Journal of Diseases and Medicinal Plants
2021; 7(2): 30-34
http://www.sciencepublishinggroup.com/j/jdmp
doi: 10.11648/j.jdmp.20210702.11
ISSN: 2469-8202 (Print); ISSN: 2469-8210 (Online)

Antimicrobial Properties of the Hydroethanolic Extract of *Bauhinia rufescens* L. and *Euphorbia hirta* L., Two Plants of the Traditional Chadian Pharmacopoeia

Emmanuel Issa[1, *], Adoum Fouda Abderrazzack[2], Kokou Anani[1], Ameyapoh Yaovi[1]

[1]Laboratory of Microbiology and Food Quality Control, Department of Medical and Biological Analysis, University of Lomé, Lomé, Togo

[2]Department of Biomedical Sciences, University of Health Sciences and Sanitation Toumai, Ndjamena, Chad

Email address:
issanuel175@gmail.com (E. Issa), razzackadoum@yahoo.fr (A. F. Abderrazzack)

[*]Corresponding author

To cite this article:
Emmanuel Issa, Adoum Fouda Abderrazzack, Kokou Anani, Ameyapoh Yaovi. Antimicrobial Properties of the Hydroethanolic Extract of *Bauhinia rufescens* L. and *Euphorbia hirta* L., Two Plants of the Traditional Chadian Pharmacopoeia. *Journal of Diseases and Medicinal Plants*. Vol. 7, No. 2, 2021, pp. 30-34. doi: 10.11648/j.jdmp.20210702.11

Received: March 11, 2021; **Accepted**: March 23, 2021; **Published**: April 7, 2021

Abstract: *Objective*: To evaluate the antimicrobial properties of hydroethanol extracts of *Bauhinia rufescens* L. and *Euphorbia hirta* L. *Methodology and results*: The hydroethanol extracts of *Bauhinia rufescens* L. and *Euphorbia hirta* L.; two plants of the Chadian traditional pharmacopoeia used in the treatment of infantile diarrhoea and gastroenteritis in adults were submitted to in vitro tests in order to highlight their antibacterial and antifungal properties. The method of microdilution in liquid medium coupled with spreading on agar medium was used for the tests. The microbial strains used consisted of 13 hospital bacterial strains and 6 reference strains including a yeast strain of the *Candida albicans* ATCC 90028 species. The results obtained confirm the antimicrobial properties of *Bauhinia rufescens* L. and *Euphorbia hirta* L. because at 50 mg/ml, they inhibit 100% the growth of gram positive and Gram-negative bacilli tested. On the other hand, for *Candida albicans* ATCC 90028, it is only the extract of Euphorbia hirta that was active at 50 mg/ml. *Conclusion*: In view of these results, we can say that these plants have an antibacterial activity and that their use in traditional phytotherapy is justified.

Keywords: *Bauhinia rufescens*, *Euphorbia hirta*, Anti-microbial Properties

1. Introduction

In developing countries, problems of access to quality medicines remain a concern. In Chad, the shortage of medicines in terms of quality and quantity is constant in the various health structures. Some peripheral health structures are inaccessible during the rainy season because of the poor state of the roads. The World Health Organization (WHO) estimates that approximately 80% of the population uses traditional herbal preparations [7]. Natural substances derived from plants are of great interest in industry, food, cosmetics and pharmacology. The increase in the resistance of microorganisms to the antimicrobial agents used is due to the misuse and inappropriate use of antibiotics, which is currently posing very serious problems for scientists and clinicians. Diseases caused by microorganisms are increasingly difficult to treat with existing drugs [16]. Thus, scientists have turned their attention to the search for new drugs of natural origin, but for this traditional medicine to be effective, it must provide indisputable scientific proof. Through this study, we want to contribute to the valorization of Chadian medicinal plants by evaluating the antimicrobial properties of two plants (*Euphorbia hirta* L. and *Bauhinia Rufescens L.*) used in traditional medicine in Chad for the treatment of childhood diarrhea and infectious gastroenteritis in adults.

2. Materials and Methods

2.1. Plant Material

The leaves of *Bauhinia rufescens* and the whole plant of *Euphorbia hirta* were harvested in September 2020 25 km from the city of Ndjamena (Chad) in a village called Marra. The harvested plant material was authenticated at the

herbarium of the Botany Department of the Faculty of Exact and Applied Sciences of Farcha of the University of Ndjamena (Chad).

2.2. Preparation of Extracts

The leaves of *Bauhinia rufescens* and the whole plant of *Euphorbia hirta* were dried at room temperature away from the sun and dust, and then crushed in a clean mortar before being reduced to a fine powder using an electric mill. 500 g of *Bauhinia rufescens* powder and 300 g of *Euphorbia hirta* powder were macerated in an ethanol/water mixture (70/30). The resulting mixture was incubated for 48 hours at laboratory temperature and frequently agitated. The macerate was then successively filtered with absorbent cotton before being filtered on Whatman N° 1 filter paper under vacuum pumping. The solvent was evaporated with Rotavapor and the total hydroethanol extracts obtained were used to prepare solutions with a concentration of 100 mg/ml which were sterilized by vacuum filtration on 0.45 µm millipore membrane. The recovered extracts were stored at 4°C in the refrigerator prior to testing.

2.3. Extract Yield

The yield of the extraction was determined by the ratio between the mass of the dry extract obtained after evaporation and the mass of the starting plant material, which is given by the following formula: Yield (%) = M1/ M0 X 100. M1= mass of the extract after evaporation, M0= mass of the vegetable starting material.

2.4. Microbial Strains

The microbial strains used are made up of pathogenic bacteria isolated and identified in a medical environment at the Bacteriology Laboratory of the Polyclinelle Wossinu-Gbogbode Lomé and reference strains.

Table 1. *Microorganisms tested.*

microorganisme	Famille	Gram	Provenance
Escherichia coli	Enterobacteriaceae	negative	Polyclinelle Wossinu
Salmonella enteritidis,	Enterobacteriaceae	negative	Polyclinelle Wossinu
Shigella dysenteriae	Enterobacteriaceae	negative	Polyclinelle Wossinu
Klebsiella pneumoniae,	Enterobacteriaceae	negative	Polyclinelle Wossinu
Pseudomonas aeriginosa	Pseudomonacea	negative	Polyclinelle Wossinu
Acinetobacter spp.	Enterobacteriaceae	negative	Polyclinelle Wossinu
Salmonelle typhi ATCC 14028	Enterobacteriaceae	negative	LAMICODA
Staphylococus aureus ATCC 25922	Enterobacteriaceae	positive	LAMICODA
Klebsiella pneumoniae ATCC 700603	Enterobacteriaceae	negative	LAMICODA
Escherichia coli ATCC 25922	Enterobacteriaceae	negative	LAMICODA
Pseudomonas aeruginosa ATCC 27853	Pseudomonacea	negative	LAMICODA
Candida albicans ATCC 90028	Saccharomycetacea		LAMICODA

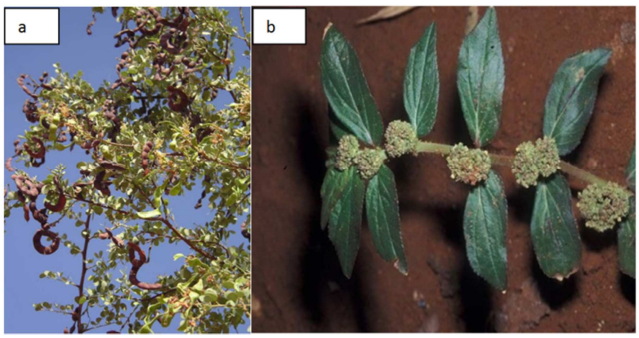

Figure 1. *Bauhinia rufescens (a) et Euphorbia hirta (b).*

2.5. Evaluation of Antimicrobial Activity

2.5.1. Preparation of the Microbial Suspension

The strains were transplanted on nutrient agar to have young 24-hour bacterial colonies.

2.5.2. Micro Dilution Technique

Microdilution in liquid medium is the reference method for the determination of MIC (Minimum Inhibitory Concentration). It consists in inoculating with bacterial strains a range of wells containing hydroethanol extracts to be tested at increasing concentrations. The MIC corresponds to the first dilution for which no bacterial growth is visible to the naked eye after 18 hours of incubation. The handling is done in a microtiter plate. The culture medium is a Müller Hinton broth. Different concentrations are prepared with dilutions (of gradient 2) starting from a concentration of 100 mg / ml of each extract. 100 µl of the bacterial suspension are distributed in test wells of the microplate containing the extracts. The control wells consist of: Broth alone, Broth + bacterial suspension and Broth + gentamycin. The plates are then incubated at 37°C for 24 hours. After incubation, possible growth is revealed by the presence of cloudiness at the bottom of the well. The MIC is defined as the minimum concentration of extract for which no growth visible to the naked eye is observed. Cups that have shown no visible microbial growth from the MIC and the next well diluted at ½ are re-isolated on nutrient agar. Seeding is done by spreading on the surface of the agar. After 24 h incubation in an oven at 37°C, the culture media are evaluated for (BMC). Thus, the action of an extract will be considered as bactericidal if the ratio CMB/CMI is equal to 1. The action is said to be bacteriostatic if the CMB/CMI ratio is greater than 1 [7].

2.6. Data Processing and Analysis

The data was analyzed using Microsoft office Excel 2007.

3. Results and Discussion

3.1. Result of Extraction

The extraction yield is summarized in the table below. It is expressed as a percentage in relation to the mass of the initial powder.

Table 2. *Yield of hydroethanolic extracts of Bauhina rufescens L. and Euphorbia hirta L.*

Plantes	Plants Quantity of plant powder	Quantity of extract after evaporation Yield	Rendement
Bauhinia rufescens (leaves)	500 g	46 g	9,2%
Euphorbia hirta (whole plant)	300 g	30 g	10%

Rdt (%)= M1/ M0 X 100. M1= mass of the extract after evaporation, M0= mass of the starting plant material.

3.2. Inhibitory Activities of the Hydroethanol Extract at 50 mg/ml

At a concentration of 50 mg/ml, the hydroethanolic extract of the leaves of *Bauhinia rufescens* L. and the whole plant of *Euphorbia hirta* L. totally inhibited the in vitro growth of all bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* ATCC 14028, *Salmonella enteritidis*, *Shigella dysenteriae*, *Klebsiella pneumoniae, Pseudomonas aeriginosa* and Acinetobacter spp)

Table 3. *Effect of hydroethanol extract on microbial growth of reference strains.*

Hydroethanol extract	Microorganism (reference strains)					
	***S. aureus* ATCC 25922**	***E. coli* ATCC 25922.**	***S.typhi* ATCC 14028**	***P. aeruginosa* ATCC 27853**	***Candida albicans* ATCC 90028**	***K.pneumonia* ATCC 700603**
Bauhinia rufescens (50 mg/ ml)	-	-	-	-	+	-
Bauhinia rufescens (25 mg/ ml)	-	-	+	-	+	+
Euphorbia hirta (50 mg/ ml)	-	-	-	-	-	-
Euphorbia hirta (25 mg/ ml)	-	-	-	-	-	+

+ = microbial growth
-= absence of microbial growth

Table 4. *Effect of hydroethanol extract on microbial growth of hospital strains.*

	Microorganism (hospital strains)						
	S.aureus	***E. coli***	***S. enteritidis***	***P.aeruginosa***	***K. pneumoniae***	***S.dysenteriae***	***Acinetobacter Spp***
Bauhinia rufescens (50 mg/ ml)	-	-	-	-	-	-	-
Bauhinia rufescens (25 mg/ ml)	+	-	+	-	+	+	+
Euphorbia hirta (50 mg/ ml)	-	-	-	-	-	-	-
Euphorbia hirta (25 mg/ ml)	+	+	+	-	+	-	+

+ = microbial growth
-= absence of microbial growth

At a concentration of 25 mg/ml of the hydroethanol extract of *Bauhinia rufescens*, the bacterial strains showed variable data. This extract of *Bauhina rufescens* completely inhibited the growth of the following hospital strains: *Pseudomonas aeruginosa, Staphylococcus aureus*, *Escherichia coli* and *Klepsiella pneumoniae*. On the reference strains, the 25 mg/ml extract inhibited 100% growth of Pseudomonas aeruginosa ATCC 27853, *S.aureus* ATCC 25922, *Klebsiella pneumonia* ATCC 700603 and *E. coli* ATCC 25922.

The hydroethanol extract of *Euphorbia hirta* at 25 mg/ml completely inhibited the growth of Pseudomonas aeriginosa, *Staphyloccoccus aureus* and *Shigella dysenteriae*. On reference strains, the 25 mg/ml extract inhibited 100% growth of *Pseudomonas aeruginosa* ATCC 27853, *S. aureus* ATCC 25922, *E. coli* ATCC 25922, *Salmonella typhi* ATCC 14028 and *Candida albicans* ATCC 90028.

Table 5. *Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of the different extracts and their interpretation.*

		Microorganism (reference strains)					
		***Staphylococcus aureus* ATCC 25922**	***E. coli* ATCC 25922.**	***Salmonella typhi* ATCC 14028**	***Pseudomonas aeruginosa* ATCC 27853**	***Candida albicans* ATCC 90028**	***K.pneumonia* ATCC 700603**
Bauhinia rufescens	CMI	12,5	12,5	50	12,5	100	50
	CMB	25	25	50	25	100	50
	CMB/CMI	2	2	1	2	1	1
Euphorbia hirta	CMI	12,5	12,5	12,5	12,5	12,5	50
	CMB	25	25	25	25	25	50
	CMB/CMI	2	2	2	2	2	1

Table 6. *Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of the different extracts and their interpretation.*

		Microorganism (hospital strains)						
		S. aureus	***E. coli***	***S.enteritidis***	***P.aeruginosa***	***K. pneumoniae***	***S.dysenteriae***	***Acinetobacter spp***
Bauhinia rufescens	CMI	50	12,5	50	12,5	50	50	50
	CMB	50	25	50	25	50	50	50
	CMB/CMI	1	2	1	2	1	1	1
Euphorbia hirta	CMI	50	50	50	12,5	50	12,5	50
	CMB	50	50	50	25	50	25	50
	CMB/CMI	1	1	1	2	1	2	1

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) as well as the type of action of the extracts exerted on the bacteria tested.

It should be noted that the evaluation of the antimicrobial activity of these plants was carried out on the hydroethanolic extracts. The plant powders were macerated on a mixture (methanol and water in a 70/30 ratio). This extraction method has the advantage of extracting a large quantity of the active ingredients but also these solvents have no effect on the germs tested.

The results of our work indicate that the hydroethanolic extract of the leaves of *Bauhinia rufescens*. and the whole plant of *Euphorbia hirta*. inhibit microbial growth. These plants exert their effect both on gram-positive cocci and gram-negative bacilli tested and also on yeasts (*Candida albicans* ATCC 90028*)*. These results corroborate with those of other researchers who have highlighted the antibacterial potential of *Bauhinia rufescens* and *Euphorbia hirta* extracts [1, 4, 2].

3.3. Sensitivity of Staphylococcus aureus

The hospital strain of *Staphylococcus aureus* and the reference strain (*Staphylococcus aureus* ATCC 25922) were all sensitive to the hydroethanolic extract of *Bauhinia rufescens* with a MIC of 12.5 mg/ml. Other work has also highlighted the sensitivity of *Staphylococcus aureus* to *Bauhinia rufescens* extracts with MICs varying according to the nature of the solvent used and my extraction method (14, 10, 5).

For the hydroethenolic extract of *Euphorbia hirta,* we obtained a MIC of 12.5 mg/ ml. These data corroborate with those obtained by Mahamat et al, 2010 in India with a MIC equal to 12.5 mg/ml. A lot of work has been carried out worldwide on *Euphorbia hirta* using different solvents to demonstrate the sensitivity of *Staphylococcus aureus* to *Euphorbia hirta* extract [1, 13, 6, 3, 11].

3.4. Sensitivity of Salmonella typhi ATCC 14028 and Salmonella enteritidis

Salmonella typhi is responsible for most gastroenteritis, especially in low-income countries. This bacterium was sensitive with a MIC of 50 mg/ ml with hydroethenolic extract of *Bauhinia rufescens* and with extract of *Euphorbia hirta*, the bacterium was even more sensitive with a MIC of 12.5 mg/ml. Studies carried out in India, Thailand and northern Sudan confirmed the sensitivity of *Salmonella typhi* to ethanolic and aqueous extract of *Euphorbia hirta* with MIC and MBCs that vary according to the part of the plant used, the nature of the solvent and also the technique used [1, 3, 8, 13, 12, 2, 11].

3.5. Sensitivity of Shigella dysenteriae

Shigella dysenteriae was susceptible to *Bauhinia rufensis* with a MIC of 50 mg/ ml. Work carried out by H Husain et al, 2009 in Nigeria found MICs of 12.5 mg/ml with the hexane extract and 25 mg/l with the aqueous extract. The efficacy of an extract depends on the solvent used and the extraction method used. The hydroethanolic extract of *Euphorbia hirta* allowed us to have even more interesting results with a MIC of 25 mg/ml. Similar results were obtained in a study carried out in Nigeria by El-Mahmood Muhammad Abubakar 2009, whereas in work carried out in Thailand on methanolic extract of *Euphorbia hirta,* the authors obtained a MIC of 0.5 mg/ ml with the ethanolic extract of *Euphorbia hirta* [15]. Variations in the data observed in relation to the results would be partly related to the method and the nature of the solvent used as well as the isolates tested. In addition, the origin of the germs tested (isolation site) may determine their behaviour towards the extracts as observed with classical antibiotics.

3.6. Sensitivity of Escherichia coli

The reference strain (*E. coli* ATCC 25922) was sensitive with a MIC of 12.5 mg/ml with the hydroethanolic extract of *Bauhina rufescens* L. but also with that of *Euphorbia hirta* L. This finding was made in India (15). For hospital strains the MIC varies from 25 to 50 mg/ml depending on the part of the plant used. Many studies carried out throughout the world have confirmed the sensitivity of Escherichia coli to extracts of *Bauhinia rufescens* and *Euphorbia hirta* [3, 15, 13, 14, 10, 5].

3.7. Sensitivity of Pseudomonas aeruginosa

Pseudomonas aeruginosa was sensitive to the hydroethanol extract of *Bauhinia rufescens* L and *Euphorbia hirta* L with a MIC of 12.5 mg/ ml. These data are confirmed by the studies performed in Nigeria in 2009 [4] with a MIC of 12.5 mg/ml respectively.

3.8. Sensitivity of Klebsiella Pneumoniae and Acinetobacter spp.

Klebsiella pnuemonia and Acinetobacter spp. were sensitive to hydroethanolic extract of *Bauhinia rufescens* L and *Euphorbia hirta* L with a MIC of 50 mg/ml.

3.9. Sensitivity of Candida albicans

With the hydroethanolic extract of *Bauhinia rufescens, Candida albicans* was only sensitive to a concentration of 100 g/ml. Work carried out in Sudan also obtained a MIC of 100 mg/ ml respectively. In addition, a very low MIC of 1.25 mg/ ml was obtained with the methanolic extract in Nigeria in 2009 [4]. In view of these results we could say that the sensitivity of a microbial germ to medicinal plant extracts is a function of the solvent. Tested with the hydroethanolic extract of *Euphorbia hirta*, Candida albicans was very sensitive with a MIC of less than 12.5 mg/ ml.

4. Conclusion

This work on the evaluation of the antimicrobial activities of the hydroethenolic extract of *Bauhina rufescens* L. and *Euphorbia hirta* L. allowed to high light their antibacterial and antifungal properties. These results then justify their use in traditional medicine against infectious diseases of bacterial or fungal origin. These results can be exploited for the purification of the active ingredient (s) of the plants used and then contribute to the preparation of improved forms of effective remedies against infectious diseases based on these two plants.

References

[1] Bhuvaneshwar Upadhyay*, K. P. Singh and Ashwani Kumar (2010): Pharmacognostical and antibacterial studies ofdifferent extracts of *euphorbia hirta* l. *Journal of Phytology, 2 (6): 55–60.*

[2] EK. Elumalai, T. N. V. K. V. Prasad, J. Hemachandran, S. Viviyan Therasa, T. Thirumalai, E David 1 (2010): Extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta* and their antibacterial activities *J. Pharm. Sci. & Res. Vol. 2 (9), 2010, 549-554.*

[3] El-Mahmood Muhammad Abubakar (2009): Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. *Journal of Medicinal Plants Research Vol. 3 (7), pp. 498-505.*

[4] H. Usman, M. Sc., F. I. Abdulrahman, Ph. D., A. H. Kaita, Ph. D, and I. Z. Khan, Ph. D.(2009): Antibacterial Assays of the Solvents Partitioned Portions of Methanol Stem Bark Extract of *Bauhinia rufescens* Lam [Leguminosae-Caesalpinioideae]. *The Pacific Journal of Science and Technology* Volume 10. Number 2.

[5] Hassan, H. S.,* Sule, M. I., Usman, M. A., Usman, M. and Ibrahim, A. (2009): Preliminary phytochemical and antimicrobial screening of the stem bark extracts of bauhinia rufescens lam using some selected pathogens *bajopas volume 2 Number 2 December, 2009.*

[6] J. N. Ogbulie, C. C. Ogueke, I. C. Okoli and B. N Anyanwu (2007): Antibacterial activities and toxicological potentials of crude ethanolic extracts of *Euphorbia hirta* African Journal of Biotechnology Vol. 6 (13), pp. 1544-1548.

[7] Karou D, Dicko1 M H, Simpore J et Traore A S., 2005. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. African Journal of Biotechnology, 4,823-828.

[8] K V. Bhaskara Rao, L. Karthik1, E K. Elumalai, K. Srinivasan, Gaurav Kumar (2010): Antibacterial and antifungal activity of *Euphorbia hirta* l. Leaves: A comparative study. *Journal of Pharmacy Research 2010, 3 (3), 548-549.*

[9] Keita Y, Koné O, Ly K A et Häkkinen V., 2004. Etude chimique et l'activité antibactérérienne des distillats de quelques variétés de mangue de Guinée. *Comptes Rendus chimie, 7, 1095-1100.*

[10] Mamoudou Hamadou, Bakari Daoudou, Baane Martin-Paul, Salamatou Mohamadou and Djoulde Darman Roger (2020): Inhibitory Effect of Methanolic and Methanolic- Aqueous Mixture Extract of Leaves of *Plectranthus neochilus Schltr* (Lamiaceae) *and Bauhinia rufescens Lam* (Fabaceae) on Two Strains of Enterobacteria Producing Beta-lactamases*JAMB, 20 (7): 11-20, 2020; Article no. JAMB. 58420.*

[11] Mohammad Abu Basma Rajeh, Zakaria Zuraini, Sreenivasan Sasidharan, Lachimanan Yoga Latha and Santhanam Amutha (2010): Assessment of *Euphorbia hirta* L. Leaf, Flower, Stem and Root Extracts for Their Antibacterial and Antifungal Activity and Brine Shrimp Lethality. *Molecule*, *15*, 6008-6018.

[12] Mohammed I. Garbi, Ahmed S. Kabbashi, Elbadri E. Osman, Mahmoud M. Dahab, Waleed S. Koko and Ibrahim F. Ahmed (2015): Antioxidant activity and phytochemical screening of methanolic leaves extract of *Bauhinia rufescens* (Lam) *International Invention Journal of Biochemistry and Bioinformatics (ISSN: 2408-722X) Vol. 3 (3) pp. 23-27, November, 2015.*

[13] Musaddique Hussain, Umer Farooq, Muhammad Rashid, Hazoor Bakhsh, AbdulMajeed, Imran Ahmad Khan, Samia Latif Rana, Muhammad Shafeeq-ur-Rahman, Abdul Aziz (2014): Antimicrobial activity of fresh latex, juice and extract of *Euphorbia hirta* and *Euphorbia thymifolia* – an in vitro comparative study. *International Journal of Pharma Sciences. Vol. 4, No. 3 (2014): 546-553.*

[14] Nosaiba K. Hamed and Suad A. Gadir (2018): Phytochemical Screening, Characterization and Antimicrobial Activity of a Flavonoid from Sudanese *Bauhinia rufescens* (kulkul) (Caesalpiniaceae) Roots *EJMP, 24 (2): 1-8; Article no. EJMP. 41375.*

[15] Shanmugapriya Perumala,, Roziahanim Mahmuda, Suthagar Pillaia, Wei Cai Leea, Surash Ramanathanb (2012): Antimicrobial Activity and Cytotoxicity Evaluation of *Euphorbia hirta* (L.) Extracts from Malaysia. *APCBEE Procedia 2. 80–85.* www.sciencedirect.com.

[16] Orhan D D, Ozcelik B, Zgen S et Ergun F. (2010). Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiological Research, 165, 496-50.