-6.6	-5.3	-4.5	-6.0	-5.7

More so, the calculated molecular descriptors displayed in Table 7 showed that the compounds used in this work agreed with the Lipinski rule. According to Lipinski rule, the Molecular Weight value must be less than (≤) 500, Log P ≤ 5, HBD ≤ 5, and HBA ≤ 10 [30]. Therefore, the calculated molecular weight for all the compound used in this work were in line with rule. Also, Log P, HBA and HBD values were in accordance with the standard used in this work.

Table 7. The obtained calculated molecular descriptors used in this work

Compounds	Molecular Formula	Molecular Weight (amu)	Area	Vol	PSA	HBD	HBA	POL	Log P
Annonaine	$C_{17}H_{15}NO_2$	265.312	268.35	267.04	26.685	0	3	61.94	2.99
Anomuricine	$C_{19}H_{23}NO_4$	329.396	358.85	342.54	50.355	1	5	67.90	2.80
Anomurine	$C_{20}H_{25}NO_4$	343.423	385.21	365.33	38.715	0	5	69.68	3.06
Asimilobine	$C_{17}H_{17}NO_2$	267.328	281.77	276.74	36.238	1	3	62.68	2.70
Atherosperminine	$C_{19}H_{23}NO_2$	297.398	348.87	335.34	24.580	1	3	67.32	3.31
Coclaurine	$C_{17}H_{19}NO_3$	285.345	311.17	296.93	56.461	2	4	64.20	2.66
Coreximine Real	$C_{19}H_{21}NO_4$	327.380	341.37	330.58	52.572	2	5	66.90	2.61
Nornuciferine	$C_{19}H_{19}NO_4$	325.364	324.36	321.23	40.108	0	5	66.36	2.74
Reticuline	$C_{19}H_{23}NO_4$	329.396	355.91	342.55	52.818	2	5	67.88	2.92
Stepharine	$C_{17}H_{17}NO_3$	283.327	298.09	288.11	41.147	0	4	63.69	1.91

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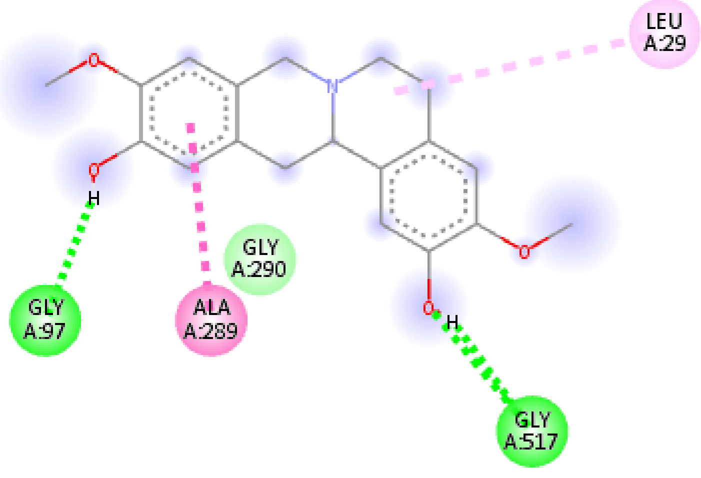

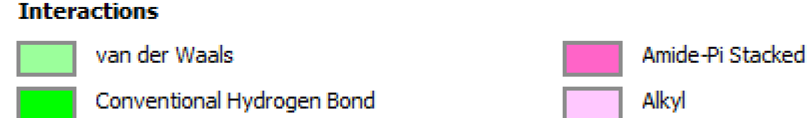

Fig. 2. Interactions of Coclaurine with the residue in the active site *Aspergillus flaws* cell line (1cf3)

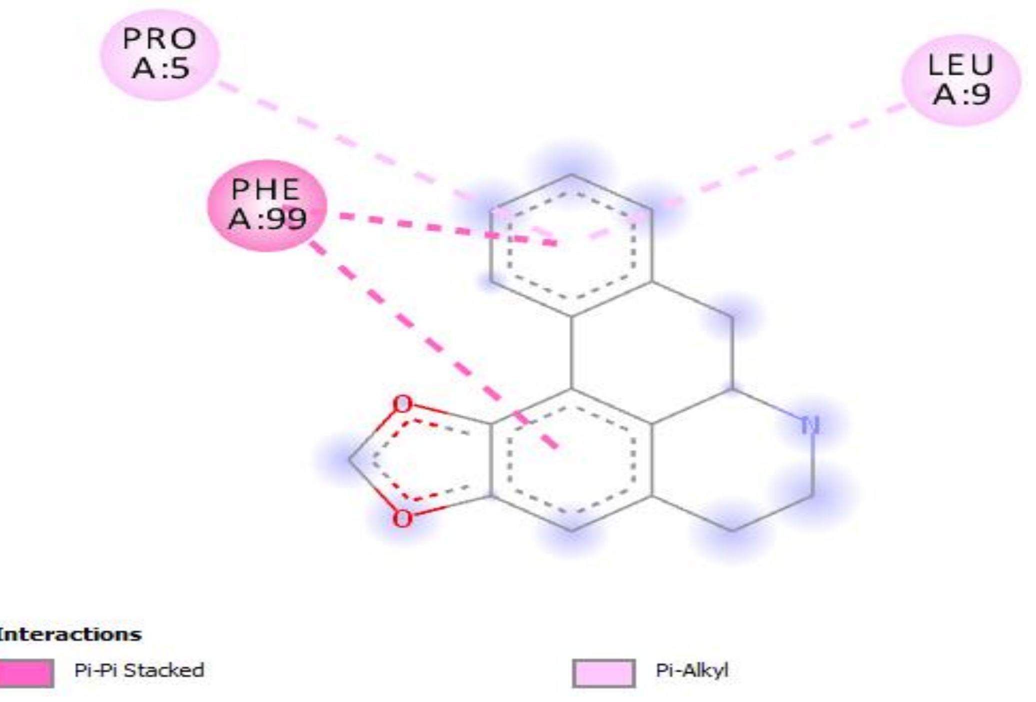

Fig. 3. Interactions of Annonaine with the residue in the active site *Candida albican* cell line (1q42)

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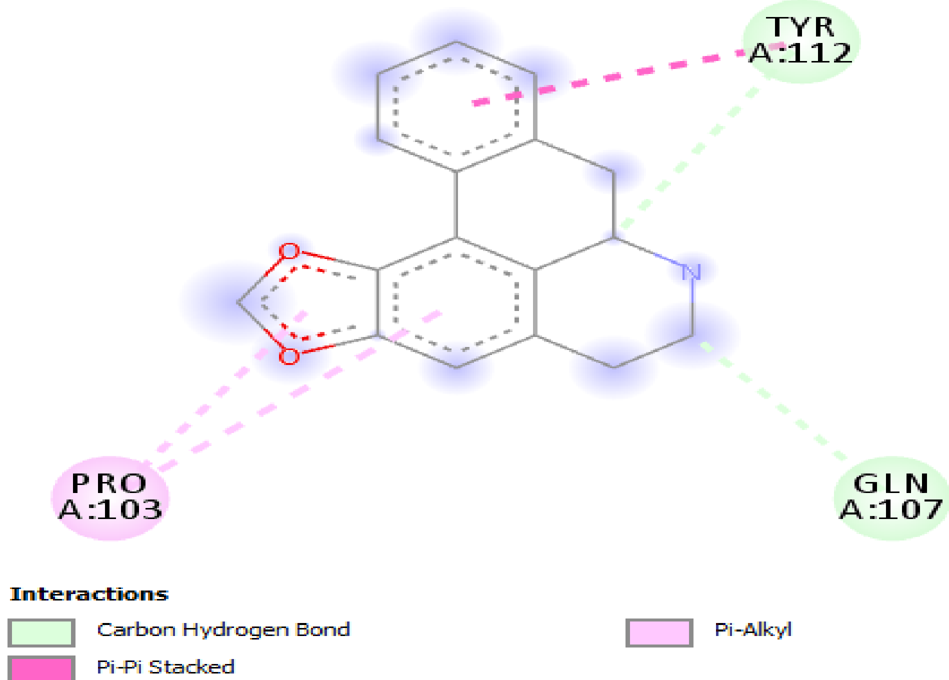

Fig. 4. Interactions of Annonaine with the residue in the active site *Fusarium oxysperium* cell line (5od4)

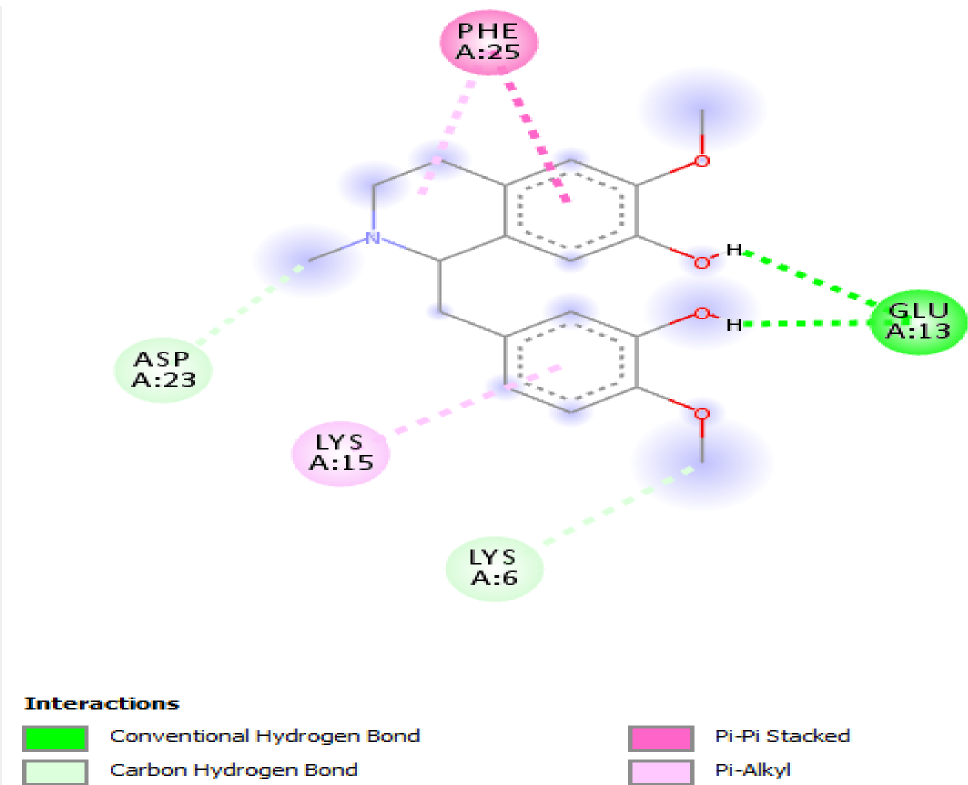

Fig. 5. Interactions of Annonaine with the residue in the active site of *Penicillium camemeri* cell line (2mhv)

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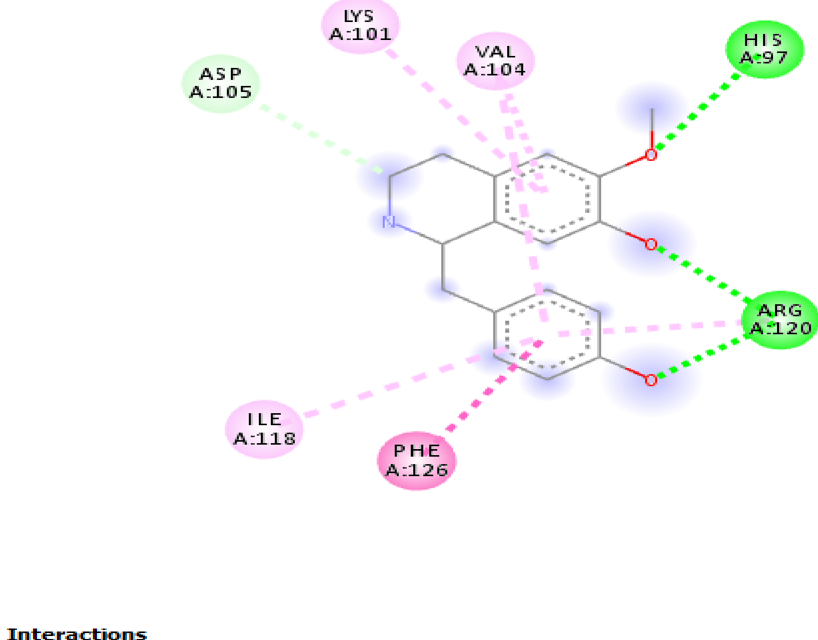

Fig. 6. Interactions of Coclaurine with the residue in the active site of *Bacillus subtilis* cell line (3zih)

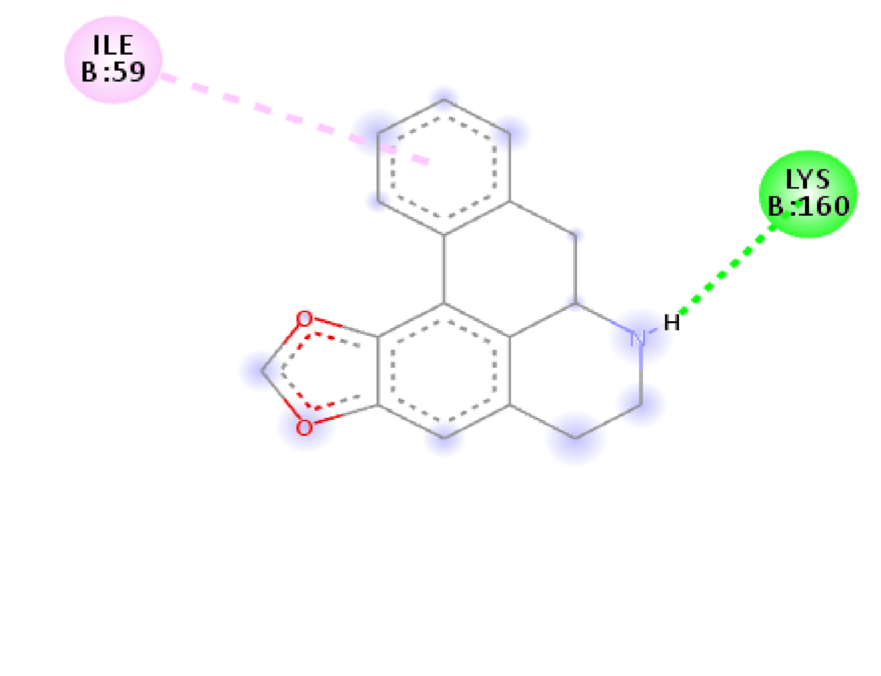

Fig. 7. Interactions of Annonaine with the residue in the active site of *Clostridium sporogenes* cell line (4q0y)

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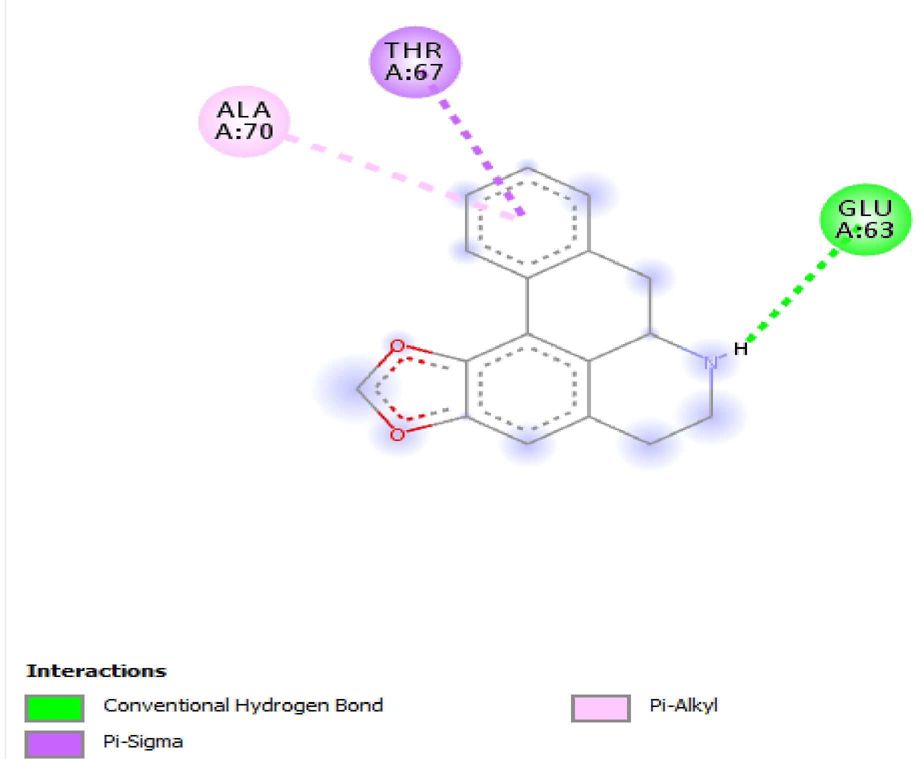

Fig. 8. Interactions of Annonaine with the residue in the active site of *Entrococcus faecalis* cell line (2n50)

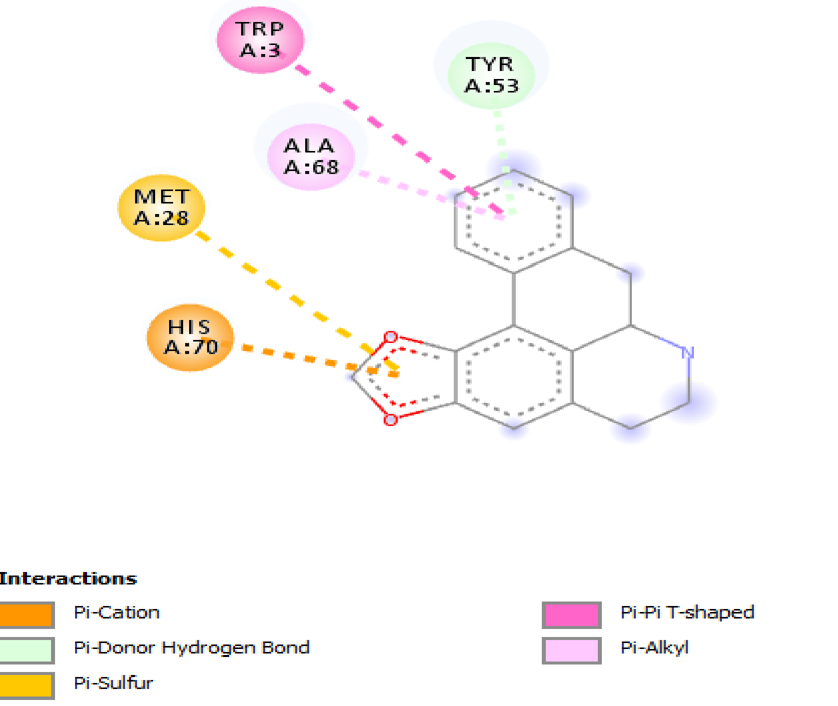

Fig. 9. Interactions of Annonaine with the residue in the active site of *Klebsiella pneumonia* cell line (2rqx)

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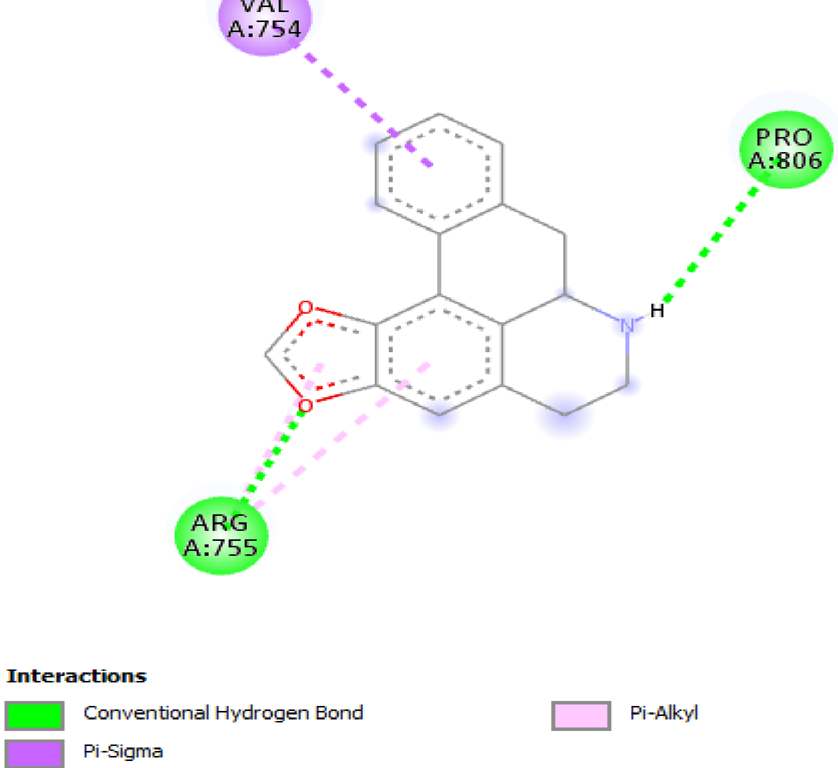

Fig. 10. Interactions of Annonaine with the residue in the active site of *Pseudomonas aeruginosa* cell line (2j5o)

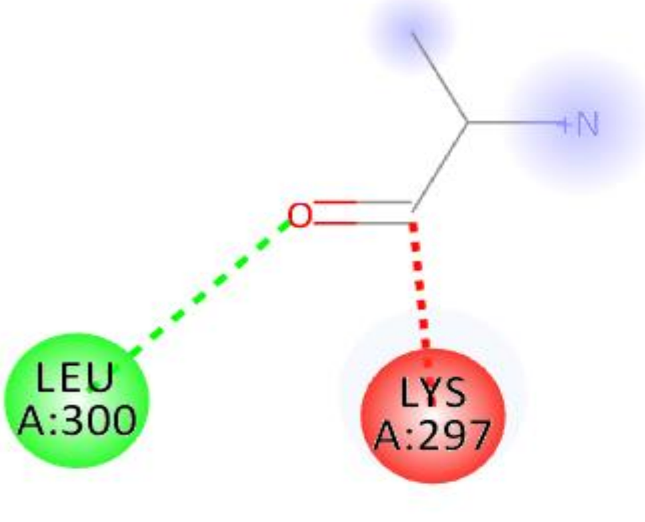

Fig. 11. Interactions of Annonaine with the residue in the active site of *Staphyloccus aureus* cell line (4g8x)

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4. Conclusions

This study shows that *Annona muricta* bark possess some valuable phytochemicals, good antifungal and antibacterial activities that can be used against the multidrug resistant pathogenic bacteria and fungi. Therefore there is a need for exploration and development of new antimicrobial agents from Annona *muricta* bark sources and to study its phytochemicals and applications in food additives. In addition, clinical trials concerning the rich pharmaceutical potential of *Annona muricta* should be encourage and investigations on the biological activities to perform to gain insight to develop new pharmaceutical agents. Furthermore, the docking study between the studied ligands and series of fungi and bacteria cell lines revealed the interactions that existed between the ligand-receptor relationships. In this work, the calculated descriptors agreed well with Lipinski rule. Also, Annonaine inhibited *Candida albican* (PDB ID: 1q42) and *Fusarium oxysperium* (PDB ID: 5od4) and Coreximine Real and Reticuline inhibited *Aspergillus flaws* (PDB ID: 1cf3) and *Penicillium camemeri* (PDB ID: 2mhv) respectively more than other studied compounds. More so, the activity of Annonaine against the bacteria cell lines used in this work reveal the effectiveness of Annonaine as an anti-bacteria agent. Annonaine inhibited *Clostridium sporogenes, Entrococcus faecalis, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphyloccus aureus* cell lines than other studied compounds and Coclaurine inhibited *Bacillus subtilis*.

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