j.Egypt.vet.med.Assoc 79, no 3. 823 – 835 (2019)

INVITRO ANTIBACTERIAL EFFECT OF SOME HERBAL PLANT EXTRACTS AGAINST *E.COLI*

By

Doaa El-Tahan[1], Heidy Abo El-Yazeed[1], Sherin I, Ibrahim[2], Barakat, A[3], and El-Hariri,M.[1]

[1]Department of Microbiology, Faculty of veterinary medicine Cairo University

[2]Department of Pharmacology, Faculty of veterinary medicine Cairo University

[3]Department of Zoonosis, National Research Center.

ABSTRACT

Many edible plants have been used since ancient time to control microbial infections. This study was designed for evaluating the antibacterial effect of the herbal plant extracts against *E.coli.* The broth microdilution method was used to determine the Minimal Inhibitory Concentrations (MIC) of the studied extracts. Methanol extraction method was applied for *Moringa* leaves, *Cucurbita pepo* fruits*, Cucurbita pepo* seeds, *Citrilluscolocynthis* seeds and *Citrilluscolocynthis* fruits. It was found that, methanolic extract of *Citrilluscolocynthis* fruits had antibacterial effect on *E.coli* at MIC 640 µg/m. On the other hand, hexane extract of *Moringa* leaves *Cucurbita pepo* fruits, *Citrillus colocynthis* seeds and *Citrillus colocynthis* fruits did not show any inhibitory zone for *E.coli*. However, *Cucurbita pepo* seed extract showed inhibition for *E.coli* at MIC 640 µg/ml. Chloroform extract of *Moringa* leaves, *Cucurbita pepo* seeds and *Citrilluscolocynthis* seeds showed inhibition for *E.coli* at MIC 640 µg/ml, but the chloroform extract of *Cucurbita pepo* fruits and *Citrilluscolocynthis* fruits didn't show any inhibitory zone for *E.coli.* In addition, it was found that butanol extract of *Moringa* leaves showed inhibition for *E.coli* at MIC 1280 µg/ml while that of *Cucurbita pepo* fruit, *Cucurbita pepo* seeds, *Citrilluscolocynthis* fruits and *Citrilluscolocynthis* seeds had effects on *E.coli* at MIC 640 µg/ml. These results confirm the traditional claims and provide promising baseline information for the potential use of the tested extracts in the fight against bacterial infections.

Keywords:

Antibacterial, *E.coli*, Extract, MIC, *Moringa*, *Citrilluscolocynthis*, *Cucurbita pepo.*

INTRODUCTION

Infectious diseases are still a major health concern, accounting for 41% of the global disease burden measured in terms of Disability-Adjusted Life Years (DALYS), close to all non-infectious diseases (43%) and far more than injuries (16%) [1]. One of the main causes of this problem is the widespread emergence of acquired bacterial resistance to antibiotics in such a way that the world is facing today, a serious threat to global public health [2] in the form of not only epidemics, but also pandemics of antibiotic resistance [3]. Several mechanisms have been accounted for, but active efflux plays an important role in this phenomenon [4]. The accumulation of different antibiotic resistance mechanisms within the same strains has led to the appearance of the so called multi-drug resistant bacteria [2]. Due to this problem of resistance to antibiotics, attention is now being shifted towards biologically active components isolated from plant species commonly used as herbal medicine, as they may offer a new source of antibacterial, antifungal and antiviral activities [5]. The potential antimicrobial properties of plants are related to their ability to synthesize several secondary metabolites of relatively complex structures possessing antimicrobial activities[6,7]. The present work was therefore designed to investigate the antibacterial effects of different solvent extracts of three herbal plants, namely *Cucurbita pepo* seeds, *Cucurbita pepo* fruits, *Moringa* leaves, *Citrilluscolocynthis* seeds and *Citrilluscolocynthis* fruits against *E.coli* bacteria.

MATERIAL ANDMETHODS

Plant material and extraction:

The collected plant materials used in this study were obtained from Faculty of Agriculture, Cairo University and identified in the Plant Identification Department, Agriculture Museum, Dokki, Egypt and included the leaves of *Moringa oleifera*, fruits and seeds of *Cucurbita pepo*, fruits and seeds of *Citrulluscolocynthis* [8].

Air dried and powdered sample (250 gm.) of each plant was extracted with methanol for 48 hr at room temperature (25°C), using Whatman Grade No.1 filter paper and concentrated under reduced pressure, then dried to give the crude extracts [9].

Fractionation of these crude extracts was done as by solubilization in water and sequential partition with hexane, chloroform and butanol. Each obtained fraction was then evaporated to dryness and subjected to biological assays. The produced crude extracts and their fractions

were kept hermetic, refrigerated and in darkness, to avoid chemical decomposition or contamination. All extracts were stored at 4°C until further use [10].

Bacterial strains and culture media:

The studied bacteria included the reference type (from the American Type Culture Collection) of *Escherichia coli* (ATCC 87). The strain used was maintained in LB medium (Lysogeny broth), and activated on Mueller Hinton Agar plates 24 h prior to any antimicrobial test. Mueller Hinton Broth (MHB) was used for all antibacterial assays [9].

Bacterial susceptibility testing:

The test samples were first emulsified in DMSO/MHB (50:50 V/V). The solution obtained was then added to MHB, and serially diluted two fold (in a 96 - wells micro plate). One hundred microliters (100 µl) of inoculum (1.5×10^8 CFU/ml) prepared in MHB was then added. The plate was covered with a sterile plate sealer then it was agitated to mix the contents of the wells using a shaker and incubated at 37°C for 18 h. The final concentration of DMSO was 2.5% and did not affect the microbial growth. Wells containing MHB, 100 µl of inoculum and DMSO at a final concentration of 2.5% served as negative control. The MICs of samples were detected after 18 h incubation at 37°C. Viable bacteria reduce this yellow dye to pink. MIC was defined as the lowest sample concentration that exhibited complete inhibition of microbial growth and then prevented this change [11, 12].

Statistical analysis:

Statistical analysis using ANOVA test was applied to evaluate the effect of different solvents and different plants on the inhibition of *E.coli* growth.

RESULTS

Plant material and extraction:

Air dried and powdered sample (250 gm) of each plant was extracted with methanol, hexane, chloroform and butanol. The yield of each one explained in (Table 1).

Table (1): The yield of different solvents used for extraction of each plant sample.

Plant	Yield / Type of solvent (gm)			
	Methanol	Hexane	Chloroform	Butanol
***Cucurbita Pepo* Fruits**	**57.3**	**0.6**	**0.2**	**3.3**
***Cucurbita Pepo* Seeds**	**12.7**	**3.9**	**0.4**	**0.65**
***Moringa* Leaves**	**15.2**	**1.2**	**0.2**	**2.2**
***Citrillus Colocynthis* Fruits**	**23.3**	**0.8**	**0.2**	**4.9**
***Citrillus Colocynthis* Seeds**	**8.6**	**0.8**	**0.2**	**1**

Antibacterial activity of the extracts:

Determination of Minimum Inhibitory Concentration (MIC) for the different plant extracts:

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial that inhibit the visible growth of a microorganism following overnight incubation.

As shown in (Table 2), Fig. (1), methanol extract of *Moringa* leaves, *Cucurbita pepo* fruits, *Cucurbita pepo* seeds, *Citrilluscolocynthis* seeds and *Citrilluscolocynthis* fruits had effects on *E.coli* with MIC 640 µg/ml.

Hexane extract of *Moringa* leaves, *Cucurbita pepo* fruits, *Citrilluscolocynthis* seeds and *Citrilluscolocynthis* fruits did not show any inhibitory zone for *E.coli.* While the hexane extract of *Cucurbita pepo* seeds showed inhibition for *E.coli* with MIC 640 µg/ml.

Chloroform extract of *Moringa* leaves, *Cucurbita pepo* seeds, *Citrilluscolocynthis* seeds and *Citrilluscolocynthis* fruits showed inhibition for *E.coli* with MIC 640 µg/ml. While, the chloroform extract of *Cucurbita pepo* fruits did not show any inhibitory zone for *E.coli.*

Butanol extract of *Moringa* leaves showed inhibition for *E.coli* with MIC 1280 µg/ml. While butanol extract of *Cucurbita pepo* fruits, *Cucurbita pepo* seeds, *Citrilluscolocynthis* fruits and *Citrilluscolocynthis* seeds had effects on *E.coli* with MIC 640 µg/ml.

Table (2): The MIC for the different solvent extracted plant on *E.coli.*

Solvent	MIC/ plant (µg/ml)				
	Cucurbita Pepo Fruits	***Cucurbita Pepo Seeds***	***Moringa Leaves***	***Citrillus Colocynthis Fruits***	***Citrillus Colocynthis seeds***
Methanol	**640**	**640**	**640**	**640**	**640**
Hexane	**R**	**640**	**R**	**R**	**R**
Chloroform	**R**	**640**	**640**	**640**	**640**
Butanol	**640**	**640**	**1280**	**640**	**640**

(R = Resistant).

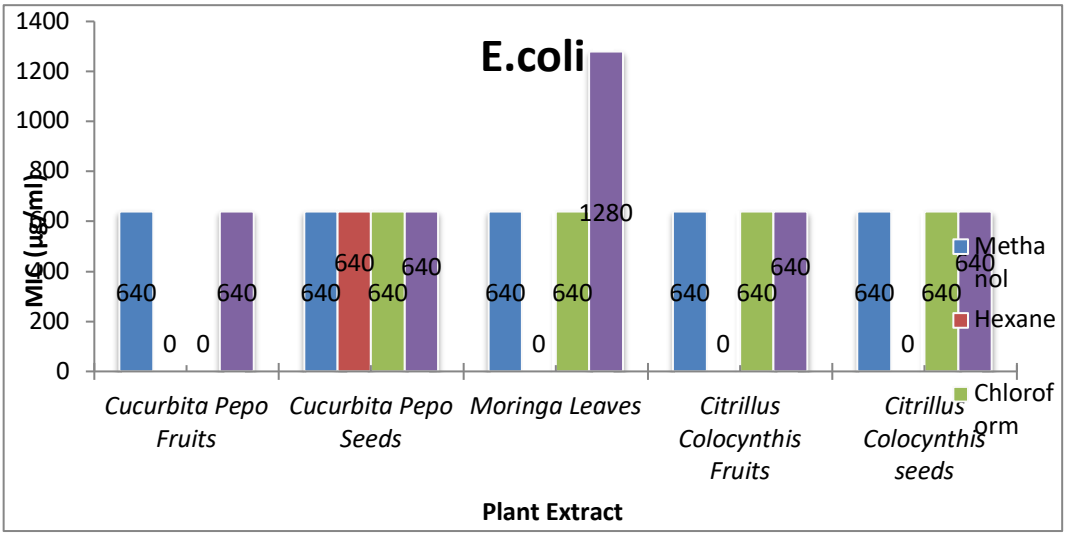

Fig. (1): The MIC for the different solvent extracted plant on *E.coli*.

Statistical analysis:

All the data obtained from ANOVA tests are shown in (Tables 3, 4) and Fig. (2, 3). Analysis of ANOVA test demonstrated that, the different solvents had significant effect on the inhibition of *E.coli* growth ($p < 0.05$). The different solvents could be ranked according to the inhibition force of *E.coli* growth as follow: methanol> butanol > chloroform > hexane Fig. (2). Subsequent post hoc test was performed and it revealed that the inhibition of *E.coli* growth by hexane extract (lowest inhibition to *E.coli*) was significantly different from that of methanol extract (highest inhibition to *E.coli*), chloroform extract and butanol extract.

On the other hand, the inhibition of *E.coli* growth by methanol, chloroform and butanol extracts was non-significant from each other.

Results also revealed that, the different plant extracts showed that there was no obvious significant effect (non-significant) on the inhibition of *E.coli* growth (Table 3). However, practically the different plant extracts could be ranked according to the inhibition effect of *E.coli* growth as follow: *Cucurbita pepo* seeds>*Citrulluscolocynthis seeds* and *Citrulluscolocynthis* fruits>*Moringa* leaves >*Cucurbita pepo* fruits Fig. (3).

Table (3): ANOVA table for the effect of plant extract on the inhibition of *E.coli* growth.

Source	Sum of Squares	DF	Mean Square	F	Sig.
Corrected Model	**9.298E6**	**7**	**1328274.286**	**3.706**	**.023**
Intercept	**2.654E7**	**1**	**2.654E7**	**74.057**	**.000***
Solvent	**7372800.000**	**3**	**2457600.000**	**6.857**	**.006***
Plant	**1925120.000**	**4**	**481280.000**	**1.343**	**.310**
Error	**4300800.000**	**12**	**358400.000**	**-**	**-**
Total	**4.014E7**	**20**	**-**	**-**	**-**
Corrected Total	**1.360E7**	**19**	**-**	**-**	**-**

DF: Degree of Freedom, F- Test, Sig.: Significant.

*** Significant at p < 0.05.**

Table (4): Pair wise comparison for the effect of solvents on the inhibition of *E.coli* growth.

(I) Solvent	(J) Solvent	Mean Difference (I-J)	Std. Error	Sig.
Methanol	**Hexane**	**-1536.0000***	**378.62911**	**.007***
	Chloroform	**-384.0000**	**378.62911**	**.745**
	Butanol	**-128.0000**	**378.62911**	**.986**
Hexane	**Methanol**	**1536.0000***	**378.62911**	**.007***
	Chloroform	**1152.0000***	**378.62911**	**.044***
	Butanol	**1408.0000***	**378.62911**	**.013***
Chloroform	**Methanol**	**384.0000**	**378.62911**	**.745**
	Hexane	**-1152.0000***	**378.62911**	**.044***
	Butanol	**256.0000**	**378.62911**	**.904**
Butanol	**Methanol**	**128.0000**	**378.62911**	**.986**
	Hexane	**-1408.0000***	**378.62911**	**.013***
	Chloroform	**-256.0000**	**378.62911**	**.904**

*** Significant at p < 0.05.**

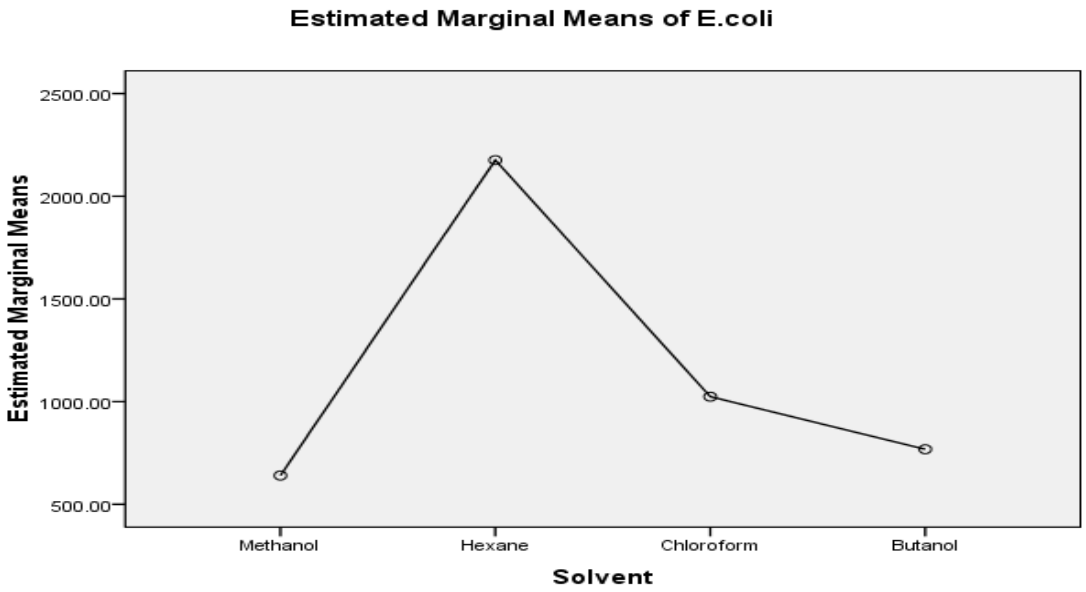

Fig. (2): Effect of the solvents on the inhibition of *E.coli* growth.

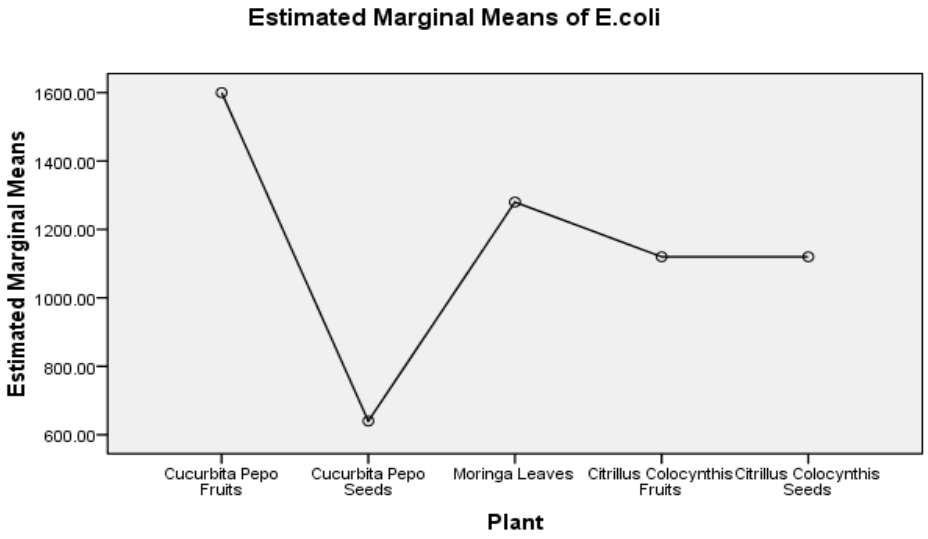

Fig. (3): Effect of the plant extracts on the inhibition of *E.coli* growth.

DISCUSSION

In plants, secondary metabolites attract beneficial and repel harmful organisms, serve as phytoprotectants and respond to environmental changes. In animals, such compounds have many beneficial effects including antibacterial and antiviral properties [13, 14]. The classes of secondary metabolites detected in the tested herbal extracts can somehow provide a preliminary explanation of their activities [15].

Phytochemical screening revealed the presence of several classes of secondary metabolites such as alkaloids, polyphenols, flavonoids, anthraquinones, coumarins, saponins, tannins, triterpenes and steroids. Several molecules belonging to these classes were found to be active on pathogenic microorganisms [13, 16-18]. The presence of such metabolites in the studied plant extracts can provide a preliminary explanation on their antibacterial activities. Differences were observed in the antibacterial activities of the extracts. These could be due to the differences in their chemical composition as well as in the mechanism of action of their bioactive constituents [13]. All the extracts are rich in secondary metabolites, especially the extract from *Moringa*; However, activity does not depend only on the presence of secondary metabolites in the plant extracts, but mostly on their concentration and the possible interactions with other constituents [19].

Methanol has polarity that was used to fit or extraction of various polar compounds, but certain groups of non-polar compounds are also soluble in methanol. The molecule of methanol consists of a single atom of a tetrahedral carbon linked to three hydrogens and a-OH group.

The-OH group is the polar group and the three hydrogen molecules are the water insoluble hydrocarbon chain. That is why methanol can dissolve polar molecules and non-polar compounds can be extracted with n-hexane. In this study, chemical contents of plant extract differ on the nature of the compounds of the solvent used in the extraction procedure. However, the pumpkin fruit has good beta-carotene, carbohydrates, vitamins, minerals; amino acids and polysaccharides, which including bound protein are the bioactive materials of pumpkin [20-22]. Moreover, **(Shah *et al,)*** reported that extraction strategy yields different phytochemical extracts. The bacteria observed differ in sensitivity and this could be due to the differences in cell wall structure. Gram-negative bacteria have lipopolysaccharides in their cell wall, which may prevent the active compounds from reaching the cytoplasmic membrane

of bacteria. Generally, the overall impact of pumpkin extracts against the microorganism was clearly shown where the effects of extracts increase with the increase of extract concentrations [20, 23].

The inhibitory activity of *M. oleifera* was previously reported against some bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella typhi* [24]. The present study confirmed the antimicrobial potential of those plants and provides additional information on its ability to inhibit the growth of multi drug resistant (MDR) bacteria. The results of this work are very important taking into account the medicinal importance of the tested MDR bacteria [25-27] and also the fact that samples used are edible plants [19].

The antimicrobial activity of different solvent extracted samples from *Moringa* leaves, *Cucurbita pepo* fruits,*Cucurbita pepo*seeds,*Citrilluscolocynthis* fruits and *Citrilluscolocynthis* seeds were investigated by the broth micro dilution method to determine the minimal inhibitory concentration (MIC).

Methanol extracted samples of *Moringa* leaves, *Cucurbita pepo* fruits*, Cucurbita pepo* seeds, *Citrilluscolocynthis* seeds and *Citrilluscolocynthis* fruits had effects on *E.coli* with MIC 640 µg/ml. Such results agree with those of **(Noumedem *et al.*)** They stated that, the methanol extract of *Cucurbita pepo* displayed the largest spectra of activity with MIC (128-512 µg/ml) when tested on *E.coli*, it's inhibitory effect has been observed in all the 29 Gram negative bacteria (100% of activity) [9]. In other study,**(Akhilesh Dubey *et al.*,)** found that, the methanol extract of fruit of *C. pepo* was evaluated for antimicrobial activity against *Escherichia coli*, The extract showed moderate to high activity against the investigated microbial strain [28].

Similarly, **(Dzotam *et al.*,)** found an antibacterial activity of methanol extract of *Moringa oleifera* leaves on *E.coli* with MIC (128-1024 µg/ml) [19]. Also, the obtained result go parallel with the report of **(El-Badri *et al.*)** who found that there was an inhibition zone (20 mm) for methanol extract of *Cucurbita pepo* fruits when tested on *E.coli* [29]. The same result was obtained by **AE Al-Snafi** who found that the maximum antimicrobial activity was exhibited by methanol extract of the *Citrilluscolocynthis* fruits against *Escherichia coli* [30].

Hexane extracted samples of *Moringa* leaves, *Cucurbita pepo* fruits*, Citrilluscolocynthis* seeds and *Citrilluscolocynthis* fruits showed no activity against *E. coil* at any concentration.

Contrary to these result hexane extracts of *Cucurbita pepo* seeds, control the growth of *E. coil* effectively at 640 µg/ml concentration.These results agree with Abdel-Rahim who stated that, the hexane extract of *Cucurbita pepo* seeds was giving a large inhibition zone (about 26.5mmdiameter) when tested on *E.coli* [31].On the other hand,Elmalik reported an inhibitory effect of hexane fraction of *Citrilluscolocynthis* fruits with inhibitory zone 15.7 mm [10].

Meanwhile, the obtained results disagree with the report of Aljuraifani who said that, the minimum concentration (MIC) for hexane extract of *Cucurbita pepo* fruits was 12.5 µg/ml for *E.coli* leading to a conclusion that, the hexane extract of pumpkin was found to be the most potent. These indicate that some active substances in *Cucurbita pepo* dissolved in varying degrees in the different solvents [23].Also,**(Hammer *et al,*).**reported that fixed oil of pumpkin failed to inhibit any organisms at the highest concentration, which was 2·0% (v/v) [31].

Chloroformextractedsamples of*Moringa* leaves,*Cucurbita pepo* seeds and *Citrilluscolocynthis* seeds showed inhibition for *E.coli* with MIC 640 µg/ml.While the chloroform extract of *Cucurbita pepo* fruits and *Citrilluscolocynthis* fruits did not show any inhibitory zone for *E.coli.* These results agree withVinoth who stated that, the antibacterial activity of chloroform extract of *Moringa olifera* showed maximum zone of inhibition (6 mm) against *Escherichia Coli* [24].

While, the current results differ from those reported by Aljuraifani who said that, the minimum concentration (MIC) for chloroform extract of *Cucurbita pepo* fruits was 12.5 µg/ml for *E.coli*. These indicate that some active substances in *Cucurbita pepo* dissolved in varying degrees in the different solvents [23]. On the other hand, this disagree with the report of **(El-Badri *et al.)*** who found that there was an inhibition zone (15 mm) for chloroform extract of *Cucurbita pepo* fruits when tested on *E.coli* [29].

Butanol extracted samples of *Moringa* leaves showed inhibition for *E.coli* with MIC 1280 µg/ml.On the other hand, butanol extract of *Cucurbita pepo* fruits, *Cucurbita pepo* seeds, *Citrilluscolocynthis* fruits and *Citrilluscolocynthis* seeds had an effect on *E.coli* with MIC 640 µg/ml. These results disagree with the data obtained by **(El-Badri *et al.,*)** who found that there was no inhibition zone (mm) for butanol extract of *Cucurbita pepo* fruits when tested on *E.coli* [29].

CONCLUSION

The overall results of the present investigation confirmed the traditional uses of the studied herbal extracts in the treatment of bacterial infections. This study also provides baseline information for the possible use of the different solvent extracted samples in the control of infectious diseases involving *E.coli*. Further researches are required to determine which active compounds responsible for these inhibitory effects.

REFERENCES

Noah, D. and G. Fidas (2000): The global infectious disease threat and its implications for the United States: National Intelligence Council Washington Dc.

Chopra, I. (2000): New drugs for the superbugs. Microbiology Today**:** 27: p. 4 - 6.

Chanda, S., Y. Baravalia, M. Kaneria, and K. Rakholiya (2000): Fruit and vegetable peels–strong natural source of antimicrobics. Curent Research, Technology and Education Topic in Applied Microbioogy and Microbial Biotechnology, 444: p. 450.

Pages, J.-M., J.-P. Lavigne, V. Leflon-Guibout, E. Marcon, F. Bert, L. Noussair, and M.-H. Nicolas-Chanoine (2009): Efflux pump, the masked side of ß-lactam resistance in Klebsiella pneumoniae clinical isolates. PLoS One. 4 (3): p. e4817.

Maiyo, Z., R. Ngure, J. Matasyoh, and R. (2010): Chepkorir, Phytochemical constituents and antimicrobial activity of leaf extracts of three Amaranthus plant species. African Journal of Biotechnology, 9 (21): p. 3178-3182.

Matasyoh, J., Z. Maiyo,R.(2009): Ngure, and R. Chepkorir, Chemical composition and antimicrobial activity of the essential oil of Coriandrum sativum. Food Chemistry. 113 (2): p. 526 -529.

Souza, E.L.d., E.D.O. Lima, K.R.d.L. Freire, and C.P.D. (2005): Sousa, Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. Brazilian archives of Biology and Technology. 48 (2): p. 245-250.

El-Said, S.I. (2008): "Some pharmacological and toxicological studies on some Egyptian plants "..

Noumedem,J.A.,M.Mihasan,S.T.(2013): Lacmata,M.Stefan, J.R. Kuiate, and V. Kuete, Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against Gram-negative multidrug-resistant bacteria.BMC complementary and alternative medicine.13(1): p. 26.

Elmalik, A.O.A.E.M., A.E.-D.M.S. Hosny, and M.A.E.-A.M. (2014): Rabeh, Study on antimicrobial, anticancer, insects larvicidal and antioxidant activities of Citrullus colocynthis.

Kuete, V., B. Ngameni, C.C.F., R.K. Tankeu, B.T. (2008): Ngadjui, J.J.M. Meyer, N. Lall, and J.R. Kuiate, Antimicrobial activity of the crude extracts and compounds from Ficus chlamydocarpa and Ficus cordata (Moraceae). Journal of ethnopharmacology, 120 (1): p. 17-24.

Wiegand, I., K. Hilpert, and R.E.W. (2008): Hancock, Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols. 3 (2): p. 163.

Cowan, M.M., Plant products as antimicrobial agents (1999): Clinical microbiology reviews, 12 (4): p. 564-582.

Kuete, V. (2010): Potential of Cameroonian plants and derived products against microbial infections a review. Planta Medica. 76 (14): p. 1479 -1491.

Voukeng, I.K., V. Kuete, J.P. Dzoyem, A.G. (2012): Fankam, J.A.K. Noumedem, J.R. Kuiate, and J.-M. Pages, Antibacterial and antibiotic-potentiation activities of the methanol extract of some cameroonian spices against Gram-negative multi-drug resistant phenotypes. BMC research notes, 5 (1): p. 299.

Awouafack, M.D., P. Tane, V. Kuete, and J.N. (2013): Eloff, Sesquiterpenes from the medicinal plants of Africa, in Medicinal plant research in Africa, Elsevier. p. 33-103.

Ndhlala, A.R., S.O. (2013): Amoo, B. Ncube, M. Moyo, J.J. Nair, and J. Van Staden, Antibacterial, antifungal, and antiviral activities of African medicinal plants, in Medicinal plant research in Africa, Elsevier. p. 621- 659.

Tsopmo, A., F.M. Awah, and V. (2013): Kuete, Lignans and stilbenes from African medicinal plants, in Medicinal plant research in Africa, Elsevier. p. 435 - 478.

Dzotam, J.K., F.K. Touani, and V. (2016): Kuete, Antibacterial and antibiotic-modifying activities of three food plants (Xanthosoma mafaffa Lam., Moringa oleifera (L.) Schott and Passiflora edulis Sims) against multidrug-resistant (MDR) Gram-negative bacteria. BMC complementary and alternative medicine. 16 (1): p. 9.

Shah, N., S. Farman, Z. Hussain, M.B. (2013): Arain, and S. Shams, Cold extraction strategy for crude dye extraction from Cucurbita Pepo leaves. Pakhtukhwa Journal of Life Science. 1: p. 130-144.

Al-Ghazal, A.T. (2012): Evaluation of antibacterial effect of Cucurbita pepo (yakten) extracts on multi-antibiotic resistance bacterial strains isolated from human urinary tract infections. RAF. J. Sci. 23 (2): p. 1-7.

Prasad, M.P. (2014): In vitro phytochemical analysis and antioxidant activity of seeds belonging to Cucurbitaceae family. Indian Journal of Advances in Plant Research. 1 (4): p. 13-18.

Aljuraifani, A. (2017): Impact of solvent types on antimicrobial activities of pumpkin (Cucurbita pepo L.) pulp extracts. The Asian International Journal of Life Sciences. 229-235.

Vinoth,B.,R.(2012): Manivasagaperumal,andS.Balamurugan,Phytochemical analysis and antibacterial activity of Moringa oleifera Lam. Int J Res Biol Sci. 2(3): p. 98-102.

Mallea, M., J. Chevalier, C. Bornet, A. (1998): Eyraud, A. Davin-Regli, C. Bollet, and J.-M. Pages, Porin alteration and active efflux: two in vivo drug resistance strategies used by Enterobacter aerogenes. Microbiology. 144 (11): p. 3003-3009.

Tran, Q.-T., K.R. (2010): Mahendran, E. Hajjar, M. Ceccarelli, A. Davin-Regli, M. Winterhalter, H. Weingart, and J.-M. Pages, Implication of porins in Î²-lactam resistance of Providencia stuartii. Journal of Biological Chemistry,. 285 (42): p. 32273-32281.

Mallea, M., A. (2003): Mahamoud, J. Chevalier, S. Alibert-Franco, P. Brouant, J. Barbe, and J.-M. Pages, Alkylaminoquinolines inhibit the bacterial antibiotic efflux pump in multidrug-resistant clinical isolates. Biochemical Journal. 376 (Pt 3): p. 801.

Dubey, A., N. (2010): Mishra, and N. Singh, Antimicrobial activity of some selected vegetables. International Journal of Applied Biology and Pharmaceutical Technology. 1(3): p. 994-999.

El Badri, A.O., O. (2010): Khalil, I.F. Ahmed, Q. Yuan, and S.E. Ibrahim, In Vitro Antimicrobial Activity of Summer Squash (Cucurbita pepo L.).

Al-Snafi, A.E. (2016): Chemical constituents and pharmacological effects of Citrullus colocynthis-A review. IOSR Journal of Pharmacy. 6 (3): p. 57-67.

Hammer, K.A., C.F. Carson, and T.V. (1999): Riley, Antimicrobial activity of essential oils and other plant extracts. Journal of applied microbiology,. 86 (6): p. 985-990.