ISSN: 2520-5234

Available online at http://www.sjomr.org

SCIENTIFIC JOURNAL
OF MEDICAL RESEARCH

Vol. 3, Issue 12, pp 128-134, Autumn 2019

ORIGINAL ARTICLE

Effect of *Cassia senna* Fruit Hexane Extract as Antibacterial Against Urinary Tract Infection Pathogens and Antioxidant Activity

Bayadir Abdul Ameer Qasim[1], **Wijdan Hussein Abd-Alsahib**[2] and **Abdullah Hamad Lafta**[3]

[1,2,3] Department of Biology, College of Science, University of Basrah, Basrah, Iraq.

ARTICLE INFORMATIONS

Article History:
Submitted: 15 October 2019
Revised version received:
10 November 2019
Accepted: 18 November 2019
Published online: 1 December 2019

Key words:
Medicinal plants
Antibacterial
Antioxidant
UTI

Corresponding author:
Bayadir Abdul Ameer Qasim
Email: jonyousef111@gmail.com
Department of Biology
College of Science
University of Basrah
Basrah
Iraq

ABSTRACT

Objectives: This study investigate the role of hexane extract of *Cassia senna* fruits as antibacterial against urinary tract infection UTI pathogens and antioxidant activity.

Methods: The chemical composition of extract was performed by GC-MS Analysis. the antibacterial potential was achieved using agar well diffusion method and Minimum inhibitory concentration method also determination of human red blood cells (RBCs) cytotoxicity whereas antioxidant performed by DPPH assay.

Results: The results of GC-MS showed linolaidic acid 25.08%, linoleic acid ethyl ester 20.04%, beta-sitosrol 14.8%, gamma- sitosterol 13.37%, hexadecanoic acid ethyl ester 13.04%, tetracosane 9.16%, Stigmasterol 6.73%, Lupeol and Betulin area was 5.66%. the extract exhibit antibacterial activity against UTI pathogens with excellent and broad spectrum activity as compared with antibiotics with no toxic effect against human(RBCs). the highest inhibition zone was 50 mm of *Staphylococcus pseudintermedius* and the lowest 18 mm for *proteus vulgaris* with MIC ranging between less than 1 and 1 mg. From the analysis of antioxidant potential of *Cassia Senna* hexane fruit extract revealed high inhibition DPPH percent 91% at high concentration 1000 ul/ml as well as ascorbic acid.

Conclusion: This study highlights that hexane extract of *Cassia senna* fruit has potential components which elicited their biological activities against UTI pathogens and antioxidant.

Citation: **Qasim B.A., Abd-Alsahib W.H.** and **Lafta A.H.** "Effect of *Cassia senna* Fruit Hexane Extract as Antibacterial Against Urinary Tract Infection Pathogens and Antioxidant Activity". Sci. J. Med. Res. 2019; 3 (12): 128-134.

INTRODUCTION

Urinary tract infection UTI is a bacterial contagion that affects any part of the urinary tract. the members of family Enterobacteriaceae are The most common cause of UTI which include Gram negative bacteria *E. coli*, *Klebsiella*, *Enterobacter* and *Proteus*. the most common bacteria capable of causing infection in humans and animals, particularly urinary tract infections is *E. coli* [1]. As well Gram positive *Staphylococcus* sp. plays a role in this infection [2]. One of the most concerning threats to global health is Antimicrobial resistance AMR, new anti-biotic are needed to overcome it [3]. AMR occurs when microorganisms are able to survive in the presence of drugs that would normally inhibit their growth [4].

At present, the selection of effective antibiotics that may be used in UTI are restricted particularly as new resistant strains protrude [5]. Thus, there Is a pressing need for novel antimicrobial agents to be used experiential or as an eventual therapy. for the management of UTI, Medicinal plants may offer an alternative [6,7]. Hence, much research to develop new antimicrobial agents were attracting.

one the most important medicinal plant is *Cassia senna*, A small herbaceous shrub reaches a height of 1.5 meters. flower are racemes, yellow, fruit are legume, greenish brown [8, 9].

Cassia species have been of medical interest because their good therapeutic value in folk medicine [10]. The plant sap can act against microorganisms by stop the growth of microbial colony [11].

many active compounds are found in *Cassia senna* including alkaloids, lecithin sappronin, glycoside, Anthraquinone glycosides A, B, C, D, Sennoside, glycoside Aloe emodin and Rhein in addition to sterols, gelatinous substances, dyes yellow flavonite resins, cyanose, isoflavonide and phytoestrogens [12]. aromatic plants contain Natural products are conceders to be one of the potent sources for the screening of free radical scavenging agents, antioxidant and antimicrobial [13]. Hence the present study focused to screening the fruits of *Cassia senna* hexane solvent extract to find more active antioxidant and antimicrobial agents against pathogens of UTI.

MATERIALS AND METHODS

Collection of plant: *Cassia senna* fruits was purchased from the market in the town of Basra during January 2019, brought to the laboratory stored in bags until use.

Extraction technique: The fruits of *Cassia Senna* have been taken and then grinds into powder by an electrical grinder. The powder was kept in a closed container at 4 ℃ until the time of use.

Preparation of Hexane extract: The method of Bobby *et al* [14] was Followed, a twenty- five 25 gm of dried powder was extracted with 500 ml of hexane by Soxhlet continuous Extraction for 24 hours. The solution was filtered by using whatmann No.13 filter paper then the filtrate was concentrated under reduced pressure on a rotary evaporator at 50 ℃ and dried at room temperature, the extract were collecting in sterilized glass tubes until use.

Gas Chromatography-Mass Spectroscopy: Gas Chromatography-Mass Spectroscopy GC-MC analysis was achieved with an auto Mass Hunter/GCMS instrument using a SPB 5 (30m/0.25mm/0.5mm) capillary column. 1 ml of each extract dissolved in methanol was injected in the following conditions: injector temperature 300℃; carrier gas, helium; pressure,11.962 psi. compound were identified on the basis of their mass spectral data.

Microorganisms: The pathogenic bacteria isolated from urinary tract infections was used in this study are details in Table 1. All microbial isolates were provided by department of biology collage of science, university of Basra.

Table 1: **Frequency and occurrence Percentage of fungal isolates isolated from oil-rich soils.**

Microorganisms	Details
Staphylococcus aureus	Bacteria GM^+
Proteus vulgaris	Bacteria GM^-
E.coli	Bacteria GM^-
Pseudomonas aeruginosa	Bacteria GM^-
Proteus mirabilis	Bacteria GM^-
Staphylococcus pseudintermedis	Bacteria GM^+
Staphylococcus hominies	Bacteria GM^+
Acientobacter baumanii	Bacteria GM^-
Acientobacter baylyi	Bacteria GM^-
Streptococcus pyogenes	Bacteria GM^+
Streptococcus pneumonia	Bacteria GM^+

Determination of antibiotics resistant and fruits hexane extract activity: All bacterial strain were cultured and maintained on either sterile nutrient broth or nutrient agar, the extracts of fruits *Cassia Senna* was evaluated against Eleven selected pathogenic of UTI bacterial isolates. 100 mg/ml of hexane extract were dissolved in Dimethyl Sulfoxide (DMSO). The antibacterial assay was done by agar well diffusion. The bacteria was grown on Mueller Hinton agar Petri dishes via spreading at concentration of 10^8 Colony Forming Unit (CFU)/mL (0.5 McFarland turbidity standards) by sterile cotton swab and allowed to remain in contact for 1 min, wells were made on the plates using sterile core borer with a diameter of 6 mm. 50 ul of extract with a concentration of 100 mg/ml was pipetted gently in to wells, DMSO were also examined following the pervious steps as a control. The plates were incubated at 37 ℃ for 24 h. inhibition zone around each well was measured in mm [15]. Also Susceptibility of isolates to Five types of antibiotics were achieved by disc diffusion method [16]. Commercially available antibiotics disc (Bioanalyes company) were used 30 mg FEP Cefepime, 10mg CN Gentamicin, 15 mg ER Erythromycin , 5mg CIP Ciprofloxacin ,30mg AK Amikacin measured the diameter of the inhibition zone in mm inclusive of the diameter of the discs.

Cytotoxicity assay: Human red blood cells (RBC) was tested according to [17] with some modification. The process was performed by preparing the blood solution 1 ml of blood was added to 20 ml sterile phosphate buffer saline PBS solution) the concentration of extract (5, 10, 25, 50, 100, 250 and 500 mg/ml) were obtained using a DMSO as a solvent. Followed 100 microliters of each

concentration was added to 2 ml of blood solution, tubes were incubated at 37 ℃. The solution was monitored after 3h and 24 h. to observe the positive result by turning the solution into clear, while the hydrolysis of red blood cells turns the blood solution into turbid indicating the cytotoxicity . At the same time ,positive control (blood solution with water)and negative control (blood solution with phosphate buffer) were used as control to compare the results.

Antioxidant activity

DPPH assay: 2, 2-diphenyl-1-picrylhydrazyl method was performed as described by Mohanty [18] With minor modification The assay involves the measurement of disappearance of the colored free radical DPPH by spectrophotometric determination. The free radical scavenging activity of *Cassia senna* Fruits hexane extracts and standard vitamin C was determined, 1 ml of different concentrations (1000,750-500-250-100-75-50-25 and 10 ug/ ml) of extract and standard were mixed with 1 mL of freshly prepare DPPH in methanol (0.004%) vortexed thoroughly, the solution was incubated at room temperature in the dark for 30 min. the absorbance was recorded at 517 nm using UV - Vis spectrophotometer methanol was used as a blank solution. The free radical scavenging activity was expressed as the percentage of inhibition which was determined using the following equation:

RSA=[(DPPH−*A*Sample)/*A*DPPH] *100

Were %RSA is the percentage of inhibition, ADPPH is the absorbance of DPPH (t=0 min) and A sample is the absorbance of the extract.

RESULTS

Gas Chromatographic-Mass Spectrometry (GC-MS) Analysis: GC-MS chromatogram analysis showed many peaks, indicating the Organic compounds which classified by structural criteria Figure as following: were rich in terpenoids and fatty acids and other lipophilic compounds such as saturated and unsaturated free fatty acid, methyl and ethyl esters of saturated and unsaturated fatty acids, saturated triglycerides and diglycerides, sterols ,triterpenes, mono- and sesquiterpenes, unsaturated monoglycerides, phytosterols. The GC-MS study of *cassia senna* fruits hexane extract has shown 43 chemical constituents, the highest peak area was of linolaidic acid 25.08% ,linoleic acid ethyl ester 20.04%, beta-sitosrol 14.8%, gamma-sitosterol 13.37%,hexadecanoic acid ethyl ester was 13.04%, tetracosane was 9.16%,Stigmasterol 6.73% ,Lupeol and Betulin area was 5.66%. The chemical nature of compounds was identified on the basis of molecular formula, retention time (RT), molecular weight and compound name Table 2.

Antibacterial Activity test

The antibacterial potential of *Cassia senna* fruits hexane extract and five antibiotics were tested against common gram negative and positive urinary tract pathogens, Table 3. After proper incubation the results were recorded and represented in Figure 4. The results showed that hexane extract has broad spectrum activity against UTI pathogens , the highest inhibition zone of hexane extract was 50 mm of *Staphylococcus pseudintermedius* followed by *Staphylococcus hominies*, while there was no effect on *Streptococcus* sp. and *Acientobacter baumanii* .

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

The MIC values for antibacterial activity of *Cassia senna* fruits hexane extract were presented in Table 4. inhibited the species were less than 1 mg/ml, while it was 3, and 10 mg/ml for the Gm- strains E. coli and *Acientobacter baylyi* respectively. The highest MBC value was 25mg/ml for *Acientobacter baylyi*.

DPPH scavenging assay

The antioxidant activity assay employed the inhibition of free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) because its stability, simplicity and reproducibility so widely used for evaluating natural antioxidants. The results of percentage inhibitions for DPPH assay are given in Figure 3. Antioxidant activity of *Cassia senna* fruits hexane extract to increase with increase in concentration from 100 µg/ml to 1000 µg/ml , and showed strong inhibition activity 91% at 1000 ug/ml as well as ascorbic acid.

Determination of the cytotoxicity of *Cassia senna* fruits hexane extract

The cytotoxicity of *Cassia senna* fruits hexane extract was tested on human RBCs. The result showed in Table 5 the extract was non-toxic at all concentrations after 3 h and 24 h of incubation at 37℃ Measuring the level of extract cytotoxicity based on hydrolysis of RBCs.

DISCUSSION

This results of GC-MS chromatogram analysis confirm with [19]. The presence of these constituents confirms the pharmacological activity of this extract because many of them have reported biological activity such as fatty acids which exhibited strong antibacterial activity against various oral bacteria [20]. One study was pointed to the presence of oil extract had an effect on gram-positive bacteria [21]. Also linoleic are known to have potential antibacterial and antifungal agents [22, 23]. Other Study was reported the bioactive fractions linoleic acid Possessed antibacterial activity against *Mycobacterium aurum* and *M. phlei* [24]. One study was reported that betulin and its derivatives have been reported bioactivity against bacteria [25].

The antimicrobial activity may due to bioactive compounds such as steroids has antimicrobial properties and used to treat infection caused by bacteria [26]. Steroid conjugate with poly amines which bind in DNA so effected on gram negative and positive bacteria , fungi , and protozoa [27]. Steroid substances such as gama and beta-sitosterol, and stigmasterol inhibited *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pasteurella multocida* and *Pseudomonas aeruginosa* [28]. The results compatible with [29] that hexane extract of *Cassia senna* leaves showed a moderate antibacterial activity, also agreed with [30].

Table 2: **Bioactive Compounds of *Cassia senna* fruits Hexane Extracts.**

No.	Compound Name	Formula	Area%	RT
1	Neophytadiene	C20H38	3.61	21.278
2	Octadecyne	C18H38	3.3	21.278
3	Bicyclo[3.1.1]heptane, 2,6,6-trime thyl	C10H18	0.64	21.278
4	2-Hexadecene, 3,7,11,15-tetramethy	C20H40	0.90	21.354
5	5-Ethyl-1-nonene	C11H22	0.90	21.354
6	Hexadecene,2,6,10,14 -tetramethy	C20H40	1.8	21.354
7	2 methylpropyl) ester-1,2-Benzenedicarboxylic acid, bis	C16H22O4	0.66	21.556
8	Phthalic acid, 2,4-dimethylpent-3-yl isobutyl ester	C19H28O4	0.66	21.556
9	Hexadecanoic acid, ethyl ester	C18H36O2	13.4	22.848
10	Ethyl tridecanoate	C15H30O2	12.74	22.848
11	Octadecadienoic acid, methyl ester 9,12-	C19H34O2	0.77	23.738
12	Methyl 10-trans,12-cis-octadecadie noate	C19H34O3	0.77	23.738
13	10,13 Octadecadienoic acid, methyl Ester	C19H34O2	0.77	23.738
14	Heneicosane	C21H44	3.61	23.800
15	Heptadecane	C17H36	1.89	23.800
16	3,7,11,17-Tetramethyl-2-hexadecen	C20H40O	2.97	23.932
17	Bicyclo[3.1.1]heptane, 2,6,6-trime thyl-, (1.alpha.,2.beta.,5.alpha.)	C10H18	2.97	23.932
18	Linoleic acid ethyl ester	C20H36O2	20.04	24.419
19	Linoelaidic acid	C18H32O2	25.08	24.419
20	Octadecanoic acid, ethyl ester	C20H40O2	4.42	24.662
21	1,5-Di(1-piperidinyl)pentane	C15H30N2	1.69	24.517
22	Cyclononanone	C9H16	2.69	24.517
23	Acetonitrile, isothiocyanato	C10H10NS	2.69	24.517
24	Hexacosane	C26 H 54	2.19	25.551
25	Tetracosane	C24H50	9.61	25.551
26	Eicosanoic acid, ethyl ester	C22H44O2	1.16	26.316
27	Butyl 9,12-octadecadienoate	C22H40O2	5.76	26.955
28	-Octadecadienal, (Z)-9,17	C18H32O	5.76	26.955
29	Carbonic acid, octadecyl vinyl ester	C21H40O3	1.24	27.156
30	2-Bromo dodecane	C12H25Br	1.24	27.156
31	Docosanoic acid, ethyl ester	C24H48O2	0.67	27.872
32	Nonadecanoic acid, ethyl ester	$C_{21}H_{42}O_2$	0.67	27.872
33	Hexadecanoic acid, ethyl ester	C18H36O2	0.67	27.872
34	Pentadecane, 2,6,10,14-tetramethyl	C19H40	0.93	28.352
35	Tricosene, (Z)-	C23H46	4.28	32.305
36	9- Hexacosanol	C26H54O	4.28	32.305
37	Campesterol	C28H48O	2.82	34.063
38	Stigmasterol	C29H48O	6.73	34.619
39	gamma.-Sitosterol	C29H50O	13.37	35.682
40	Beta.sitosterol	C29H50O	14.8	35.682
41	Lupeol	C30H50O	5.66	37.017
42	Betulin	C30H50O2	5.66	37.017
43	Testosterone cypionate	C27H40O3	0.93	38.128

One researcher was notice that *senna alexandrina* extracts have a wide spectrum of antimicrobial activity[31]. For antibiotics all isolates were sensitive to Ciprofloxacin except *proteus vulgaris* which was resistance to all antibiotics. In addition, some isolates were resistant to Cefepime and Erythromycin, other isolate showed different susceptibilities to the following antibiotics Gentamicin and Amikacin. Results of our research highlight the fact that the hexane extracts revealed potent antimicrobial activity, suggest that is promising antimicrobial agents.

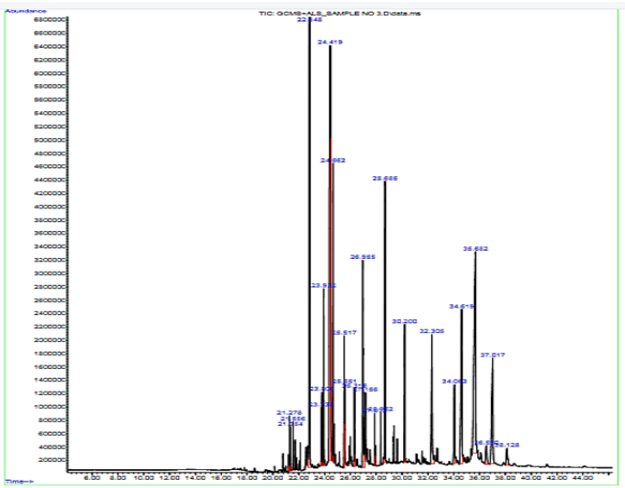
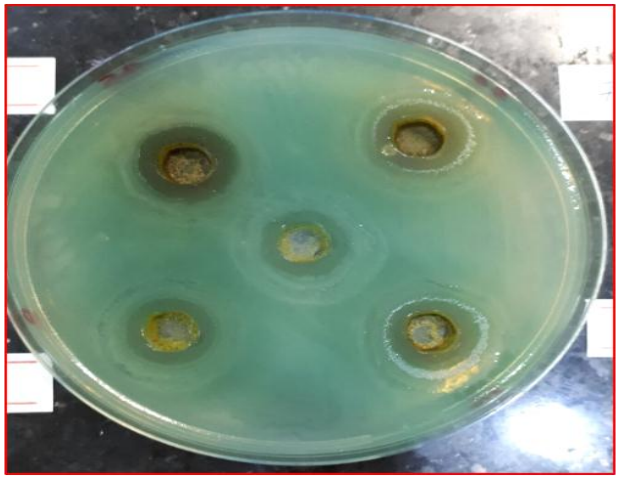


Figure 1: **Chromatogram of chemical compounds of *Cassia senna* fruits hexane extract.**

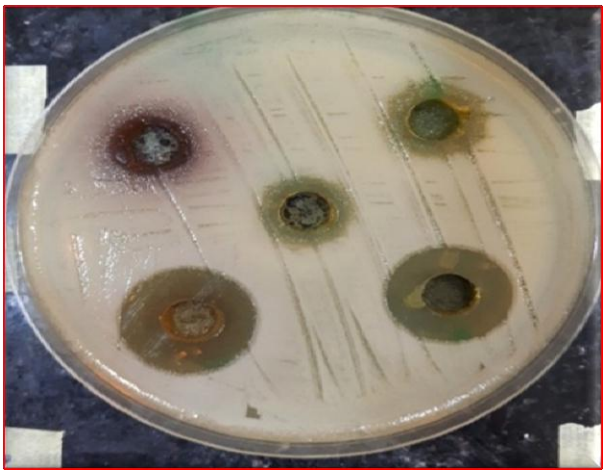
Table 3: **Results of antibacterial activity on pathogen UTI bacteria of *cassia senna* fruits hexane extract compared with antibiotics.**

Isolates	Hexane 100 mg/m	Antibiotic				
		CIP	FEP	Er	GE	Ak
Staphylococcus aureus	25	22	7	25	12	10
Proteus vulgaris	18	0	0	0	0	0
E.coli	27	20	0	0	17	25
Pseudomonas aeruginosa	25	20	0	10	15	18
Proteus mirabilis	25	25	30	0	25	25
Staphylococcus pseudintermedius	50	30	25	20	18	20
Staphylococcus hominies	28	18	0	20	14	15
Acientobacter baumanii	0	13	0	0	0	12
Acientobacter baylyi	25	20	0	0	10	15
Streptococcus pyogenes	0	20	0	0	20	25
Streptococcus pneumoniae	0	0	0	0	0	0

Note: CIP Ciprofloxacin , GE Gentamicin, Er Erythromycin, AK Amikacin, FEP Cefepime.



Staphylococcus aureus



Pseudomonas aeruginosa

Figure 2: **Antibacterial activity of fruits of *Cassia senna* fruits hexane extract on pathogen isolates**

Table 4: **The results of MIC and MBC Values of *Cassia senna* fruits hexane extract on pathogen isolates.**

Type of bacteria	Concentration mg/ml									MIC	MBC
	1	2	3	4	5	10	25	50	75		
Staphylococcus aureus	0	0	10	12	12	15	16	18	20	2	3
Proteus vulgaris	0	7	10	10	10	12	14	15	15	1	2
E.coli	0	0	0	10	12	12	13	14	15	3	4
Pseudomonas aeruginosa	0	0	10	12	13	14	15	18	22	2	3
Proteus mirabilis	0	8	10	12	12	13	15	17	20	1	2
Staphylococcus pseudintermedius	10	12	12	14	16	18	20	28	37	1>	1
Staphylococcus hominies	8	10	12	13	15	15	16	18	22	1>	1
Acientobacter baylyi	0	0	0	0	0	0	15	16	18	10	25

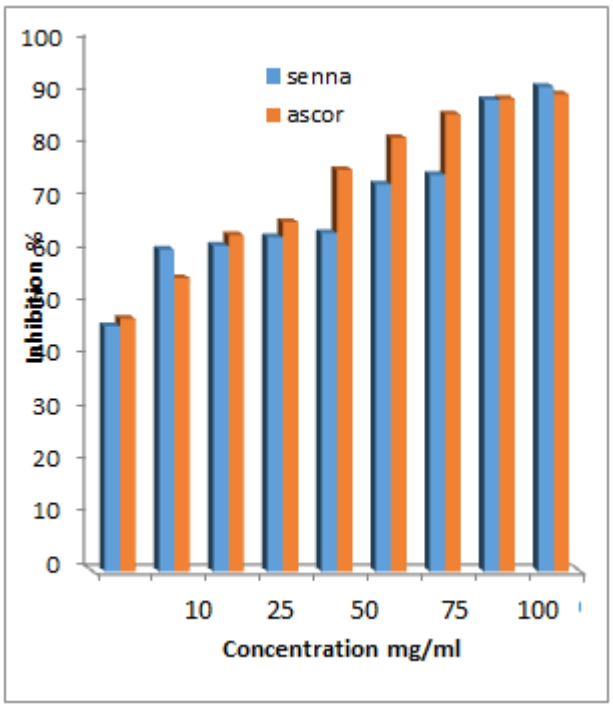


Figure 3: **Graph represents the percentage of Antioxidant (DPPH) scavenging activity of *Cassia senna* fruits hexane extract and vitamin C.**

Table 5: **Human Red Blood Cells (RBC) cytotoxicity assay in vitro of *Cassia Senna* fruits hexane extract.**

Concentration mg/ml	Incubation period	
	3 hr	24 hr
5	-	-
10	-	-
25	-	-
100	-	-
250	-	-
500	-	-

The result of antioxidant activity assay compatible with previous studies [32, 33, 34]. Anti-oxidant potentials may be due to contains Stigmasterol, β-sitosterol ,fatty acid and triterpenes with tetracyclic skeleton, The presence of electron-rich oxygen atoms in hydroxyl and carboxyl groups of compounds enhanced their ability to donate electron to the free radical, thus increased the oxidative properties [35].

REFERENCES

1. Iroha I.R., Adikwu M.U., Esimone C.O., Aibinu I. and Amadi E.S. "Extended spectrum Beta-Lactamas (ESBL) in *E. coli* isolated from a tertiary hospital in Enugu State, Nigeria". Pak. J. Med. Sci. 2009; 25(2): 279-282.
2. Kunin C. "Urinary tract infections". 5 ed., Baltimore: Williams and Wilkins. 1997; 301-304.
3. Thabit A.K., Crandon J.L. and Nicolau D.P. "Antimicrobial resistance: impact on clinical and economic outcomes and the need for new antimicrobials". Expert Opin. Pharmacother. 2015; 16(2): 159–177. DOI:10.1517/14656566.2015.993381.
4. Founou R.C., Founou L.L. and Essack S.Y. "Clinical and economic impact of antibiotic resistance in developing countries: a systematic review and meta-analysis". PLoS ONE. 2017; 12(12): e0189621. DOI:10.1371/journal.pone.0189621.
5. Al-Attar Z. "The prevalence and antimicrobial sensitivity of Esbl Escherichia Coli. in clinical isolates". Al-Kindy College Medical Journal. 2014; 10(2): 96-99.
6. Narayanan A.S., Raja S.S., Ponmurugan K., Kandekar S.C., Natarajaseenivasan K., Maripandi A., Mandeel Q.A. "Antibacterial activity of selected medicinal plants against multiple antibiotic resistant uropathogens: a study from Kolli Hills, Tamil Nadu, India." Beneficial Microbes. 2011; 2(3): 235-43. DOI:10.3920/BM2010.0033.
7. Shakya A.K. "Medicinal plants: future source of new drugs". International Journal of Herbal Medicine. 2016; 4(4): 59-64.
8. Hardman J.G.., Limbird L.E. and Gilman AG. "The Pharmacological Basis of Therapeutics". 1996; 9th ed. McGraw-Hill, USA, 925.
9. Graves G. "Medicinal Plants (An illustrated guide to more than 180 plants that cure disease and relieve pain)". 1990; 10th ed. Bracken Book Publisher. London ,6.
10. Abo K.A., Adeyemi A.A. and Jegede I.A. "Spectrophotometric estimation of Anthraquinone content and antimicrobial potential of extracts of some Cassia species used in herbal medicine in Ibadan". Sci. Forum. 2000; 3(2): 57–63.
11. Hammer K.A., Carson C.F. and Riley T.V. "Antimicrobial activity of essential oils and other plant extracts". Journal of Applied Microbiology. 1999; 86(6): 985-990. DOI:10.1046/j.1365-2672.1999.00780.x.
12. AL-Mayah A.A. "Medicinal Plants and Herbal Therapy". College of Science, University of Basra. Dar albasaer pblishing. Lebanon. 2013; 36sp.(In Arabic).
13. Sahalie N.A., Abrha L.H. and Tolesa L.D. "Chemical composition and antimicrobial activityof leave extract of Ocimum lamiifolium (Damakese) as a treatment for urinary tract infection". Cogent Chemistry. 2018; 4: 1440894.
14. Bobby M.N., Wesely E. and Johnson M. "High performance thin layer chromatography profile studies on the alkaloids of Albizia lebbeck". Asian pacific journal of tropical biomedeicin. 2012; 2(1):S1-S6. https://doi.org/10.1016/S2221-1691(12)60119-1.
15. Padhi S. and Tayuung K. "In vitro antimicrobial potentials of endolichenic fungi isolated from thalli of Parmaelia lichen against some human pathogens". Beni-Suef Journal of Basic and Applied Science. 2015; 4(4): 299-306.
16. Bauer A.W., Kirby W.M.M., Sherris J.C. and Turck M. "Antibiotic susceptibility testing by a standardized single disk method". American Journal of Clinical Pathology. 1966; 45(4): 493-496.
17. Xian-guo H. and Urasella M. "Antifungal compound from Solanum nigrum". J. Ethnopharm. 1994; 43: 173-177.
18. Mohanty S.K., Mallappa K.S. and Godavarthi A. "Evaluation of antioxidant, in vitro cytotoxicity of micropropagated and naturally grown plants of Leptadenia reticulata (Retz.)Wight & Arn.-an endangered medicinal plant". Asian Pacific Journal of Tropical Medicine. 2014; 7(1): S267–S271. DOI:10.1016/S1995-7645(14)60244-3.
19. Kamagaté M., Koffi C., Kouamé N.M., Akoubet A., Yao N.A.R. and Die-Kakou H.M. "Ethnobotany, phytochemistry, pharmacology and toxicology profiles of Cassia siamea Lam". The Journal of Phytopharmacology. 2014; 3(1): 57-76.
20. Huang C.B. and Ebersole J.L. "A novel bioactivity of omega- 3 polyunsaturated acids (n-3 PUFA) and their ester derivatives". Molecular Oral Microbiology. 2010; 25:75–80.
21. Koné W.M., Kamanzi A.K., Terreaux C., Hostettmann K., Traoré D. and Dosso M. "Traditional medicinal in north Cote-d'Ivoir: screening of 50 medicinal plants for antibactirial activity". Journal of Ethno pharmacology". 2004; 93: 43-49.
22. Seidel V. and Taylor P.W. "In vitro activity of extracts and constituents of Pelagonium against rapidly growing

mycobacteria". Int. J. Antimicrob. Agen. 2004; 23(6): 613-619. DOI:10.1016/j.ijantimicag.2003.11.008.
23. McGaw L.J., Jäger A.K. and Van Staden J. "Isolation of antibacterial fatty acids from Schotia brachypetala". Fitoter. 2002; 73(5): 431-433. DOI:10.1016/s0367-326x(02)00120-x.
24. Venkatesalu V., Sundaramoorthy P., Anantharaj M., Gopalakrishnan M. and Chandrasekaran M . "Studies on the fatty acid composition of marine algae of Rameswaram coast". Seaweed Res. Util. 2004; 26: 83-86.
25. Haque S., Dorota A.N., Alakurtti S., Ghemtio L. and Tammela J.Y.K. "Screening and Characterisation of Antimicrobial Properties of Semisynthetic Betulin Derivatives". PLoS ONE. 2014; 9 (7): e102696. DOI:10.1371/journal.pone.0102696.
26. Doğan A., Farmakoloji Cilt. And Kars D.N. "Turkey: Kafkas Niversitesi Veteriner Fakültesi". KAFKAS UNIVERSITY INSTITUTE OF NATURAL AND APPLIED SCIENCE JOURNAL. 2014; (in Turkish).
27. Polat Z.A., Savage P.B. and Genberg C. "In vitro amoebicidal activity of a ceragenin, cationic steroid antibiotic-13, against Acanthamoeba castellanii and its cytotoxic potential". J Ocul Pharmacol Th. 2011; 27(1): 1-5. DOI:10.1089/jop.2010.0041.
28. Dogan A., Otlu S., Çelebi Ö., Saglam P.A.K.A.G., Dogan A.N.C. and Mutlu N. "An investigation of antibacterial effects of steroids". Turk. J. Vet. Anim. Sci. .2017; 41: 302-305.
29. Hossain K., Hassan M., Parvin M.N., Md. Mahmudul Hasan, Islam S. and Haque A. "Antimicrobial, cytotoxic and thrombolytic activity of Cassia senna leaves (family: Fabaceae)". Journal of Applied Pharmaceutical Science. 2012; 2 (6): 186-190.
30. VijayaSekhar V.E., Prasad M.S., Joshi D.S.D.S., Narendra K., Satya A.K. and Rao K.R.S.S. "Assessment of Phytochemical Evaluation and In-vitro Antimicrobial Activity of Cassia angustifolia". International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(2): 305-312.
31. Elansary H.O., Szopa A., Kubica P.B., Ekiert H., Ali H.M., Elshikh M.S., Abdel-Salam E.M., El-Esawi M. and Diaa O. "Bioactivities of Traditional Medicinal Plants in Alexandria". Hindawi Evidence-Based Complementary and Alternative Medicine. 2018; 2018: 13. https://doi.org/10.1155/2018/1463579.
32. Irshad M., Zafaryab M., Singh M. and Rizvi M.M. "Comparative Analysis of the Antioxidant Activity of Cassia fistula Extracts". International Journal of Medicinal Chemistry. 2012; 2012: 6. DOI:10.1155/2012/157125.
33. Sahu J., Koley K.M. and Sahu B.D. "Attribution of antibacterial and antioxidant activity of Cassia tora extract toward its growth promoting effect in broiler birds". Veterinary World. 2017; 10(2): 2231-0916. DOI:10.14202/vetworld.2017.221-226.
34. Anushia C., Sampathkumar P. and Ramkumar L. "Antibacterial and Antioxidant Activities in Cassia auriculata". Global Journal of Pharmacology. 2009; 3(3): 127-130.
35. Duroux P.J.L., Trouillas P., Křen P.V., Otyepka P.M., Poso P.A. and Dangles P.O. "New theoretical highlighting on the molecular interactions of natural polyphenols: penetration in lipid membranes and oxidative dimerization (Doctoral Dissertation)". Available from Université de Limoges (Section Santé) Service Commun de la Documentation. 2011.