Pharmacogn J. 2020; 12(5): 977-983
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original article

In Vitro Antibacterial and *In Silico* Toxicity Properties of Phytocompounds from *Ricinus Communis* Leaf Extract

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History

- Submission Date: 22-04-2020;
- Review completed: 19-05-2020;
- Accepted Date: 27-05-2020.

DOI : 10.5530/pj.2020.12.138

Article Available online

http://www.phcogj.com/v12/i5

ABSTRACT

Background: The rapid occurrence of multiple drug resistance and adverse side effects of aliphatic medicine threatens human health. Medicinal plants are known to possess phytocompounds with antibacterial activity and less toxic effects. **Objective:** This study aimed at determining the chemical composition of the methanolic *Ricinus communis*` leaf extract and evaluate their antibacterial and toxic effects**. Methods:** *R. communis* leaves were extracted by acetone, chloroform, ethanol and methanol. The extracts were assessed for antibacterial activity against *Bacillus cereus* (ATCC 10102), *Escherichia coli* (25922), *Staphylococcus aureus* (25923) and *Pseudomonas aeruginosa* (ATCC 27853) using agar-well diffusion and microwell dilution methods. The extracts were screened for alkaloids, flavonoids, saponins, steroids, tannins and terpenoids. The chemical constituents of the methanolic extract were analysed by gas chromatography – mass spectrophotometry (GC-MS). *In silico* toxicity of the phytocompounds were investigated using PreADMET tool. **Results:** The methanol extract showed the antibacterial activity against the bacterial strains, with the MIC values of 1.56 mg/mL against *B. cereus,* 3.13 mg/mL and 6.25 mg/mL against *P. aeruginosa* and *E. coli.* The extracts revealed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids and saponins. The GC-MS showed phytocompounds namely hexadecanoic acid, methyl ester (0.62%), tridecanoic acid (0.76%), pentafluoropropionic acid, nonyl ester (0.85%), 10-octadecanoic acid, methyl ester (2.93%) and cis-vaccenic acid (94.84%). Hexadecanoic acid, methyl ester was predicted not to have mutagenic and carcinogenic effects. Moreover, all compounds exhibited low inhibitory risks against hERG gene. **Conclusion:** *R. communis* leaf extract has potential to be used as a safe source of therapeutic compounds.

Key words: *Ricinus communis*, Chemical compounds, Antibacterial activity, Toxicity.

INTRODUCTION

The discovery of antimicrobial agents has revolutionized healthcare and prolonged life expectancy globally.[1] However, in the last decades the effectiveness of the antimicrobials decreased as new resistance mechanisms are emerging and spreading among pathogens, creating multi resistant and even extreme resistant microorganisms.[2] The resistance is mainly due to the remarkable genetic modification of the microorganisms, poor quality drugs, inadequate dosing, poor patient compliance and the increased mobility of the world population.[3] Without effective antimicrobials, there would be a significant increase in mortality rates and economic meltdown, globally. Therefore, the need to search for alternative remedy from natural origin has become of paramount importance.

Medicinal plants have been acknowledged as potential sources of new compounds of therapeutic value and as sources of prime compounds for drug design and development.[4] They have ability to produce a wide variety of secondary metabolites that include; alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins.[5] These biomolecules possess antimicrobial, antioxidant, anti-quorum sensing and anti-inflammatory activities.[6,7] Although plant-based products are recognised for their profound pharmacological activities, there are limited toxicity studies conducted. This is because plant-based products are generally perceived to be safe as they are of natural origin and not synthetic.[8] However, the biosafety evaluations are essential. Hence, one of the main priorities in drug discovery process is the identification of the toxicity profiles of the lead compounds.[9]

Computational *in silico* methods have gained attention for drug safety assessment. This is because these methods are cost-effective and time saving compared to the conventional methods (*in vivo* and *in vitro* methods).[10] Moreover, computational models provide advantages for practical findings and help in making cost-effective assessments prior to the costly process of drug development.[11] Although *in silico* toxicity prediction methods are well established in medicinal synthetic chemistry, their application in the field of natural compounds is still not being explored.[12]

Ricinus communis is a well-recognized medicinal plant that belong to *Eurphobiceae* family. It is abundant in the northern KwaZulu Natal, South Africa. The plant is commonly used as a decoction to increase milk secretion, treat tumours and as an

Cite this article: Mboyazi SN, Nqotheni MI, Maliehe TS, Shandu JS. *In Vitro* Antibacterial and *In Silico* Toxicity Properties of Phytocompounds from *Ricinus Communis* Leaf Extract. Pharmacogn J. 2020;12(5):977-83.

eye lubricant.[13] It is reported to possess therapeutic properties such anticancer, antidiabetic, antimicrobial, antioxidant, laxative, antiulcer, anticancer, antiasthmatic, anti-inflammatory and wound healing.[14-19] Phytochemical screening has revealed the presence of flavanols, glycosides, alkaloids, flavonoids, saponin and steroid in different parts.[20] Biologically active compounds such as rutin, gentistic acid, quercetin, gallic acid, kaempferol-3-O-β-D-rutinoside, ricin-A, ricin, *Ricinus* agglutinin, α-pinene and α-thujone have being extracted different parts of *R. communis*.[21] Although there are many studies conducted on this plant, we assumed that harvesting the same plant at different geographic location, season and soil type can still yield novel phytochemicals of pharmacological importance.

The study aimed at evaluating the *in-vitro* antibacterial and *in silico* toxicity properties and the chemical composition of *Ricinus communis`* leaf extract. The *in vitro* antibacterial activity was investigated using agar-well diffusion and microwell dilution methods. The chemical constituents were identified using the standard biochemical techniques and gas chromatography - mass spectrophotometry (GC-MS). Lastly, the *in silico* toxicity of the identified phytocompounds were investigated using PreADMET online tool.

MATERIALS AND METHODS

Chemicals and media

All the chemicals and media used in this study were of analytical grades and were procured from Sigma Aldrich Co. Ltd (Steinheim, Germany) and Merck (Ltd) Pty.

Microorganisms

The bacterial strains (*Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 10102), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853)) were obtained from the Microbiology Laboratory, University of Zululand, South Africa.

Ricinus communis preparation and extraction

Healthy green *Ricinus communis* leaves were harvested from Felixton, Empangeni in KwaZulu-Natal and transported to the University of Zululand, South Africa prior to authentication at Department of Botany Herbarium. The plant sample was allocated specimen number SNM01 and stored at the herbarium. The leaves were washed with gentle running tap water, dried at room temperature and ground to a fine powder using a mechanical grinder. Ten grams of the powdered sample was extracted with 100 mL of acetone, chloroform, ethanol and methanol respectively, then subjected to shaking incubator at 25 °C, 110 rpm for 72 h. The extract was filtered using Whatman No.1 filter paper, transferred into pre-weighed watch glass plates. The extract was then evaporated under a stream of air in at room temperature for efficiency enumeration of extraction.[22,23]

Antibacterial activity

Agar-well diffusion assay

The extract was evaluated for its antibacterial activity against two Gram positive bacteria: *S. aureus* (ATCC 25923) and *B. cereus* (ATCC 10102) and two Gram negative bacteria: *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853). The fresh bacterial cultures were grown to exponential phase and were adjusted to MacFarland standard (1.5 X 10^6 cf/mL) using spectrophotometer (Spectroquant-Pharo 100). The bacterial lawns were made on Mueller-Hinton agar using sterile swabs and 4 wells were bored. Thereafter, 100 µL of the methanolic extract was pippetted into the wells. The plates were incubated at 37 °C overnight and zones of inhibition were recorded.[24]

Minimum inhibitory concentration (MIC)

The MIC of the extract was determined using broth dilution method in a sterile 96-well plate in accordance with Eloff.[25] Briefly, 100 µL of Mueller-Hinton broth was added into the wells, followed by addition of 100 µL of the extract (100 µL) in the first row. The extract was serially diluted to vary the concentration. About 100 µL of fresh bacterial cultures which were adjusted to MacFarland standard (1.5 X10^6 cf/mL), were pipetted into the wells. Ciprofloxacin served as a positive control while 10 % DMSO was a negative control. The plates were incubated at 37 °C. After 24 h of incubation, 40 µL of p-iodonitrotetrazolium violet (0.2 mg/mL) was added into the wells and incubated at 37 °C for 20 min. The MIC was considered as the lowest concentration that inhibited bacterial growth.

Minimum bactericidal concentration (MBC)

The wells that demonstrated no visible bacterial growth during MIC evaluation were streaked on nutrient agar for MBC evaluation. The plates were incubated at 37 °C for 24 h. The lowest concentrations that completely killed the bacteria was considered as the minimum bactericidal concentration of the extract.[26]

Phytochemical analysis

Preliminary phytochemical screening of *R. communis* crude extract was conducted for detection of phytocompounds.[27] The analysis of volatile compounds from the methanolic extract was conducted using gas chromatography-mass spectrometry (THERMO Gas Chromatography TRACE ULTRA VER: 5.0.). Firstly, the helium gas` flow rate was set to 1 mL min^{-1}, with 1:50 spit ratio. The injector temperature was adjusted to 250 °C with the detector temperature fixed at 280 °C. The column temperature was kept at 40 °C for 1 min predated by linear programming increasing the temperature from 40 - 120 °C. Two microlitres of the methanolic extract was injected for analysis. Mass spectra required in the scan mode was 70 eV in the range of 50 - 50 m/z.[28]

Toxicity of the compounds

The toxicological properties of the compounds were calculated using an online server PreADMET (https://preadmet.bmdrc.kr/). The compounds were first drawn and then subjected for evaluation of toxicity by selecting ToxAlert option. Thereafter, mutagenicity and carcinogenicity profiles and inhibition of the human ether-a-go-go related gene (hERG) by the of the compounds were noted.[29]

Statistical analysis

Experiments were all done in triplicate and data was expressed as mean ± standard deviation. The statistical analyses were performed by one-way analysis of variance and considered to be significantly different at $p < 0.05$.

RESULTS

Agar-well diffusion assay

The antibacterial activity of *R. communis* leaf extracts was determined through agar-well diffusion assay and the results are displayed in Table 1. The methanolic extract exhibited the highest antibacterial activity in comparison with other extracts, with the inhibitory zones of 24.33, 24.00 25.33 and 25.7 mm against *B. cereus* (ATCC 10102), *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853), respectively. The acetone extract was the second most active extract, exhibiting inhibitory activity against *B. cereus* (ATCC 10102) (21.7 mm), *S. aureus* (ATCC 25923) (22.7 mm), *E. coli* (ATCC 25922) (23.0 mm) and *P. aeruginosa* (ATCC 27853) (20.3 mm). The ethanolic extract also showed antibacterial activity ranging from 20.00 - 22.33 mm against the bacterial strains. The chloroform extract was the least

active extract against the selected bacteria giving zones of inhibition in a range of 0.0 to 18.7 mm.

MIC and MBC of the methanolic extract

The minimum inhibitory concentration of the most active extract during agar-well diffusion method (methanolic extract) was evaluated by serial dilution. The lowest MIC values of 1.56 was obtained against *S. aureus* (ATCC 25923) while highest MIC value of 6.25 mg/mL was observed against *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) (Table 2).

Preliminary phytochemical screening of *R. communis* crude extract

The preliminary evaluation of the classes of the chemical constituents of *R. communis* leaf material were done and the results are presented in Table 3. The *R. communis`* leaves showed the presence of alkaloids, flavonoids, glycosides, saponins, steroids, tannins and terpenoids.

GC-MS analysis of the *R. communis* methanolic left extract

The methanolic leaf extract under GC-MS chromatogram gave a yield of 5 volatile compounds (Table 4). The compounds are hexadecanoic acid, methyl ester (0.62%), tridecanoic acid (0.76%), pentafluoropropionic acid, nonyl ester (0.85%), 10-octadecanoic acid, methyl ester (2.93%) and cis-vaccenic acid (94.84%).as the major component.

In silico toxicity of the phytocompounds

In silico mutagenicity, carcinogenicity and hERG inhibition of the phytocompounds are presented in Table 5. Hexadecanoic acid, methyl ester was the only compound estimated to be non-mutagenic. Tridecanoic acid is the only compound which did show the ability to cause carcinogenic effect in mice. All the identified compounds are predicted not to possess carcinogenic effects on rats and showed low risks of blocking hERG.

DISCUSSION

The occurrence of multi-drug resistance by bacteria against conventional antimicrobial agents raises the need for a search for alternative and potent drugs. Plants derived compounds are recognised for their pharmacological properties that include antibacterial activities. In the present study, the extract derived from the leaves of *R. communis* were evaluated for their antibacterial potencies and toxicity.

Antibacterial activity

The antibacterial activity of the different leaf extracts was evaluated using agar well diffusion assay. The extracts displayed broad spectrum

Table 1: Antibacterial activity of *Ricinus communis* leaf extracts.

Bacteria	Antibacterial activity (mm)			
	Acetone	Chloroform	Ethanol	Methanol
B. cereus (ATCC 10102)	23.0	18.7	22.7	24.3
E. coli (ATCC 25922)	21.7	0.0	20.0	24.0
S. aureus (ATCC 25923)	22.7	13.0	23.0	25.7
P. aeruginosa (ATCC 27853)	20.3	0.0	22.3	25.3

Table 2: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the methanolic extract.

Bacteria	Extract		Ciprofloxacin	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
P. aeruginosa (ATCC 27853)	6.25 ± 00	> 50 ± 00	25 ± 00	> 50 ± 00
E. coli (ATCC 25922)	6.25 ± 00	> 50 ± 00	25 ± 00	> 50 ± 00
S. aureus (ATCC 25923)	1.56 ± 00	> 50 ± 00	25 ± 00	> 50 ± 00
B. cereus (ATCC 10102)	3.13 ± 00	> 50 ± 00	25 ± 00	> 50 ± 00

Table 3: Preliminary phytochemical screening of the crude leaf extracts.

Phytochemicals	Result
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+

Key: + denotes presence and – denotes absence

Table 4: Volatile compounds obtained from *R. communis* methanolic extract.

Number of compounds	Compounds	Area (%)
1	Hexadecanoic acid, methyl ester	0.62
2	Tridecanoic acid	0.76
3	Pentafluoropropionic acid, nonyl ester	0.85
4	10-Octadecanoic acid, methyl ester	2.93
5	cis-Vaccenic acid	94.84

Table 5: The *in silico* toxicity profiles of the identified compounds.

Compounds	Toxicity			
	Mutagenicity (Ames test)	Carcinogenicity		
		Rat	Mouse	hERG inhibition
Hexadecanoic acid, methyl ester	Non mutagen	Positive	Positive	Low risk
Tridecanoic acid	Mutagen	Positive	Negative	Low risk
Pentafluoropropionic acid, nonyl ester	Mutagen	Positive	Positive	Low risk
10-Octadecanoic acid, methyl ester	Mutagen	Positive	Positive	Low risk
cis-Vaccenic acid	Mutagen	Positive	Positive	Low risk

of antibacterial activity against all tested strains to some degree (Table 1). However, the methanolic extract exhibited the highest antibacterial activity in comparison with other extracts (acetone, chloroform and ethanolic extracts). The promising antibacterial activities observed by the methanol extract in could be due to the variation in the number of antibacterial constituents in the extract because of the polarity of methanol and solubility of the compounds in it in comparison to other solvents.[30] Similar findings were observed other studies.[31,32]

The promising results demonstrated by the methanolic extract prompted us to evaluate its MIC and MBC using microdillition method. The extract revealed antibacterial activity ranging from 1.56 - 6.25 mg/mL against the tested bacterial strains (Table 2). It should also be noted that the MIC of the methanolic extract was lower than that of ciprofloxin, indicative of the resistance of the selected bacterial strains to this drug. Rendering to the antibacterial activity results in both agar well and dilution methods, the Gram-positive strains were more susceptible to the extract in comparison to the Gram negative strains. This could be due to the differences in the cell walls of these classes of bacteria, as Gram negative bacterial strains are often resistant to most antimicrobial agents due to their outer membrane, which tends to exclude some antimicrobials from penetrating the bacterial cells by acting as a selective barrier.[33,34] Our findings are in agreement with the results from the study by Abd-Ulgadi and the colleagues, whereby the *R. communis* leaf extract displayed profound activity against Gram positive bacteria in comparison to the Gram negative bacteria.[35] The extract did not demonstrate any MBC within the tested concentration (Table 2). Therefore, it was concluded that the extract only possesses the bacteriostatic effect and not bactericidal effects within the tested concentrations.

Preliminary phytochemical screening of *R. communis* crude extract

The biological activities of chemical compounds from plants are of medical importance. The detected classes of phytocompounds have been recognised for their pharmacological properties that include antibacterial activity.[36] Moreover, their presence implies that the leaves of *R. communis* can serve as potential sources of bioactive antimicrobial compounds.

GC-MS analysis of the *R. communis* methanolic left extract

The GC-MS chromatogram profile of the methanolic leaf extract revealed a total of five volatile phytocompounds (Table 4). All the five identified volatile compounds are the prominent antimicrobial compounds and have other important pharmaceutical properties. Hexadecanoic acid, methyl ester has been reported to be abundant in most plants. It possesses antibacterial, anticancer, anti-inflammatory, haemolytic and cytotoxic activities.[37, 38] The 10-octadecanoic acid, methyl ester has antimicrobial, antioxidant, anti-arthritic, hypocholesterolemic, hepatoprotective properties.[39, 40] cis-Vaccenic acid is an omega-7 fatty acid commonly known for its antibacterial and hypolipidemic effects.[41] Tridecanoic acid and pentafluoropropionic acid, nonyl ester are also known to possess the antimicrobial activity.[42] Thus, the antimicrobial activity observed in this study was attributed to the presence of these compounds.

In silico toxicity of the phytocompounds

Mutagenic compounds are strongly linked to the occurrence of various diseases including cancer and neurodegenerative diseases.[43] The mutagenicity of the identified compounds was estimated by Ames test using PreADMET tool and the results are demonstrated in Table 5. Therefore, only these two compounds have no ability to alter the genetic makeup in biological systems, consequently causing undesirable side effects.[44] PreADMET was also used to evaluate the carcinogenicity of the compounds on mice and rats. According to PreADMET, the negative prediction results translate carcinogenic activity whereas positive means the compound does not have carcinogenic activity. Tridecanoic acid is the only compound which did show the ability to cause carcinogenic effect in mice whereas the other compounds are predicted not to possess carcinogenic effects on rats (Table 5). This implies that the compounds without the potential ability to induce carcinogenic effects are safe for use.[45] Pharmacological inhibition of the hERG channel can results in poor repolarisation and prolonged QT interval, consequently leading to sudden heart failure.[46] All the identified compounds showed low risk of blocking hERG (Table 5), which is an implication for potential commercial advantage as therapeutic agents.

CONCLUSION

The leaf extract showed the antibacterial activity against the tested bacterial strains, with the profound activity against the selected Gram positive bacteria. The extract revealed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, terpenoids and saponins. The GC-MS showed phytocompounds such as hexadecanoic acid, methyl ester, tridecanoic acid, pentafluoropropionic acid, nonyl ester, 10-octadecanoic acid, methyl ester and cis-vaccenic acid. The antibacterial activity was attributed by the presence of these phytocompounds. The *in silico* toxicity predictions reveal hexadecanoic acid, methyl ester not to have mutagenic and carcinogenic effects. Moreover, all compounds exhibited low inhibitory risks against hERG gene. For future study, the *in vitro* and *in vivo* studies of the identified compounds are recommended.

ACKNOWLEDGEMENTS

The authors are appreciative to the National Research Foundation of South Africa, the University of Zululand for providing financial support and necessary laboratory facilities.

CONFLICTS OF INTEREST

The authors declare/ no conflicts of interest.

ABBREVIATIONS

DMSO: dimethyl sulfoxide; rpm: revolution per minute; INT: p-iodonitrotetrazolium violet; µg/mL: microgram/milliliter; g: gram; mL: millimeter; °C: degree Celsius; µL: microliter; %: percent; EF: extract; hERG: human ether-a-go-go related gene; GC-MS: gas chromatography-mass spectrometry; GC: gas chromatography; MS: mass spectrometry; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; v: volume; *R. communis: Ricinus communis*; *S. aureus*: *Staphylococcus aureus*; *B. cereus*: *Bacillus cereus*; *E. coli*: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; ATCC: American Type Culture Collection.

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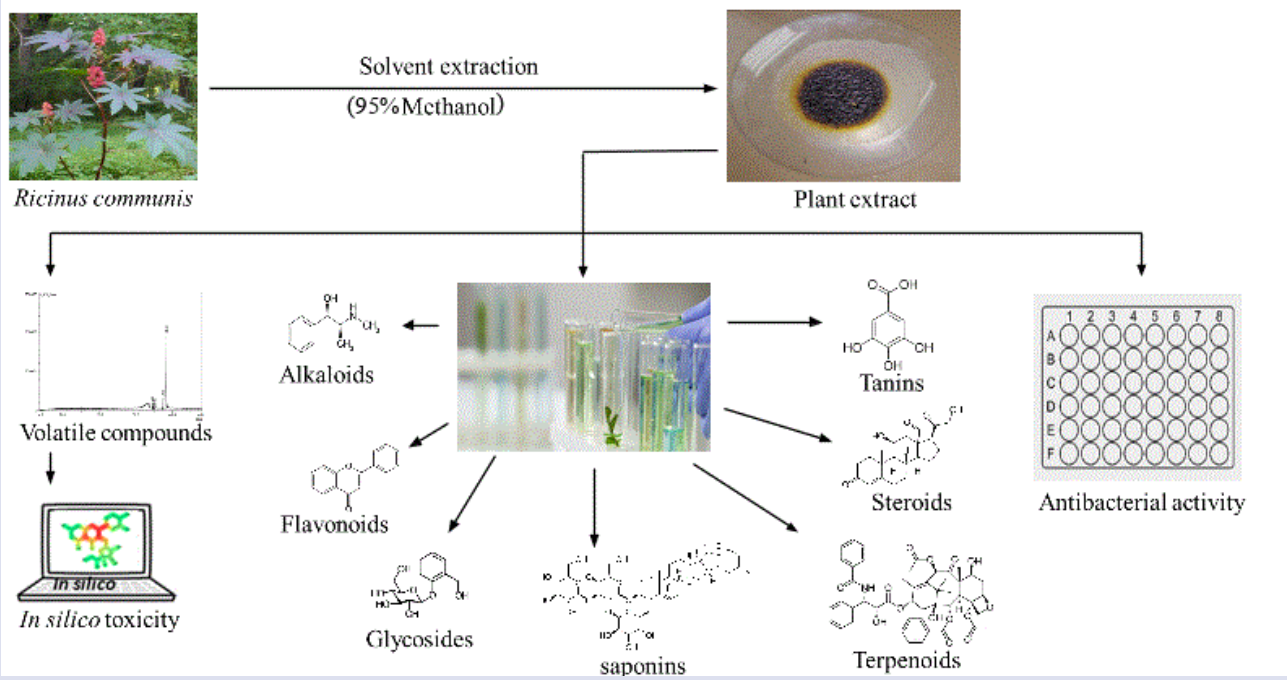
GRAPHICAL ABSTRACT



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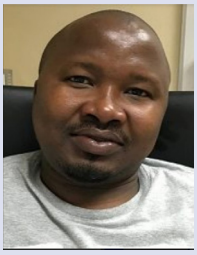

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Cite this article: Mboyazi SN, Nqotheni MI, Maliehe TS, Shandu JS. *In Vitro* Antibacterial and *In Silico* Toxicity Properties of Phytocompounds from *Ricinus Communis* Leaf Extract. Pharmacogn J. 2020;12(5):977-83.