

**ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACTS OF NEEM
(AZADIRACHTA INDICA), GARLIC (ALLIUM SATIVUM), GINGER (ZINGIBER
OFFICINALE) AND TURMERIC (CURCUMA LONGA) AGAINST GROWTH
INHIBITION OF HOSPITAL STRAINS**

Student Study Project 2020-21

To

TARA Govt. Degree College & PG (A) College



By

**Department of Microbiology
TARA Government Degree & PG (A) College
Sangareddy**

TARA GOVERNMENT COLLEGE[A] , SANGAREDDY-502 001

DEPARTMENT OF MICROBIOLOGY
CERTIFICATE

This is to certify that the project work entitled with the “Antibacterial Activity of Aqueous Extracts of Neem (Azadirachta Indica), Garlic (Allium Sativum), Ginger (Zingiber Officinale) And Turmeric (Curcuma Longa) Against Growth Inhibition Of Hospital Strains

“Antibacterial Activity Of Aqueous Extracts Of Neem (Azadirachta Indica), Garlic (Allium Sativum), Ginger (Zingiber Officinale) And Turmeric (Curcuma Longa) Against Growth Inhibition Of Hospital Strains”, which has been submitted by B.Sc (Microbiology) Students (2020-2021) to TARA Govt. Degree College is the original work done by them.

The results embodied in this report have not been to any other University or Institution for the award of any degree.

Dr.K. Jyothi
Assistant Professor of Microbiology

Project supervisor

PLACE: SANGAREDDY

Date:

TARA GOVERNMENT COLLEGE, SANGAREDDY – 502001

[AUTONOMOUS]

BONAFIDE CERTIFICATE

Certified that the project report” is the bonafide work of

1. Afsha Naaz , MZC III year -6058-19-457-001
2. S.Shivaleela , MZC III year -6058-19-457-024
3. N . Nikitha , MZC III year -6058-19-457-021
4. P.Mamatha , MBC III year -6058-19-458-011
5. G.Sai Bhargavi, MBC III year -6058-19-458-006

Who carried out the project work under supervision of Dr.K.Jyothi, Assistant Professor of Microbiology TARA Govt.Degree College.

Dr . K. JYOTHI ,
Assistant Professor of Microbiology

M. PRAVEENA
Principal

DECLARATION

We hereby declare that the project report entitled Antibacterial Activity Of Aqueous Extracts Of Neem (Azadirachta Indica), Garlic (Allium Sativum), Ginger (Zingiber Officinale) And Turmeric (Curcuma Longa) Against Growth Inhibition Of Hospital Strains” , is the work done in the campus at Tara Government College during the academic year 2021.

1. Afsha Naaz , MZC III year -6058-19-457-001
2. S.Shivaleela , MZC III year -6058-19-457-024
3. N . Nikitha , MZC III year -6058-19-457-021
4. P.Mamatha , MBC III year -6058-19-458-011
5. G.Sai Bhargavi, MBC III year -6058-19-458-006

ACKNOWLEDGEMENT

We would like to express our profound sense of gratitude and indebtedness to our project adviser Dr.K.Jyothi, Asst. Prof. Of Microbiology, TARA Government Degree & PG College, Sangareddy, for her valuable guidance, untiring cooperation at each and every phase of this Project work .

We also express our sincere thanks to Smt. M. Praveena, Principal of the college. We are thankful to the department of Microbiology faculty members for providing us the required facilities to complete our Project work.

We take it as a privilege to thank our guide Dr. K. Jyothi, Assistant Professor of Microbiology, for her guidance.

Presented By :

1. Afsha Naaz , MZC III year -6058-19-457-001
2. S.Shivaleela , MZC III year -6058-19-457-024
3. N . Nikitha , MZC III year -6058-19-457-021
4. P.Mamatha , MBC III year -6058-19-458-011
5. G.Sai Bhargavi, MBC III year -6058-19-458-006

TITLE OF THE PROJECT

**ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACTS OF NEEM
(AZADIRACHTA INDICA), GARLIC (ALLIUM SATIVUM), GINGER (ZINGIBER
OFFICINALE) AND TURMERIC (CURCUMA LONGA) AGAINST GROWTH
INHIBITION OF HOSPITAL STRAINS**

.

Introduction

Antimicrobials agents are important to reduce infectious diseases. Indiscriminate use of antibiotics resulted in development and spread of multi drug resistant pathogens in developing countries. Misuse of antibiotics is one of the reasons for the increasing rates of resistance, especially in rural areas (27). There is strict need for novel alternative antimicrobial strategies.

In developing countries, Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with side effects. The effective treatment of infectious diseases is possible by natural plant extracts with new source of antimicrobial activity and novel mode of action. When compared with conventional antimicrobials natural plant extracts have fewer side effects. Therefore the need for novel alternative antimicrobial strategies has renewed interest in natural products like turmeric, garlic, neem and ginger.

In developing countries like India and backward areas like Mahabubnagar district, Telangana State, people with low income such as farmers, people of small isolated villages and native communities use folk medicine for the treatment of common infectious diseases.

Aims and Objectives

To find out the antimicrobial activity of locally available Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*) and Turmeric (*Curcuma longa*) against growth inhibition of bacterial strains isolated from MNR Medical College.

Review of Literature

As per the classification reports of World Health Organization 15 million people die every year from infectious diseases worldwide and the infectious diseases remain leading cause of death [1]. Antibiotics, the most effective drugs against microbial infections in the 1950s, are recently losing their efficacies as most microorganisms have an acquired resistance [2].

Misuse of antibiotics is one of the reasons for the increasing rates of resistance, especially in rural areas [3]. Therefore, the need for novel alternative antimicrobial strategies has renewed interest in natural products like turmeric, garlic, neem, ginger etc. Exhibiting antibacterial properties.

Plants are the main source of medications for human, since they appear on earth and have abilities to synthesize endless secondary metabolites known as phytochemical compounds which serve as plant defense mechanism against macro and micro-organisms [4]. Compounds derived from plants are being used more and more widely for their potential chemotherapeutic and nutritional value [27, 28].

Most of the recent drugs are initially obtained or semi-synthesized from the plant sources, particularly from those which are prescribed in traditional medicine [5]. Apart from the negative side effects of antibiotics on human organs, the intensive use of antibiotics has led to emerging

of what is called multidrug resistant (MDR) bacteria which are now raising remarkably all over the world and become an international public health threat [6]

Allium sativum, commonly known as garlic, belongs to the Alliaceae family, which also includes leek, onion and shallot. Garlic is used widely in food and medicine [9, 10, 11], like other herbs and spices [14]. Use of *A. sativum* in alternative medicine has increased over the years [11–15]. Garlic can be prepared in various forms, namely oil, powder, raw juice and extracts [9–20]. The therapeutic effect of garlic has been attributed to its organosulfur constituents, which also are responsible for its typical flavour and odour [9-20]. Other studies have implicated thiosulfinates in the antibiotic activity of garlic [9-20].

Zingiber officinale is a rhizome commonly known as ginger or gingerroot. It can be consumed as a delicacy, medicine or spice. It lends its name to its genus and family (Zingiberaceae). Other notable members of this plant family are turmeric, cardamom and galangal [26-27]. Ginger cultivation began in South Asia and then spread to East Africa and the Caribbean. Historically, the traditional medical form of ginger was called Jamaica ginger, which was classified as a stimulant and carminative [26]. Ginger has also frequently been used to disguise the taste of medicines [27]. Studies indicate that ginger may provide short-term relief of pregnancy-related nausea and vomiting [26-27].

Research Methodology

Antibacterial Assay:

1. Bacterial cultures

Test bacterial cultures were obtained from MNR Medical College, Sangareddy. Clinical isolates of the bacteria *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and *Proteus vulgaris* were used in this study. All the cultures were sub cultured and maintained in nutrient agar slants. Each cultures was inoculated into nutrient broth and incubated for were prepared for each strain.

2. Preparation of Discs

Discs were prepared by cutting Whatman filter paper No. 1 in size of about 6 mm in diameter with the paper puncturing machine. Blank discs were sterilized by dry heat (Hot air oven).

3. Testing antimicrobial activity of extracts against bacterial cultures by Agar Disc Diffusion Method:

Locally available Neem (*Azadirachta indica*) leaves, Garlic (*Allium sativum*), Ginger (*Zingiber officinale*) and Turmeric (*Curcuma longa*) cloves were obtained and sterilized. Aqueous Extracts of each component was prepared by grinding the leaves and cloves in sterile distilled water with mortar and pestle. Sterilized Discs were soaked in the aqueous solution for 24h and air dried.

Each bacterial strain was inoculated into nutrient broth and incubated at 37⁰C for 18h in order to reach exponential phase. Each culture broth was swab inoculated on to the nutrient agar plates. Agar surface was allowed to dry for few minutes. Four filter paper discs that were soaked in test solutions were placed on each nutrient agar plate along with standard antibiotic discs susceptible for that particular organism aseptically. The standard antibiotic discs used were supplied by HiMedia. They are Erythromycin for *Staphylococcus aureus*, Tetracycline for *E.coli*, Ciprofloxacin for *Pseudomonas aeruginosa*, Tetracycline for *Klebsiella pneumoniae* , Pencillin for *Proteus vulgaris*.

Plates were incubated at 37⁰C for 24 hours, after incubation zone of inhibition of bacterial growth was observed in terms of the diameter of inhibition zone.

Findings/Results

In the present study the aqueous extracts of Neem (*Azadirachta indica*) leaves, Garlic (*Allium sativum*), Ginger (*Zingiber officinale*) and Turmeric (*Curcuma longa*) showed significant antibacterial property against clinical isolate of *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and *Proteus vulgaris*.

Turmeric (*Curcuma longa*), Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) showed zone of inhibition in the range of (1.1) to (1.5) mm inhibited the growth of *Pseudomonas aeruginosa*. The relative percentage of inhibition of growth of *Pseudomonas* against standard antibiotic Ciprofloxacin was slightly high (2.7). Neem (*Azadirachta indica*) has no significant effect on growth inhibition of *Pseudomonas*.

Turmeric (*Curcuma longa*), Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) showed zone of inhibition in the range of (0.7) to (1.8) mm inhibited the growth of *Klebsiella pneumonia*. The relative percentage of inhibition of growth of *Klebsiella* against standard antibiotic Tetracycline was slightly high (2.0). Neem (*Azadirachta indica*) has no significant effect on growth inhibition of *Klebsiella*.

Neem (*Azadirachta indica*) leaves, Garlic (*Allium sativum*), Ginger (*Zingiber officinale*) showed zone of inhibition in the range of (1.4) to (1.8) mm inhibited the growth of *Escherichia coli*. The relative percentage of inhibition of growth of *E.coli* against standard antibiotic Tetracycline was almost same (1.7) Turmeric (*Curcuma longa*) has less effect on growth inhibition of *E.coli*.

Garlic (*Allium sativum*), Ginger (*Zingiber officinale*) and Neem (*Azadirachta indica*) leaves showed zone of inhibition in the range of (1.0) to (2.0) mm inhibited the growth of *S.aureus*. The relative percentage of inhibition of growth of *S.aureus* by aqueous extract of garlic is higher than the standard antibiotic Erythromycin (1.7).

Turmeric (*Curcuma longa*), Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*), Neem (*Azadirachta indica*) leaves showed zone of inhibition in the range of (0.9) to (1.2) mm inhibited the growth of *P.vulgaris*. The relative percentage of inhibition of growth of *P.vulgaris* by aqueous extract of garlic is higher than the standard antibiotic Penicillin (1.1).

Figure 1. The results are shown in the Table 1 and Picture 1. **Picture 1. Zone of inhibition of growth of Bacteria against Neem, Garlic, Ginger and turmeric Extracts**

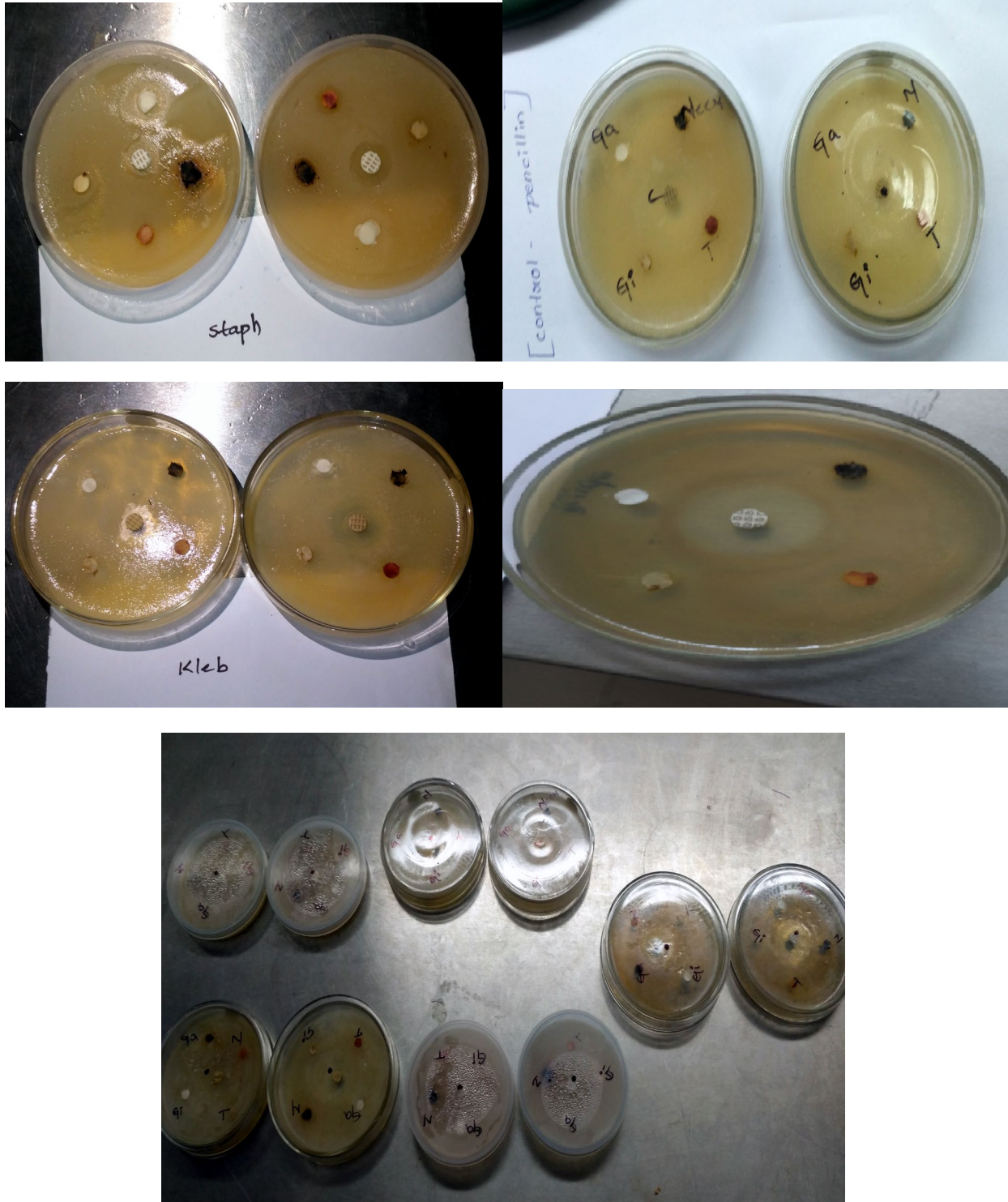


Table.1

Test organism	Aqueous Extract	Diameter of Zone of Inhibition (mm)	
		Original	Duplicate
<i>P. aeruginosa</i>	<i>Curcuma longa</i> (Turmeric)	1.3	1.5
	<i>Zingiber officinale</i> (Ginger)	1.1	0.8
	<i>Allium sativum</i> (Garlic)	1.2	0.7
	<i>Azadirachta indica</i> (Neem)	0.6	0.6
	<i>Ciprofloxacin(c)</i>	2.7	2.8
<i>K.pneumoniae</i>	<i>Curcuma longa</i> (Turmeric)	0.7	1.9
	<i>Zingiber officinale</i> (Ginger)	1.0	0.8
	<i>Allium sativum</i> (Garlic)	1.3	1.0
	<i>Azadirachta indica</i> (Neem)	0.7	0.7
	<i>Tetracycline (c)</i>	2.0	2.0
<i>E.coli</i>	<i>Curcuma longa</i> (Turmeric)	0.9	1.0
	<i>Zingiber officinale</i> (Ginger)	0.9	1.1
	<i>Allium sativum</i> (Garlic)	1.6	0.8
	<i>Azadirachta indica</i> (Neem)	1.4	1.0
	<i>Tetracycline(c)</i>	1.7	2.1
<i>S.aureus</i>	<i>Curcuma longa</i> (Turmeric)	0.7	0.8
	<i>Zingiber officinale</i> (Ginger)	0.6	1.0
	<i>Allium sativum</i> (Garlic)	1.7	2.0
	<i>Azadirachta indica</i> (Neem)	1.3	1.0
	<i>Erythromycin (c)</i>	1.3	1.3
<i>P.vulgaris</i>	<i>Curcuma longa</i> (Turmeric)	1.0	0.7
	<i>Zingiber officinale</i> (Ginger)	0.9	0.6
	<i>Allium sativum</i> (Garlic)	0.8	1.2
	<i>Azadirachta indica</i> (Neem)	0.6	0.9
	<i>Penicillin (c)</i>	1.1	1.0

--	--	--	--

Conclusions and suggestions

According to present study locally available Neem (*Azadirachta indica*) leaves, Garlic (*Allium sativum*), Ginger (*Zingiber officinale*) and Turmeric (*Curcuma longa*) can be used as substitute source for synthetic antibiotic drugs that are used against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and *Proteus vulgaris* Hospital strains.

Aqueous Extracts of the compounds were selected for testing the antibacterial property in their native form that has been used in day to day life. Natural products are excellent remedy for synthesis of new drugs with fewer side effects.

References:

1. Abat C, Chaudet H, Rolain JM, Colson P, Raoult D. Traditional and syndromic surveillance of infectious diseases and pathogens. *Int J Infect Dis* 2016; 48: 22-8.
2. Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *J Control Release* 2011; 156(2): 128-45.
3. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999; 12(4): 564-82.
4. Okeke IN, Lamikanra A, Edelman R. Socioeconomic and behavioral factors [2]leading to acquired bacterial resistance to antibiotics in developing countries. *Emerging Infectious Diseases*. 1999;5(1):18-27.
5. Munuswamy H, Thirunavukkarasu T, Rajamani S, Erusan KE, Ernest D. A review on antimicrobial efficacy of some traditional medicinal plants in Tamil Nadu. *J Acute Dis* 2013; 2: 99-105.
6. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18(3): 268-81.
7. J. Hogg, Garlic Supplements. *Complementary Medicines Summary*, UK Medicines Information, National Health Service, 2002.
9. I. Durak, H.S. Oztürk, E. Olcay, C. Güven, Effects of garlic extract supplementation on blood lipid and antioxidant parameters and atherosclerotic plaque formation process in cholesterol-fed rabbits, *J. Herb. Pharmacother.* 2 (2002) 19–32.
10. E. Lissiman, A.L. Bhasale, M. Cohen, Garlic for the common cold, *Cochrane Database Syst. Rev.* 3 (2012) 3.
11. T. Yamasaki, R.W. Teel, B.H. Lau, Effect of allixin, a phytoalexin produced by garlic, on mutagenesis, DNA-binding and metabolism of aflatoxin B1, *Cancer Lett.* 59 (1991) 84–94.
12. B.A. Iwalokun, A. Ogunledun, D.O. Ogbolu, S.B. Bamiro, J. Jimi Omojola, In-vitro anti-microbial properties of aqueous garlic extract against multidrug-resistant bacteria and *Candida* species from Nigeria, *J. Med. Food* 7 (2004) 327–333.
13. E. Block, *Garlic and Other Alliums: The Lore and the Science*, Royal Society of Chemistry, London, 2010.

14. K.Ried, O.R. Frank, N.P. Stocks, Aged garlic extract lowers blood pressure in patients with treated but uncontrolled hypertension: a randomised controlled trial, *Maturitas* 67 (2010) 144–150.
15. M.A.Jabar, A. Al-Mossawi, Susceptibility of some multiple resistant bacteria to garlic, *Afr. J. Biotechnol.* 6 (2007) 771–776.
16. T. Zeng, T.T. Guo, C.L. Zhang, F.Y. Song, X.L. Zhao, K.Q. Xie, A meta-analysis of randomized, double-blind, placebo-controlled trials for the effects of garlic on serum lipid profiles, *J. Sci. Food Agric.* 92 (2012) 1892–1902.
17. T. Yamasaki, R.W. Teel, B.H. Lau, Effect of allixin, a phytoalexin produced by garlic, on mutagenesis, DNA-binding and metabolism of aflatoxin B1, *Cancer Lett.* 59 (1991) 84–94.
18. K. Rahman, Effects of garlic on platelet biochemistry and physiology, *Mol. Nutr. Food. Res.* 51 (2007) 1335–1344.
19. N.F. Gomaa, M.H. Hashish, The inhibitory effect of garlic (*Allium sativum*) on the growth of some microorganisms, *J. Egypt. Public Health Assoc.* 78 (2003) 361–372.
20. S. Durairaj, S. Srinivasan, P. Lakshmanaperumalsamy, In vitro antibacterial activity and stability of garlic extract at different pH and temperature, *Electronic J. Biol.* 5 (2009) 5–10.
21. C. Ung-Kyu, L. Ok-Hwan, L. Seong-II, K. Young-Chan, Optimization of antibacterial activity of *Perilla frutescens* var. *acuta* leaf against *Pseudomonas aeruginosa* using the evolutionary operation-factorial design technique, *Int. J. Mol. Sci.* 11 (2010) 3922–3932.
22. A.L. Hopkins, M.G. Lamm, J.L. Funk, C. Ritenbaugh, *Hibiscus sabdariffa* L. in the treatment of hypertension and hyperlipidemia: a comprehensive review of animal and human studies, *Fitoterapia* 85 (2013) 84–94.
23. H. Mozaffari-Khosravi, B.A. Jalali-Khanabadi, M. AfkhamiArdekani, F. Fatehi, M. Noori-Shadkam, The effects of sour tea (*Hibiscus sabdariffa*) on hypertension in patients with type II diabetes, *J. Human Hypertens.* 23 (2008) 48–54.
24. H.A. Wahabi, L.A. Alansary, A.H. Al-Sabban, P. Glasziou, The effectiveness of *Hibiscus sabdariffa* in the treatment of hypertension: a systematic review, *Phytomedicine* 17 (2010) 83–86.

25. D.L. McKay, C.Y.O. Chen, E. Saltzman, J.B. Blumberg, Hibiscus sabdariffa L. tea (tisane) lowers blood pressure in prehypertensive and mildly hypertensive adults, *J. Nutr.* 140 (2009) 298–303.
26. J. Hogg, Garlic Supplements. Complementary Medicines Summary, UK Medicines Information, National Health Service, 2002
27. T. Zeng, T.T. Guo, C.L. Zhang, F.Y. Song, X.L. Zhao, K.Q. Xie, A meta-analysis of randomized, double-blind, placebo-controlled trials for the effects of garlic on serum lipid profiles, *J. Sci. Food Agric.* 92 (2012) 1892–1902.
28. I.M. Gull, M. Saeed, H. Shaukat, S.M. Aslam, Z.Q. Samra, A.M. Athar, Inhibitory extract, *Afr. J. Biotechnol.* 6 (2012) 771–776.
29. Methods for in vitro evaluating antimicrobial activity: A review Mounyr Balouiri n , Moulay Sadiki, Saad Koraichi Ibsouda *Journal of Pharmaceutical Analysis* 6 (2016) 71-79.