



USING AQUEOUS EXTRACT OF *MALVA SYLVESTRIS* AS INHIBITOR FOR THE GROWTH OF SOME MICROORGANISMS THAT CAUSE URINARY TRACT INFECTIONS

Ibtisam Fareed Ali Karm

Market Researches Center and Consumer Protection, University of Baghdad/ Baghdad/ Iraq

*Corresponding author email: ifak_77@yahoo.com

ABSTRACT

Pharmacological properties of *Malva sylvestris* that had been studied and tested. Our study investigations showed that there are a lot of rich antimicrobial and antioxidants active compounds of *M. sylvestris* and they are due to the presence of many unique compounds such as anthocyanin in plant parts precisely in leaves. It was clear that the three different bacterial strains *Escherichia coli*, *Staphylococcus saprophyticus* and *Pseudomonas aeruginosa* were obtained and isolated from infected people were the most available bacteria that cause Urinary tract infections (UTIs). In this study percentage of *Escherichia coli* was (80%) followed by *Staphylococcus saprophyticus* (14%) and *pseudomonas areugenosa* was (6%) respectively. Three type of aqueous extracts with fact of four treatments (M1, M2, M3 and M4) were done to get synergistic activity, cold water extract for fresh leave plant sample (M1), hot water extract for fresh leave plant sample (M2), hot water extract for dry power of leave plant sample (M3), the mixed extracts M4 was done from the three water extracts (M1+M2+M3) and three concentrations were done for each treatment and they were 25%, 50%, 100%. There was a significant deference among all concentrations of treatments M1, M2, M3 and M4 toward each pathogenic bacterium. Antimicrobial activity was done by using disc diffusion method. Active compounds were detected and phenolic content was determined by spectrophotometer with absorbance values measured at 765 nm. The results showed that the inhibition zones were increased with the increasing of concentrations of *Malva sylvestris* extract treatment. Results of this study encourage as to use alternative sours for curing like some plants to heal the human illness and to be more responsible towards our self and nature by using natural, cheaper, non-chemical non poisoning drugs, and give great attention for plants as alternative sours of antimicrobial activity.

KEY WORDS: aqueous extracts, Urinary tract infections (UTIs), *Malva sylvestris*, synergistic activity.

INTRODUCTION

Urinary tract infections (UTIs) are defined as the presence of microbial pathogens in the urinary tract with associated symptoms. UTIs are one of the most common bacterial infections; Urinary Tract Infections (UTIs) is an infection caused by the presence and growth of microorganism anywhere in the urinary tract and is perhaps the single commonest bacterial infection of mankind^[1,2]. When bacteria from the rectal area enter the urinary tract via the urethra to the bladder and multiply in the urine, an infection occurs^[3]. Most infections arise from one type of bacteria, *E. coli* which normally lies in the colon. The organisms most commonly responsible for catheter-associated UTIs are *E. coli*, *Proteus mirabilis*, *P. aeruginosa*, and *Streptococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *Actinomycetes*, *Nocardia*, *Candida etc.* can cause UTI. In addition *Mycoplasma* and *Chlamydia* may be associated with sexually transmitted UTI^[4,5]. New research has shown the promising use of medicinal plants: *in vitro* studies with hydroalcoholic extract from many plants for example *Myracrodruon urundeuva*, *Psidium guajava* and *Malva sylvestris* and they showed potential

antimicrobial activity against the microorganisms, antifungal activity on *Candida* strains^[6].

Malva sylvestris belong to the family of Malvaceae and it is species for the genus. Known as common mallow in English speaking^[7,8,9,10]. The pharmacological properties of *Malva sylvestris* are especially due to the presence of anthocyanins in its leaves. *Malva sylvestris* is justified by the complexity of its composition, which consists of tetrahydroxylated sesquiterpenes and diterpenes, two monoterpenes, six normal-C13 terpenes and eleven aromatic compounds^[11]. Similarly to the anthocyanin in the leaves, which has a natural potential for degrading free radicals, it serves as antioxidant, reducing total cholesterol, triglycerides in the blood and preventing thrombosis and cardio-cerebral angiopathy^[12].

Malva sylvestris extracts are reported for their radical scavenging effect^[13]. Previous chemical investigations have shown that there are a lot of rich antimicrobial and antioxidants presence at the active compounds of *M. sylvestris* such as flavonols, ferulic acid, hydroxycinnamic acids, sterols, sesquiterpenes, mono and diterpenes in leaves and stems of^[14,15,16,17].



Scheme 1: *Malva sylvestris* plant widespread in Baghdad

MATERIALS & METHODS

Preparation of plant material

Plants were collected during (February-may) from different parts in Baghdad. Parts of fresh samples were washed and shade dried to obtain 50 gram fresh dried sample. Ground plant materials were used for extraction.^[18]

Preparing Bacterial strains

Three different bacterial strains, *Escherichia coli*, *Staphylococcus saprophyticus* and *Pseudomonas aeruginosa* were obtained and isolated from infected people. The isolated bacteria were identified and their characteristic form were done include Gram stain test then the microscopic examination, motility test and biochemical tests were examined according to Cheesbrough^[19, 20]. The isolates were identified^[21]. The strains were maintained on Nutrient agar slants.

WATER EXTRACTION

Three type of extracts were done cold water extract for fresh leave plant sample (M1), hot water extract for fresh leave plant sample (M2), hot water extract for dry power of leave plant sample (M3).also three concentrations were done for each extract and they were 25%, 50%, 100%.

a)Preparing treatment (M1)

Cold extraction was done by adding 500 ml of Distal Water (DW) for 100 gm of fresh leave plant sample then incubate it for 24 hr. in shaker incubator at 35°C then filter the extract by filter paper and then centrifuged the pure liquid for 3000 r\10 min, after that the supernatant was concentrated by rotary evaporator at 45°C and then the extract was dried by oven at 37°C for 24 hr.

b)Preparing treatment (M2)

Hot extraction was done by adding 500 ml of hot Distal Water (DW) for 100 gm of fresh leaves plant sample then incubate it for 30 min. in shaker incubator at 35°C then the extract was filtered by filter paper and then centrifuged the pure liquid for 3000 r\10 min, after that the supernatant was concentrated by rotary evaporator at 45°C and then the extract was dried by oven at 37 °C for 24 hr.

c)Preparing treatment (M3)

Hot extraction was done by adding 500 ml of boiling Distal Water (DW) for 100 gm of dried leaves of plant samples then incubate it for 30 min. in shaker incubator at 35 °C then the extract was filtered by filter paper and then centrifuged the pure liquid for 3000 r\10 min, after that the supernatant was concentrated by rotary evaporator at 45 °C and then the extract was dried by oven at 37 °C for 24 hr .

Preparing the control treatment (C)

The ciprofloxacin antibiotic was considered to be the control at all experiments against all the pathogenic bacteria in the study.

Preparing the mixed treatment M4 (M1+M2+M3)

Best antimicrobial activity against every pathogenic studied bacteria of extract concentration was chosen for each one and then mixed extractions were done from the both Extractions (the two hot extractions and the cold extraction for the plant leaves). The mixed was (M1:M2:M3), (1:1:1), to get a synergistic activity for the mixed treatment.^[22]

Antimicrobial activity measuring

Disc diffusion method was done for this purpose. The *Malva sp.* extract and ciprofloxacin antibiotic stocks were also made at 25 µg \ml the concentration of the Oflaxacin served as positive controls. Autoclaved discs were loaded with 10µL of the respective plants extract, or ciprofloxacin antibiotic only were prepared then all disks air dried for 5 minutes. The nutrient agar plates were spread with 100 µL of respective culture with the help of glass spreader and the loaded discs were placed onto the surface of agar. The plates were left to dry for 5 min and kept in incubator at 37°C for 24 h. The results were seen as zone of inhibition which was measured in millimeters was determined.^[23, 22]

Active compounds Detecting and determination of phenolic content.

Chemical compounds analysis of the *Malva sp.* extracts were carried out by using the chemical classic methods according to Harborn^[24] total phenolic content was determined according to the method of Singelton^[25, 26]. Standard curve was made using Gallic acid as a standard. Different concentrations of Gallic acid were prepared in distilled water, and their absorbance values were measured at 765 nm. Samples measurement as in:

5 ml (phenol reagent) +900 ml (DW) + 100 ml (extract)_____then after 5 min we add 4 ml (Na2CO3 15%) _____incubated 120 min>>>>>then samples measured at Abs. 765 nm

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study, all data measuring as triplicate measures^[27].

RESULTS & DISCUSSION

Bacteria isolated caused Urinary tract infections (UTIs)

UTIs are caused by many microorganisms, including gram positive like *Staphylococcus* and gram negative such as *E coli* and *pseudomonas sp.* at this study *Escherichia coli*

was (80%) current and isolate followed by *Staphylococcus saprophyticus* (14%) and *pseudomonas areugenosa* (6%) respectively. This finding is similar to many reports which indicated that gram negative bacteria are mostly appeared and also the commonest pathogens isolated from patients with urinary tract infections (Figure 1).

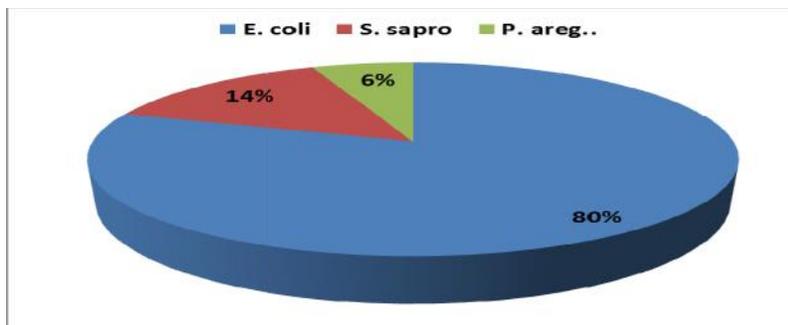


FIGURE 1: Bacteria isolated from patients with urinary tract infections. *Escherichia coli* (80%), *Staphylococcus saprophyticus* (14%) and *pseudomonas areugenosa* (6%)

Active compounds Detecting

Aqueous extracts of *Malva* flowers showed a lower radical scavenging ability compared to the leaves, contrary to being more active against the tested pathogenic microorganisms. Finally, the kind of mallow analysed here can be considered as good sources of some phenolic and antioxidant compounds [28].

Active substance in fruits and vegetables such as phenolic compounds has antioxidant activity as it shown in table (1) the M1 extract containing Flavonoids (fla), Tannins (tan) and Phenols (phe). M2 contane alk, fla, tan, ter and phe. Also we found that M3 had alk and phe. But M4 was the rich extract in studied active compound except terpenes (ter). Total phenolic contents of *Malva sylvestris* extracts samples are presented in Table (2).

TABLE 1: the active chemicals compounds in *Malva sylvestris* extracts (treatments)

<i>Malva sylvestris</i> Extracts	Alkaloids (alk)	Flavonoids (fla)	Tannins (tan)	Terpenes (ter)	Phenols (phe)
M1	-	++	+	-	++
M2	+	+	+	+	++
M3	+	-	-	-	+
M4	++	++	++	-	++

TABLE 2: Total phenols in the extracts of *Malva sylvestris* sample concentration (µg/ml) determined by using standard curve of Gallic acid

The study treatments	M1	M2	M3	M4
Phenols concentrations(µg/ml)	160.14	120.21	83.51	220.30

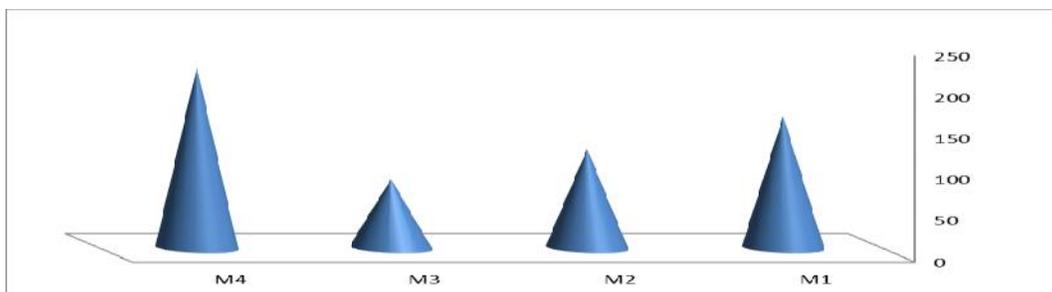


FIGURE 2: Total phenols in the extracts of *Malva sylvestris* sample concentration (µg/ml) determined by using standard curve of Gallic acid.

Malva sp. extracts antimicrobial activity and measuring inhibition zones

The inhibition zones were determined for each extract in the study and also the inhibition zones were measured for

antibiotic activity against UTIs bacteria which considered to be the control treatment. There is a relation between the type of the extract and the active compounds abundance that we can get from the plant parts (Table-1). The

pharmacological properties of *Malva sylvestris* are especially due to the presence of anthocyanins in its leaves.

Parts of *Malva* plant extracts were found to be more active toward pathogenic microorganisms specially gram positive and negative bacteria but no one of the extracts showed an

inhibition against Fungi (mold and yeast) Thus, the *M. sylvestris* extracts showed no antifungal activity. These results were comparable to the one reported by Fatima *et al.* (2013) considering the antimicrobial activity of *M. sylvestris* work in widespread on pathogenic bacteria^[29].

TABLE 3: The inhibition zones (mm) of cold fresh leave extracts of *M. sylvestris* (M1) Against UTIs microorganisms.

(UTIs) bacteria	Inhibition zone (mm) of M1 25%	Inhibition zone(mm) of M1 50%	Inhibition zone(mm) of M1 100%	Inhibition zone(mm) of C (control)	LSD
<i>E. coli</i>	5.0	8.0	10.0	15.0	3.071 *
<i>P. areugenosa</i>	6.5	9.4	11.5	16.0	3.668 *
<i>S. saprophyticus</i>	7.2	9.6	12.0	18.0	4.052 *

* (P<0.05)

TABLE 4: The inhibition zones (mm) of hot fresh leave extracts of *M. sylvestris* (M2) Against UTIs microorganisms.

(UTIs) bacteria	Inhibition zone(mm) of M2 25%	Inhibition zone(mm) of M2 50%	Inhibition zone(mm) of M2 100%	Inhibition zone(mm) of C (control)	LSD
<i>E coli</i>	3.0	4.0	6.0	15.0	2.983 *
<i>P. areugenosa</i>	3.4	4.5	5.5	16.0	3.056 *
<i>S. saprophyticus</i>	4.0	5.0	7.5	18.0	3.921 *

* (P<0.05)

TABLE 5: The inhibition zones (mm) of hot dry leave extracts of *M. sylvestris*(M3) Against UTIs microorganisms.

(UTIs) bacteria	Inhibition zone (mm) of M3 25%	Inhibition zone (mm) of M3 50%	Inhibition zone (mm) of M3 100%	Inhibition zone (mm) of C (control)	LSD
<i>E. coli</i>	3.0	4.8	7.0	15.0	3.169 *
<i>P. areugenosa</i>	4.0	6.0	8.0	16.0	3.553 *
<i>S. saprophyticus</i>	6.0	8.0	9.5	18.0	3.703*

* (P<0.05)

In table (1) there was a significant deference among all concentrations of M1 treatment toward each pathogenic bacteria, in the treatment M1 the results showed that the inhibition zones were increased with the increasing of concentrations of the M1 extract treatment. The best concentration was at 100% against *E coli*, *P. areugenosa*, *S. saprophyticus* with inhibition zones of (10, 11.5, 12) mm respectively (table 3). There was a significant deference noticed among all concentrations at these two

treatment. Inhibition zones of the treatment M2 and M3 were also increased with the increasing of extract concentration (Table 4, 5), but it appeared that the studied bacteria were less sensitive to these two treatments because the amount of active compound especially total phenols which considered to be the most important compound that is responsible for the activity of the plant extracts table (2).

TABLE 6: The inhibition zones (mm) of mixed extracts of *M. sylvestris* (M4) Against UTIs microorganisms.

(UTIs) bacteria	Inhibition zone (mm) of M4 25%	Inhibition zone (mm) of M4 50%	Inhibition zone (mm) of M4 100%	Inhibition zone (mm) of C (control)	LSD
<i>E coli</i>	6.2	8.6	11.6	15.0	3.416 *
<i>P. areugenosa</i>	6.0	10.0	12.5	16.0	2.963 *
<i>S. saprophyticus</i>	9.6	11.4	15.5	18.0	3.375 *

The result of treatment M4 gave the maximum inhibiting diameter towards the pathogenic studied bacteria which were sensitive to this treatment because of the interaction and synergetic activity among these three kinds of extracts also the phenols compound was 220.30 µg/ml this result was the highest among all extracts and the highest inhibitions zones were for the 100% concentration (11.5, 12.5 and 15.5) mm against *E. coli*, *P. areugenosa* and *S. saprophyticus* respectively (Table 6). The results of this study was similar to the results of a study by (aljanabi *et*

al., 2011) who connect the activity of plant antimicrobial with present of phenolic acids and they are play a very important role of causing damage in DNA preventing diseases^[30,31]. This study was agree with study by^[32,33] and those who showed the importance of the leave aqueous extract as a treatment for many states. There are a lot of plant used as antimicrobial such as a study by^[34] which was agree with the aim with present study by giving the importance and a site of the using of some selected plants that have been widely interred in the management of

various human illnesses in the past and present time. A study by Meena *et al.* (2014)^[35] share the opinion with this study and they revealed that phytochemical analysis shows the presence of alkaloids, tannins, flavonoids and saponins in various extracts derived from leaves, stem and seeds of plant these active components were behind the anti-sickling and antimicrobial activity possessed by leaves^[36]. A result of the study was in corresponding with a study by Sabri *et al.*, 2012^[37]. Revealed that extracts of *Malva sylvestris* L. contain alkaloids, flavonoids, tannins, starch, saponins, sterols and steroids and anthocyanosides which give the Malva healing properties. *Malva sylvestris* is a good source for natural foods supplements, pharmaceutical industry purposes and for organic food rich with antioxidant compounds^[38]. All these studies and experiments encourage as to use alternative sources of cure like some plants to heal the human illness and to be more responsible towards our self and nature by using natural, cheaper, non-chemical non poisoning drugs, and give great attention for plants as alternative sources of antimicrobial activity.

REFERENCES

- [1]. Ebie, M.Y., Kandakai-Olukemi, Y.T., Ayanbadejo, J. and Tanyigna, K.B. (2001) Urinary tract infections in a Nigerian military hospital. *Nigerian Journal of Microbiology* 15, 31-37.
- [2]. Okonko, I.O., Ijandipe, L.A., Ilusanya, A.O., Donbraye-Emmanuel, O. B., Ejembi, J., Udeze A. O., Egun O.C., Fowotade A. & Nkang A.O. (2010) Detection of Urinary Tract Infection (UTI) among pregnant women in Oluyoro Catholic Hospital, Ibadan, South-Western Nigeria. *Malaysian Journal of Microbiology*, Vol. 6(1) 2010, pp. 16-24.
- [3]. Poonam U., Sharma and Ulka Bidwai (2013) Isolation and identification of bacteria causing urinary tract infections in pregnant women in vidarbha and their drug susceptibility patterns in them. *ISSN:2319-7706.Vol.2(4)*,pp. 97-103.
- [4]. Gupta, K., Hooton, T.M., Naber, K.G. (2011) International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases, *J Clin Infect Dis*, 52(5), 103-120.
- [5]. Komala, M., Sampath Kumar, K.P. (2013) urinary tract infection: causes, symptoms, diagnosis and its management. *Indian Journal of Research in Pharmacy and Biotechnology*. Volume 1(2). P: 226-233.
- [6]. Alves, P.M., Queiroz, L.M.G., Pereira, J.V., Pereira, M.S. Atividade antimicrobiana, antiaderente e antifúngica in vitro de plantas medicinais brasileiras sobre microrganismos do biofilme dental e cepas do gênero *Candida*. *Revista da Sociedade Brasileira de Medicina Tropical*, vol. 42, n.2, p. 222-224, 2009.
- [7]. Chevallier, A. (1996): *The Encyclopedia of Medicinal Plants*. New York: DK Publishing, New York.
- [8]. Blumenthal, M., Goldberg, A. and Brinckmann, J. (2000): *Herbal Medicine: Expanded Commission E Monographs*. Marshmallow leaf. Austin, TX: American Botanical Co uncil; Newton, MA: Integrative Medicine Communications.
- [9]. Milin, V. and Kustrak, D. (2003) Official and unoffical polysaccharide containing drugs (Mucilaginous drugs) . *Farm Glas* . 59: 57-67.
- [10]. Yeole, N.B., Sandhya, P., Chaudhari, P.S. and Bhujbal, P.S. (2010): Evaluation of *Malva sylvestris* and *Pedalium murex* Mucilage as Suspending Agent. *International Journal of Pharm Tech Research*, Vol. 2(1).pp.385.
- [11]. Cutillo, F., D'Abrosca, B., DellaGreca, M. Terpenoids and phenol derivatives from *Malva sylvestris*. **Phytochemistry**, vol. 67, p.481-485, 2006.
- [12]. Wang, Z. Impact of anthocyanin from *Malva sylvestris* on plasma lipids and free radical. **Journal Forest Research**, vol.16, n.3, p.228-232, 2005.
- [13]. Karakaya, S. (2004) : Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet. *International Journal of Food Sciences and Nutrition*, 55 (1): 67-74. *International Journal of Pharm Tech Research*, 2, (1), 385-9.
- [14]. Mas, T., Susperregui, J, Berker, B.R., Cherze, C., Moreau S, Nuhrich A, Vercauteren J (1999) DNA triplex stabilization property of natural anthocyanins. *Phytochem*. 53:679-687.
- [15]. Cutillo, F., D'Abrosca B., Della Greca M, Fiorentino A, Zarelli A (2006) Terpenoids and phenolderivatives from *Malva sylvestris*. *Phytochem*. 67:481-485.
- [16]. Quave, C.L., Plano, L.R.W., Pantuso, T., Bennett, B.C. (2008b) Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*. *J. Ethnopharmacol*. 118:418-428.
- [17]. Mohammed Choukri Beghdad, Chahid Benammar, Fatima Bensalah, Fatima-Zohra Sabri, Meriem Belarbi and Farid Chemat (2014) Antioxidant activity, phenolic and flavonoid content in leaves, flowers, stems and seeds of mallow (*Malva sylvestris* L.) from North Western of Algeria. *African Journal of Biotechnology*, Vol. 13(3), pp. 486-491.
- [18]. Okonko, I.O., Ijandipe, L.A., Ilusanya, A.O., Donbraye-Emmanuel, O.B., Ejembi, J., Udeze A. O., Egun O.C., Fowotade A. and Nkang A. O. (2010) Detection of Urinary Tract Infection (UTI) among pregnant women in Oluyoro Catholic Hospital, Ibadan, South-Western Nigeria. *Malaysian Journal of Microbiology*, Vol 6(1) 2010, pp. 16-24.
- [19]. Cheesbrough, M. (2002) *Medical laboratories manual for tropical countries*. Cambridge University Press, pp. 479.
- [20]. Cheesbrough, M. (2004). *District laboratory practice in tropical countries*. Cambridge University Press. pp. 357.
- [21]. Buchanan, R.E. and Gibbons, N.E. (1974) *Bergey's Manual of Determinative Bacteriology* (8th edition). Williams & Wilkins Co. Baltimore USA.
- [22]. Karm I.F.A.(2016) The effect of some fresh water alga extracts in the inhibition of the growth of some

- microorganism that cause food spoilage. The Iraqi Journal of Agricultural Sciences – 74(4):1118-1123.
- [23]. Bauer, A.W. Kirby, W.M.M., Sherris. and M. Turck (1966) Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.*, 45: 493-496.
- [24]. Harborne, B. (1998) *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd Edition. Chapman & Hall Pub. London, UK.
- [25]. Singleton, V.L., Colorimetry of total phenolics with phosphomolybdenum dichromate reagent. *Amer. J. Enol. Viticult.* 16: 144-58 (1965).
- [26]. Kazazic, M., Djapo, M. and Ademovic, E.. (2016) Antioxidant activity of water extracts of some medicinal plants from Herzegovina region. *Int. J. Pure App. Biosci.* 4 (2): 85-90. DOI: <http://dx.doi.org/10.18782/2320-7051.2251>.
- [27]. SAS (2012) *Statistical Analysis System, User's Guide*. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary, N.C. USA.
- [28]. Mihaylova D., Popova A., Denkova R., Alexieva I., Krastanov A. (2015) In Vitro Antioxidant And Antimicrobial Activity Of Extracts Of Bulgarian *Malva Sylvestris* L. *Annuaire de l'Université de Sofia "St. Kliment Ohridski" Faculte de Biologie*. First National Conference of Biotechnology, Sofia 2014. volume 100, livre 4, pp. 41-48.
- [29]. Fatima Zohra Sabria, Meriem Belarbi, and Samira Sabri, 2013, Fatty acids profile and antimicrobial activities of the seed oil of *Malva sylvestris* L. from Algeria, *IJCEBS* Volume 1, Issue 2 (2013) ISSN 2320-4087.
- [30]. Al Janabi, Nidhal Mohammed Hindi Mazin Jamil & Al Shirifi, Rahim Hassan (2011) The inhibiting activity of the aqueous and alcoholic extracts of *Al jinebra hwera* and watercress towards some microorganisms. *A Anbar Magazine for Agronomy*, (3) 9: 304-14.
- [31]. Emad Hamdi Jassim, Aliaa Saadoon Abdulrazaq & Zina Hashem Shehab (2015) The effect of aqueous and alcoholic extracts of some plants from cruciferae family against microorganisms growth. *International journal of advanced biological research*, V.5 (2). pp 1-4.
- [32]. Arbonnier, M. (2004) *Trees, Shrubs and Lianas of West Africa dry zones*. CIRAD, MNHN, Margraf Publishers GmBH, France, P. 573
- [33]. [33] Banzouzi JT, Prost A, Rajemiarimiraho M, Ongoka P (2008a). Traditional uses of the African *Militia* species (*Fabaceae*). *Int. J. Bot.*, 4(4): 406-420 (cite or delete)
- [34]. [34] Temitope Israel Borokini and Felix Oluwafemi Omotayo. 2012. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. *Journal of Medicinal Plants Research* Vol. 6(7), pp. 1106-1118. DOI: 10.5897/JMPR09.430.
- [35]. Meena Sahu, Devshree Vermaand, K.K. Harris. (2014) Phytochemical analysis of the Leaf, Stem and Seed Extracts of *Cajanus Cajan* L. (Dicotyledoneae: Fabaceae) *World Journal of Pharmacy and Pharmaceutical Sciences*, vol 3(8), pp 694-733.
- [36]. Ibrahim, H., Sani, F.S., Danladi, B.H. and Ahmadu, A.A. (2007) Phytochemical and Antisickling studies of the leaves of *Hymenocardiaacida* Tul (Euphorbiaceae). *Pakistan Journal of Biological Sciences*, 10(5): 788-91.
- [37]. Sabri Fatima Zohra, Belarbi Meriem, Sabri Samira, Alsayadi Muneer M.S. (2012) Phytochemical Screening and identification of some compounds from Mallow, *J. Nat. Prod. Plant Resour.* 2 (4):5, pp12-516.
- [38]. Nidal Amin Jaradat*, Murad Abualhasan, Iyad Ali. (2015) Comparison of Anti-Oxidant Activities and Exhaustive Extraction Yields between Wild and Cultivated *Cyclamen persicum*, *Malva sylvestris* and *Urtica pilulifera* Leaves. *Journal of Applied Pharmaceutical Science* Vol. 5 (04), pp. 101-106. DOI: 10.7324/JAPS.2015.50417