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INFLUENCE OF ANTIBIOTICS AND STICK SWEET CHERRY (*PRUNUS AVIUM*) ON PATHOGENIC BACTERIA AND EVALUATION OF TISSUES BIOAVAILABILITY, BIOACTIVE PHYTOCHEMICAL COMPOUND AND FUNCTIONAL PROPERTIES

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Abstract

Infectious diseases pose one of the challenges greatest health in the medical world. Though many antimicrobial drugs are available commercially, they often lack recently effectiveness against developed multidrug resistant microorganisms. Recent study carried for the first time, the scope of the experiments to investigate the effect of three types of antibiotic sensitivity Erythromycin, Amoxicillin, Trimethoprim, for inhibition of microorganism which include 8 species of pathogenic bacteria and to estimate the effect of antimicrobial activity. Strains of bacteria are*: Klebsiella pneumoniae, Salmonella typhi, , Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Citrobacter freundii, Lactobacillus, Proteus mirabilis.* There is no report on bioactive phytochemical compound and functional properties of stick cherry extract, and on the effect of the extract on animal tissues. Also no data on inhibition of pathogenic bacteria growth by the extract. Extract used for inhibition of some types of pathogenic bacteria, process was done by using four different solvents; chloroform, methanol, ethanol, and hot water at concentration of 75 mg\ml to investigate growth inhibition of pathogenic bacteria which involves: *Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Lactobacillus*, *Candida albicans*. Antibacterial activity for stick cherry extract was illustrated by using diffusion agar-well assay. Extract of stick cherry were used for determination of the minimum inhibitory concentration of some types pathogenic bacteria. Analysis of the bioactive phytochemical compound and functional properties were illustrated by using, Fourier transform infra-red spectra (FTIR spectra) and gas chromatography (GC-Mass techniecs).Result of antibiotic sensitivity test illustrate that erythromycin and Amoxicillin has no influence on pathogenic bacteria only trimethoprim affected on the different strains of microorganism except *Staphylococcus aureus* and *Pseudomonas aeruginosa*. There was difference in the inhibition zone for pathogenic bacteria growth by the extract. FTIR spectral analysis for the extract investigate the appearance of different phytochemical bioactive compound, such as aromatic carbonyl of aldehyde, hydroxyl functional groups, aliphatic amines, polyphenols, indicate characteristic of carbonyls group. Analysis by GC-Mass chromatography illustrated the presence of 28 different phytochemical compounds of polyphenol compound and 13 sugars, organic acid. Also the study revealed histological analysis of liver and kidney tissues by using laboratory animals at three different concentration. The result shows no difference in morphological shape in tissues of liver and kidney in comparison with control group of tissues animals.

***Keywords*:** Antimicrobial activity, FTIR, antibiotic resistance, *Pseudomonas aeruginosa*, GC-Mass Chromatography.

Introduction

Many different plants are claimed to possess in the traditional system the antibiotic properties and are also used by the tribal people worldwide extensively. Plants are a source of large amount of drugs comprising to different groups such as antispasmodics, emetics, antimicrobials etc. (Prashant *et al*., 2011). Herbal medicine are widely used for the prevention and treatment of various infectious diseases, and contain a number of valuable substances, their chemical composition differences in mixture, active substances biologically exhibit different type of activity than acting separately, which is a result of antagonism or synergism of their different components (Monika *et al.*, 2017) Products derived Plant contain diversity a great phytochemicals, secondary metabolites the main component of polyphenol compound have antimicrobial properties such as steroids, alkaloids, phenolic acids, flavonoids and flavonols, tannins, lignin, terpenoids, quinones, phenolic (berberine) (Borges *et al.,* 2015). Mechanism of action in vivo and in vitro ,anti-inflammatory inhibition of enzyme activity (phospholipase oxygenase) i.e. hydrosulfide groups binding and inactivation of proteins bacteria (Bidlack *et al.,* 2000).The natural potent antioxidants flavonoids, scavengers free radical and chelators metal; they exhibit various physiological activities inhibit peroxidation of lipid, including antiallergic anti-inflammatory, antimicrobial and antiarthritic (Del Rio *et al.,* 2013). antimicrobial flavonoids activity is dependent probably on unsubstituted flavones and structure, it is characterized by lower flavanones and highest antifungal activity The of methyl groups or hydroxyl introduction to these compounds reduces their antifungal properties (Małolepsza and Urbanek. 2000).

Cherry sweet (*Prunus avium L.*), fruit of genus Prunus, adrupe fleshy (stone fruit) and is also one of the most appreciated fruit in the world due to sensory properties and unique taste (McCune *et al.,* 2011). In some studies in vitro and Epidemiological, it was observed involvement phenolic reducing risk of arthritis, cancer, effect of protective on neuronal cells and another diseases. Sweet Cherry contain sensible amounts of important compound such as anthocyanins, catechins, phenolics, flavonal glycosides, melatonin, hydroxyl cinnamates cyanidins, and vitamins B, A(beta carotene) and C (dehydroascorbic acid) (Kirakosyan *et al.,* 2015) Glucose Sugars, fructose, sorbitol and sucrose are major components of sweet cherries, affecting soluble solids and sweetness of fruits. Cherry provide potassium, magnesium, iron, folate, fiber & other phytonutrients bioflavonoid, as gallic acid, chlorogenic acid kaempferol, quercetin, p-coumaric acid carotenoids, (Guillermo *et al.,* 2016). More specifically, both sweet and tart cherries are rich

generous dietary fibers, reduces developing risk of the atherosclerosis, lowers blood pressure, disease of heart coronary, improve function of immunity and other syndrome of metabolic process (Saleem *et al.,* 2010; Whelton *et al.,* 2005) Also disease cells from cell-damaging oxidative stress. This may be due to dietary polyphenols, which are formed by at least one aromatic ring with one or more hydroxyl groups attached (Kim *et al.,* 2005; Seymour and Ou, 2011). After consumption of cherry bioflavonoid anthocyanin transferred to body human, play vital role in protection form cell damaging and prevent formation of free radical, also and helps to generate essential amino acid (Jan *et al.,* 2016; Hayaloglu and Demir 2015).

The development of bacteria resistant antibiotic is associated highly with rising in usage antibiotic and is one of the recent health global problems. Though numerous antimicrobial drugs are commercially available for infectious and chronic diseases. This (Eze *et al.,* 2013). The era of resistance antibiotic is a cause of increasing bacteria concern as to continue develop countermeasures against adaptive current antibiotics at an alarming rate (Vimbela *et al.,* 2017). The expanding of antibiotics resistance bacteria has concern become a growing worldwide. Extracts of medicinal plant offer potential development of new agents influence infections which difficult for treatment. Life-threatening agent and serious are potentially bacteria, capable of diseases infectious promoting (Alviano and Alviano, 2009, Mahady, 2005). The emergence of bacteria antibiotic-resistant has been effect several action dynamic mechanism consistent with roles of bacteria against defense antimicrobial host (Mary *et al.,* 2018). Moreover, pathogens bacteria have also effectively evolved countermeasures significantly against agents antibacterial from overexposure to antibiotics stemming, such as pumps efflux that remove antibacterial agent before its target site reaching and effect exert (Tenover, 2006). The emergence of these strains resistant methods for overcoming delved into the new drugs antibiotic development diversity chemical boasting. Treatment with synthetic antibiotics in some countries is not always possible due to their adulteration and high cost (Vimbela *et al.,* 2017). Conventional antibiotics cause depression of bone marrow and the liver severe damage and other causes neurotoxic, nephrotoxic or hypertensive and importantly; infectious pathogens have developed resistance to all known antibiotics (Chong and Pagano, 1997).

Although there is a number of publication on plants extracts which have antimicrobial activity against different species fungal, accordingly there is one study which concerned evaluation of sweet cherry stick extracts antimicrobial activity was demonstrated inhibition zone of bacteria growth pathogen, using five different types of bacteria which involve; (*Escherishia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, *Proteus vulgaris* (Maysoon, 2018). Extraction has done by using two solvents chloroform and ethanol with different concentration; it was observed existences of inhibition for pathogenic bacteria. The objective of the recent study was to investigate the effect of three types of antibiotic sensitivity Erythromycin, Amoxicillin, Trimethoprim, for inhibition of eight isolated pathogen bacteria strains and to estimate the effect of antimicrobial activity. Strains of bacteria are: *Klebsiella pneumonia*e, *Salmonell*a, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Citrobacter freundii, Lactobacillus, Proteus mirabilis*). Also to estimate the influence of stick sweet cherries extract on inhibition zone of pathogenic bacteria to find antimicrobial activity. A few colonies of the organisms were used :(*Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Lactobacillus,* and *Candida albicans.* Extraction was done by using four different solvent: chloroform, methanol, ethanol and hot water at same concentration 75 mg/ml. Bioactive phytochemical compound and functional properties of stick cherry extract was analysed by Fourier transform infra-red spectrum (FTIR) evaluation and GC–Mass chromatography. Also histological analysis of liver and kidney tissues were done by using laboratory animals after oral administration of the stick cherry extract at three different concentration.

Materials and Methods

Antibiotic sensitivity test The bacterial suspension was streaked evenly with a swap on the surface of agar plates Mueller Hinton and was dried for 3 to 5 minutes, then tested for Antibiotic sensitivity. The disks (Trimethoprim, Amoxicillin, Erythromycin) are placed on cultured agar and incubate overnight at 37 ºC. (Sivakumari and Shanthi, 2009). Eight bacterial strains isolated used for antibiotic sensitivity test, they are *Klebsiella pneumonia*e, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* , *Citrobacter freundii, Lactobacillus, Proteus mirabilis*). Bacterial samples obtained from (Biotechnology Branch/University of technology) where testing was conducted used for the assays.

Stick Cherry solvents Extracts Preparation Stick sweet cheery (*Prunus avium*) were collected during summer from cherries fruit and purified, rinsed with H_2O and air dried at 37 °C for three days, then and grinding to powder. The extraction was done by using four different solvents, weighting of 22 gm of crushed stick sweet cherries for each solvent: chloroform, methanol, ethanol and hot water at the same concentrations .The extraction by using chloroform, 300 ml of 70% methanol, 300 ml of 70% ethanol, and hot water in soxhlet extractor at boiling degree for 8 hours and then left it to be cooled with continuous slow mixing solution in the rotary evaporation solution was filtered until thick solution getting. At room temperature the solution was dried for 2-3 days until it becomes a dried crushed a then take it and stored in the refrigerator at 4°C (Maysoon, 2014). The dried stick extracts were re-suspended in (Distilled water for methanol, ethanol extract and for chloroform extract in ethanol) to obtain the final concentration 75 mg/ml for testing antimicrobial activity.

Estimation of stick cherry extract for antimicrobial activity:

The antibacterial activity of the plant extract was determined by using an agar-well diffusion assay (Felipe *et al.,* 2009). Against five strains pathogenic microorganism growth these are *Proteus mirabilis Escherichia coli, Pseudomonas aeruginosa, Lactobacillus*, *Candida albicans* (yeast). It was obtained from biotechnology division (university of technology). Each bacterial strain onto Muller-Hinton agar was transferred and sabouraud dextrose agar for yeast by using a sterile cotton swab. The suspension of bacteria was adjusted against 0.4-0.5 McFarland in comparison with standard scale. The target strain suspensions of (0.1 ml) were pour on to each cell (8mm diameter using gel puncture method)on all plates of nutrient agar (Felipe *et*

al., 2009). Control group by applying distilled water used for ethanol, methanol and boiling water extract**.** Incubation of plates for 24 hrs, over night at 37 ºC, then appearing of the inhibition zone around measured well in millimeter of triplicate experimental result.

FTIR Analysis:

Fourier transform infra-red spectrum (FTIR), spectrometer Perkin-Elmer FTIR Spectrum one of FTIR in the range 4000–400 cm^{-1} at resolution used was 4 cm^{-1}. The KCl mixed with powder extract from Sigma procured. Disc sample was prepared by pressing with the machine preparing disc and placed in FTIR spectroscopy.

Gas Chromatography-Mass Spectrometry analysis

The extract was analyzed by using (Model; QP2010 Plus, shimadzu, Tokyo, Japan) equipped with a VF-5ms capillary column fused silica (30m length, 0.25mm, 0.25mm film thickness. The temperature of column oven was programmed from 80°C -310°C for 2°C min^{-1}. In electron mode impact (EI70 eV) ionization of sample component was performed. The injector temperature was fixed at 270°C and of dectors one 230 °C. Helium (00.9995%)was the fixed carrier gas with flow rate of 1.21ml min^{-1}. The mass range from 40-650 m/z was scans. 2.0 microliter of stick cherry ethanolic extract with Hamilton syringe was injected to GC-MS manual for total analysis ion chromatography injection technique split. Total running time was 56mins of GC-MS. Each extract constituents was expressed as relative percentage with normalization of peak area percentage. The ethanolic extract of bioactive compound were identified by comparing patterns of mass spectra and their retention indices with reference to wiley Registry of Mass spectral Data. New York (Wiley 8) and fatty acid methyl ester Library version 1.0 (FAME library) sources.

Histological analysis

Administration Dose

The doses prepared with three different concentrations of (125 mg/ml, 250 mg/ml and 500 mg/ml) the animals were orally administrated of 0.4ml\day of the stick cherry extract daily for 8 days at each concentration.

Treatment of animals

Eight adult male mice (25-30 gm, age 2 months) were purchased from Biotechnology division, Technology University, treated and control mice and were provided with same feed and water. Randomly the animals divided into 4 groups, each composed of 2 mice. The first group was treated with 125 mg/ml; the second group was treated with 250 mg/ml and the third group was treated with 500 mg/ml orally administered daily for 8 days and the fourth group was not treated as a control group.

Preparation of liver and kidney tissues

The perfuse-fixed liver and kidney placed in fluid Bouin overnight and routinely processed for embedding paraffin. The liver and kidney were cut into sections 5-µm. Three serial sections were mounted on slides per liver and kidney, de-paraffinized, re-hydrated and with hematoxyline - eosin stain were stained. Sections of the liver and kidney were examined by light microscopy (Arti *et al.,* 2003).

Result and Discussion

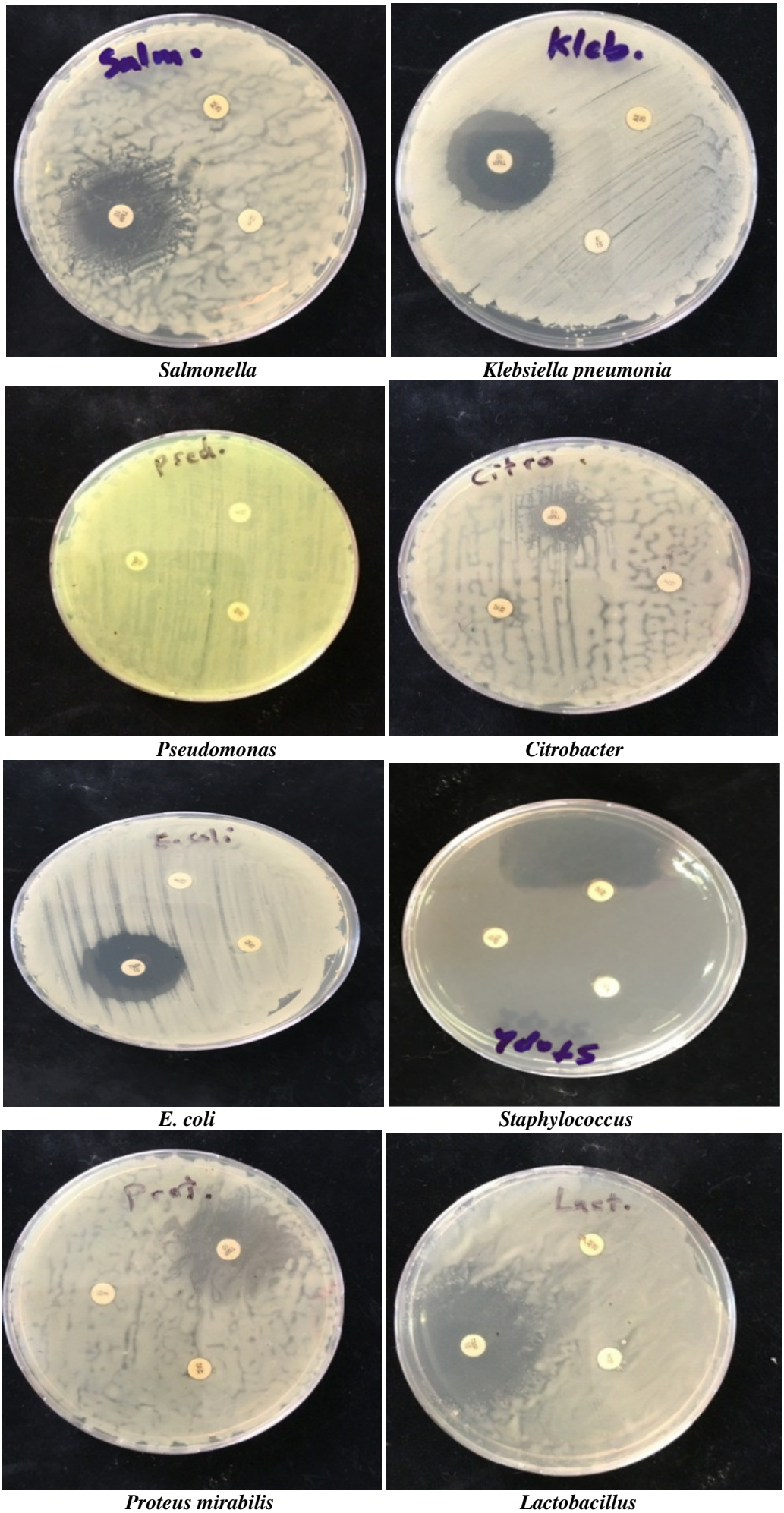
Herbal medicines have confirmed antibacterial activity, they are not yet commercially available, until their more of action and dosage is confirmed (Monika *et al.*, 2017). Compared with used commercial antibiotics most of the currently, natural antibacterial compounds appears to have lesser side effects and toxicity benefits as well as a higher stability (Michael *et al.,* 2000). Result represent antibiotic level of resistance or sensitivity to pathogenic bacteria, antibiotic sensitivity test was performed for different microorganism strains to see the pattern of antibiotic sensitivity. It showed that there is no effect for erythromycin nor Amoxicillin only trimethoprim affected on the different strains of microorganism except *Staphylococcus aureus* and *Pseudomonas aeruginosa* as in table 1 and figure 1. This may be probably that the strains was used from patient in the hospital and may be these bacteria have a kind of mutation make it resistant to the antibiotics. Some of the organisms have resistant capacity against few antimicrobial drugs it means that multi-drug resistant microorganisms with lower susceptibility to antibiotics are raised constantly for the treatment of infectious diseases. Extracts from plants may serve antimicrobial and antioxidant activity to influence the body system (Kalaimagal and Umamaheswari, 2014).

Treatment with synthetic antibiotics is not always possible due their high cost and adulteration. Antimicrobial resistance threatens effective prevention and treatment of an ever increasing range of infections caused by bacteria, parasites, virus, and fungi (Onwa *et al.,* 2016).

Table 1 : Comparison of inhibition zone of different pathogenic bacteria by using 3 different antibiotic

No.	Types of microorganism	Erythromycin	Amoxicillin	Trimethoprim
1	*Klebsiella pneumoniae*	0	0	10
2	*Salmonella typhi*	0	0	10
3	*Escherichia coli*	0	0	10
4	*Staphylococcus aureus*	0	0	0
5	*Pseudomonas aeruginosa*	0	0	0
6	*Citrobacter freundii*	0	0	5
7	*Lactobacillus*	0	0	10
8	*Proteus mirabilis*	0	0	10

Inhibition zone in mm



Salmonella ***Klebsiella pneumonia***

Pseudomonas ***Citrobacter***

E. coli ***Staphylococcus***

Proteus mirabilis ***Lactobacillus***

Fig. 1 : Effect of different antibiotics on pathogenic bacteria

Effect of different Cherry stick extracts

Medicinal plant are widely used for treatment of different disease and important source of potentially useful structures for new chemotherapeutic development. Minimum inhibitory concentration and antibacterial activity of plant extracts were determined by using agar well diffusion method and well plate method respectively. Susceptibility test of antimicrobial could be used to identify extracts effectiveness against pathogens bacterial infections (Kalaimagal and Umamaheswari, 2014, Mary, 2018). The result clearly illustrate the effect of by stick cherry extract (Prunus) by using four different solvents, chloroform , ethanol methanol and boiling water on the inhibition zone of pathogenic bacterial isolates . After studying the comparison of effect of different solvent of cherry stick extract (chloroform, ethanolic, methanolic and hot water) at concentration of 75mg/ml ,result showed the stick extract with chloroform influence all the types of pathogenic bacteria, but methanol and boiling water only effect on *Proteus vulgaris* at same concentration with diameter at inhibition zone, while ethanolic extract affected all types of pathogenic bacteria except *Pseudomonas aeruginosa* and also there is no effect of methanol and hot water extract on *Candida albicans* as shown in table 2 and figure 2. However, the inactivity of the extracts to some organisms may be due to their cell wall which is a multilayered structure and complex, it was suggested that DNA inhibiting cell reproduction (Michael *et al.,* 2000). The phenolics compound interfere formation of free radicals also with the propagation both by inhibiting the initiation reaction of involved enzymes and chelating metals transition. It was observed that phytochemical antioxidant bioactive compound flavonoids, polyphenols, play a vital role in free radicals removing and in cytotoxic effects (Russo *et al.,* 2002). They interfere enzyme cellular metabolism and disturb the membrane cellular or act as a H+ carrier, depleting adenosine triphosphate pool. The effects of cytotoxic phenolic compounds may depend on their lipophilicity, which is very important for cells penetration. On the other hand, biological membranes content, lipids and proteins facilitate the polyphenols solubility and also differences in structure of cell membrane and chemicals metabolic activation can affect polyphenols activity It was found that resistance of antifungal may depend on genus, species, isolation source strain, as well as on the components activity in the cherry stick extracts (Marino *et al.,* 2001; Szliszka *et al.,* 2009 and Maysoon, 2019).

Table 2: The Inhibition zone of pathogenic bacteria. by using different solvents of stick cherry extracts (Prunus).

		Diameter of inhibition zone (mm)			
No	**Types of microorganism**	**Chloroform (75mg)**	**Methanol (75mg)**	**Ethanol (75mg)**	**Hot Water (75mg)**
1	*Proteus vulgaris*	17	15	10	15
2	*Escherichia coli*	15	0	20	0
3	*Pseudomonas aeruginosa*	13	0	0	0
4	*Lactobacillus*	15	0	12	0
5	*Candida albicans*	7	0	20	0

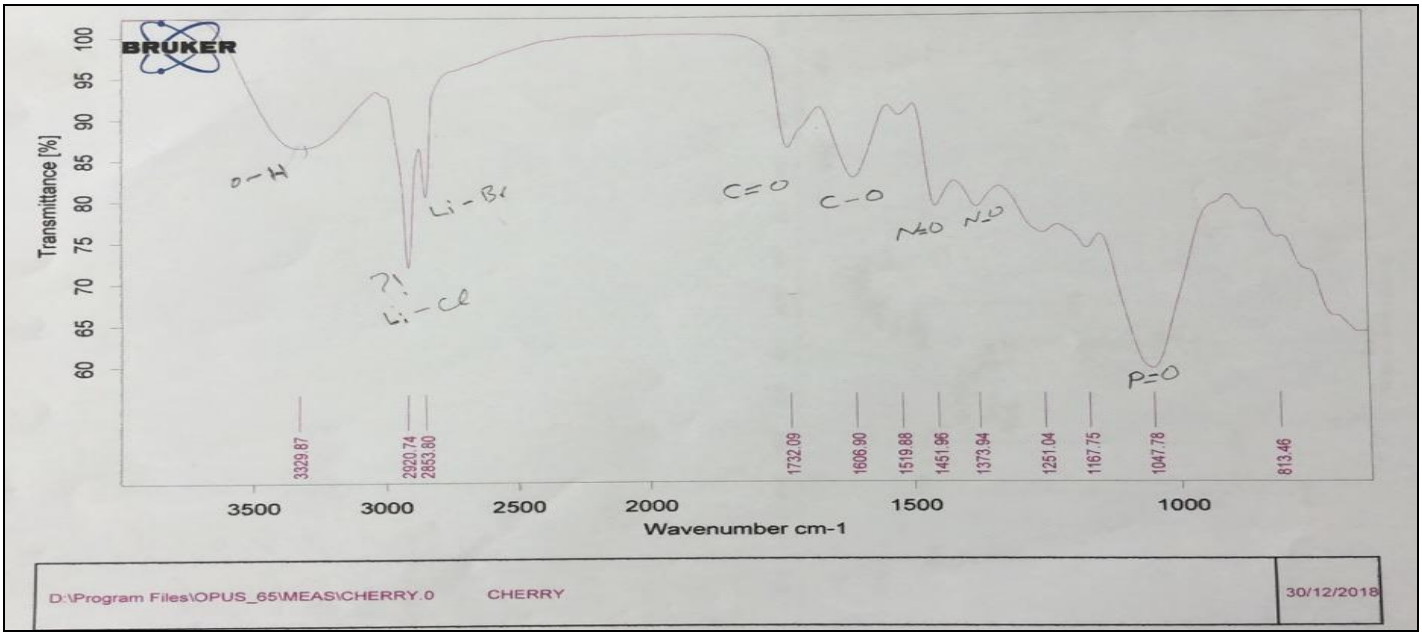


Fig. 2 : The spectra of FTIR of stick cherry extract

Fourier transform infra-red FTIR spectra (FTIR) measurements were carried out to identify the possible biomolecules responsible for the biological activity, the analysis was estimated by using washed powder of stlck sweet cherry it shows different spectum and exhibit different peaks at various functional groups present at different position as in figure 2. FTIR spectrum image of stick s cherry shows bands at 3329.87 cm^{-1} indicate hydroxyl group and polyphenol groups. At 2920.74 cm^{-1}, 2853.80 cm^{-1} indicate C-H stretching and presence of chloride, lithium and boron. Band at 1732.09 cm^{-1} (-C=O) stretch of aromatic compound carbonyls aldyhyde and ketone and functional group in acid and 1606.90 cm^{-1} represent -C-O- band (20). Also among them, the absorption peak at 1519.88 cm^{-1} may be assigned as absorption peaks of (-N=O) or (–C-O-), with protein carboxylate group. The peak at 1451.96 cm^{-1}, 1373,94 cm^{-1}

indicate stretching vibrations to aromatic C=C (-C-N) (-N=O)of aromatic amino group the absorption indicating notably enhanced residual amount of NO3 in the solution (Satyavani *et al.,* 2011; Kandakumar *et al.,* 2014). The absorbance peak at 1251.04. cm^{-1} is assigned to the amide III group (-N=O). The peak of 1167.75 cm^{-1} refer to –C=N streating, 1047.78 cm^{-1} represents the aromatic ring (–P=O) vibrations, indicate the involvement of free catechin. The peak between 700-1000 represent aromatic group and aliphatic compound (Bar *et al*., 2009, Shikuo *et al*., 2007). The FTIR analysis showed that O-H, N-H, C-H, -C-C- -C=C-, N-H, C=C, C-O, O-H and C-H are the functional groups present in the stick sweet cherry

GC-Mass Analysis

Results of GC-Mass chromatogram of the ethanolic extract of stick sweet cherry showed peaks indicating the presence of 28 and 13 compounds belongs to phenols and sugars compounds respectively, as shown in figure 2, 3. With comparison, the mass spectrum of the unknown component was compared with the database of Wiley 8 and National Institute Standard and Technology (NIST11) of the mass spectra of the constituents (Marzoqi *et al.,* 2015; Teodora *et al.,* 2014). The retention time, molecular formula, molecular weight and percentage of total ion current of various bioactive compounds are presented in Table 3, 4. The major phyto-constituent present in the stick sweet cherry were show maximum percentage are: Tetradecane, Heptadecanoic acid, Hexadecane, n-Hexadecanoic acid, Dodecanoic acid, Palmitic acid, 7-Hexadeceonic acid, 11-bromoundecanoic acid, Linalool, 9, 12, Octadecadienoic acid methyl ester, Pinostrobia Flavanoids are phenolics structure containing one carbonyl group complexes with extra cellular and soluble protein and with bacterial wall, cell thus exhibits antibacterial activity through these complexes (Thammarat *et al.,* 2018). The total ion chromatograms of stick cherry extract was analyzed using GC-Mass instrument and the constituents were quantified via the peak area normalization method. Table 3 shows the retention indices, molecular weight, total ion current and constituent identity. Total of 28 phenolic compounds and the13 sugars were identified. The data presented in Table 3 were based on the information obtained from the GC-MS analyses results in Fig 3,4 respectively. However the full scan spectra show different compound, eg. such as 2-Proponoic acid,2-methylpropyl ester. Total ion current percentage The chemical composition of the stick cherryis affected by several factors, such as species ,geographical location, plant part (Marzoqi *et al.,* 2015). The main component and the chemical composition of different compound affected by several factors, such as specie geographical location, harvest time, plant part and isolated method. The compounds: 2-Cyclohexen-1- one, 4-hydroxy-3- methyl-6-(1- methylethyl)- , 2,6-Dimethoxy phenol5. Also the presence of Linalool in high total ion current percentage (7.29 %), and other terpenoids, 1-Tetradecanol, acrylate (6.08%) has antibacterial activity (Marzoqi *et al.,* 2015, Thammarat *et al.,* 2018).

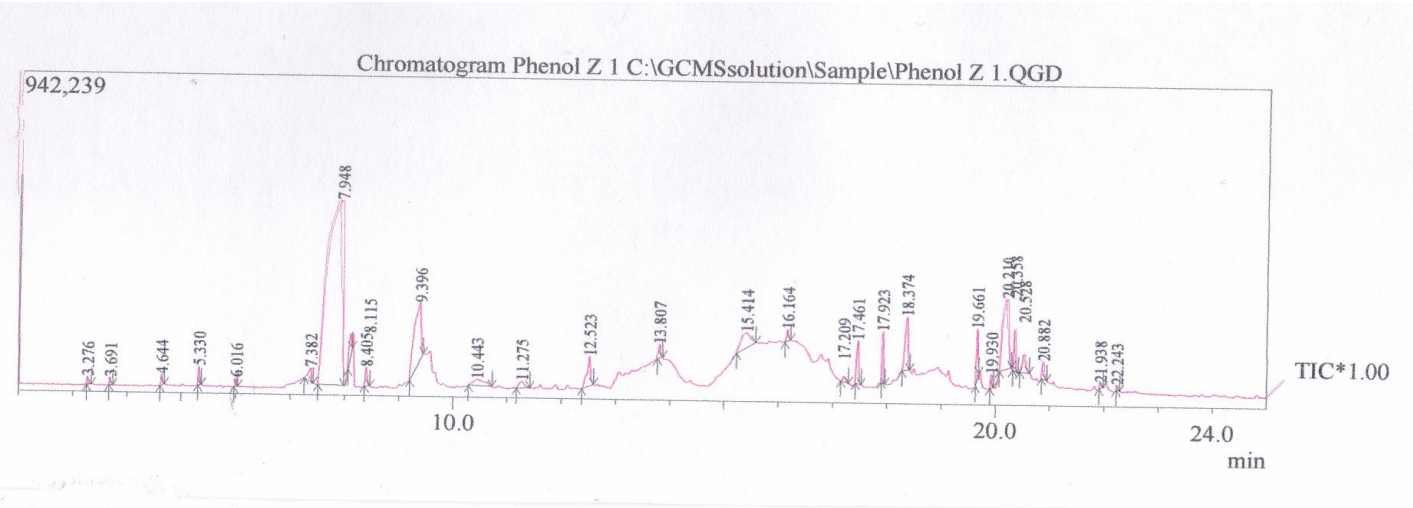


Fig. 3 : Total ion chromatogram of stick sweet cherry For Phenol

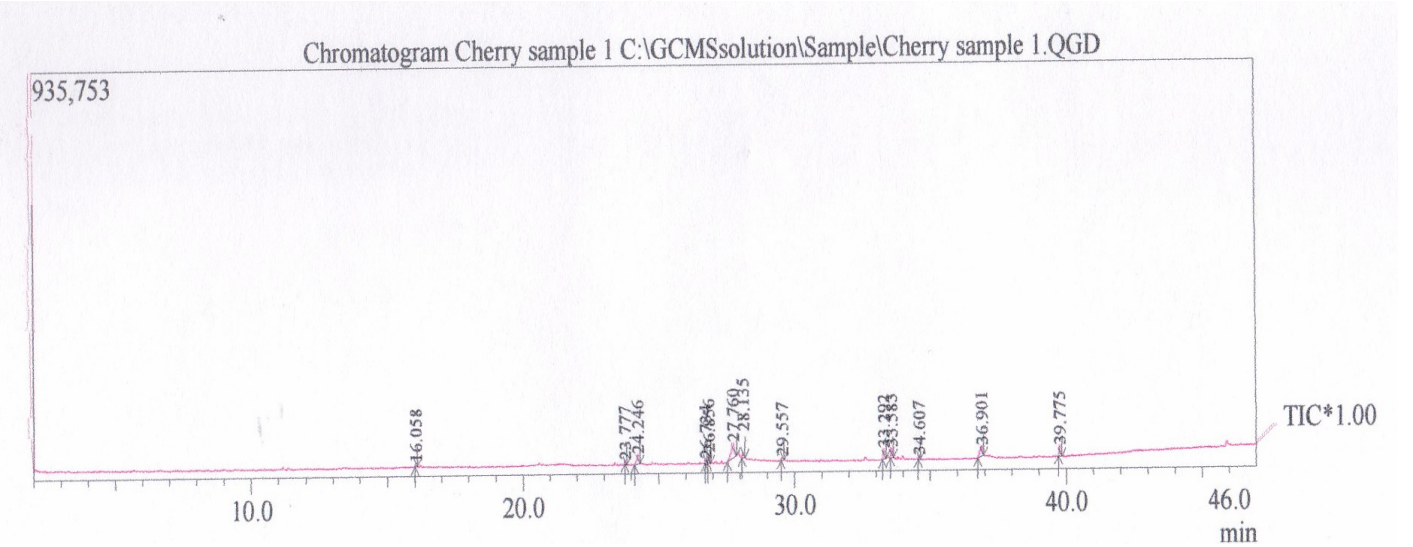


Fig. 4 : Total ion chromatogram of stick sweet cherry For sugars.

Table 3 : Chemical composition (Phenols) of stick sweet cherry extract identified by GC-MS

Compound	RT min	Molecular formula	Molecular weight	Total ion current (%) of stick
1,3,5-Triazine-2,4,6-Triamine	3.691	$C_3H_6N_6$	126.2	1.2
Octadecanoic acid	5.330	$C_{18}H_{36}O$	284	1.78
2-Cyclopenten-1-one, 2-hydroxy-	6.016	$C_5H_6O_2$	98	1.25
2-Proponoic acid,2-methylpropyl ester	8.115	$C_7H_{12}O_2$	128	2.40
Linalool	9.396	$C_{10}H_{18}O$	154.253	7.29
Tetradecane	10.443	$C_{14}H_{30}$	198	11.73
Hexadecane	11.275	$C_{16}H_{34}$	226.44	5.39
2,6-Dimethoxy phenol5	12.523	$C_8H_{10}O_3$	154	3.40
2-Cyclohexen-1- one, 4-hydroxy-3- methyl-6-(1- methylethyl)-	13.807	$C_{10}H_{16}O_2$	168	2.08
Methyl 17- methyl- octadecanoate	15.414	$C_{20}H_{40}O_2$	312	1.41
PhytolPhenol, 2,6-bis(1,1- dimethylethyl)-4- methyl (BHT)	16.164	$C_{15}H_{24}O$	220	1.08
1,4,7,10,10-Pentamethyl-2,4,6,8,9-pentaazatricyclo [5.2.1.02,6] dec-8-ene-3,5-dione	17.209	$C_{10}H_{15}N_5O_2$	237	0.53
n-Hexadecanoic acid	17.461	$C_{16}H_{32}O_2$	256	2.19
Heptamethyl6-Chloro-3,4,4a,5,6,8a-hexahydro-2Hchromene	17.923	$C_9H_{13}ClO$	172	1.40
Dodecanoic acid	18.374	$C_{12}H_{24}O_2$	200	2.86
3-Phenyl-1-(toluene-4-sulfonyl)-pyrrolidine2,5-dicarboxylic acid 2- benzyl ester 5-tertbutyl ester	19.661	$C_{30}H_{33}NO_7S$	551	1.40
Phytol	19.930	$C_{20}H_{40}O$	296	1.40
1-Tetradecanol, acrylate	20.210	$C_{17}H_{32}O_2$	268	6.08
9,12-Octadecadienoic acid (Z,Z)-	20.358	$C_{18}H_{32}O_2$	280	1.74
1,3,4,5 Tetrahydroxycyclohexanecarboxy lic acid (Quinic acid)	20.528	$C_7H_{12}O$	192	3.50
2-Allyl-5a-hydroxy-octahydro-5-oxa-2-azacyclopenta[c]inden-1-one	20.882	$C_{13}H_{19}NO_3$	237	1.99
Tetradecanoic acid	21.938	$C_{14}H_{28}O_2$	228	1.78
1-Heptadecanol (1-Eicosanol)	22.243	$C_{17}H_{36}O$	256	1.52

Table 4 : Chemical composition (sugars) of stick sweet cherry extract identified by GC-MS

Compound	RT min	Molecular formula	Molecular weight	Total ion current (%) of stick
1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	16.058	$C_{15}H_{24}$	204	5.38
Isopentyl caffcate	23.777	$C_{14}H_{15}O_4$	250.12	5.08
9,12,Octadecadienoic acid methyl ester	24.246	$C_{19}H_{34}O_2$	294.4721	9.38
n-Hexadecanoic acid	26.734	$C_{16}H_{32}O_2$	256	4.44
Pinostrobia	26.856	$C_{16}H_{14}O_4$	270.0881	6.15
Heptadecanoic acid	27.760	$C_{17}H_{34}O_2$	270	15.75
9,12,15- Octadecatrienoic acid, methyl ester (Linolenic acid, methyl ester)	28.135	$C_{19}H_{32}O_2$	292	6.88
Octadecanoic acid (Stearic acid)	29.557	$C_{18}H_{36}O$	284	8.65
Palmitic acid	33.292	$C_{16}H_{32}O_2$	256.430	6.37
7-Hexadeceonic acid	33.583	$C_{16}H_{32}O_2$	256.430	6.69
11-bromoundecanoic acid	36.901	$C_{11}H_{21}BrO_2$	265.191	12.67

Histological analysis

In this study the result of oral administration of cherry stick extract (125 mg/ml, 250 mg/ ml and 500 mg/ml) on the liver and kidney tissue, there is no effect of this extract on the kidney and liver tissue at any concentration and this disagree with Remmelt Van der Werf (Remmelt *et al.,* 2018) "Many cell nuclei were shifted from a location in the center of the hepatocyte to the periphery, apparently due to interference from normal cell structures because of the presence of numerous large fat globules".

Patient with chronic kidney disease who experienced hemodynamically mediated acute kidney injury and hyperkalemia after daily consumption of cherry concentrate. The method of injury was most likely cyclooxygenase inhibition by the compounds in cherries that mimic the mechanism of action of nonsteroidal anti-inflammatory medications. Ceasing cherry concentrate consumption led to improvements in both the patient's hyperkalemia and kidney injury. Physicians should be aware of the potentially harmful side effects of cherry concentrate and approach the use of cherry extract or concentrate with caution in patients with underlying kidney disease (Randy, 2014).

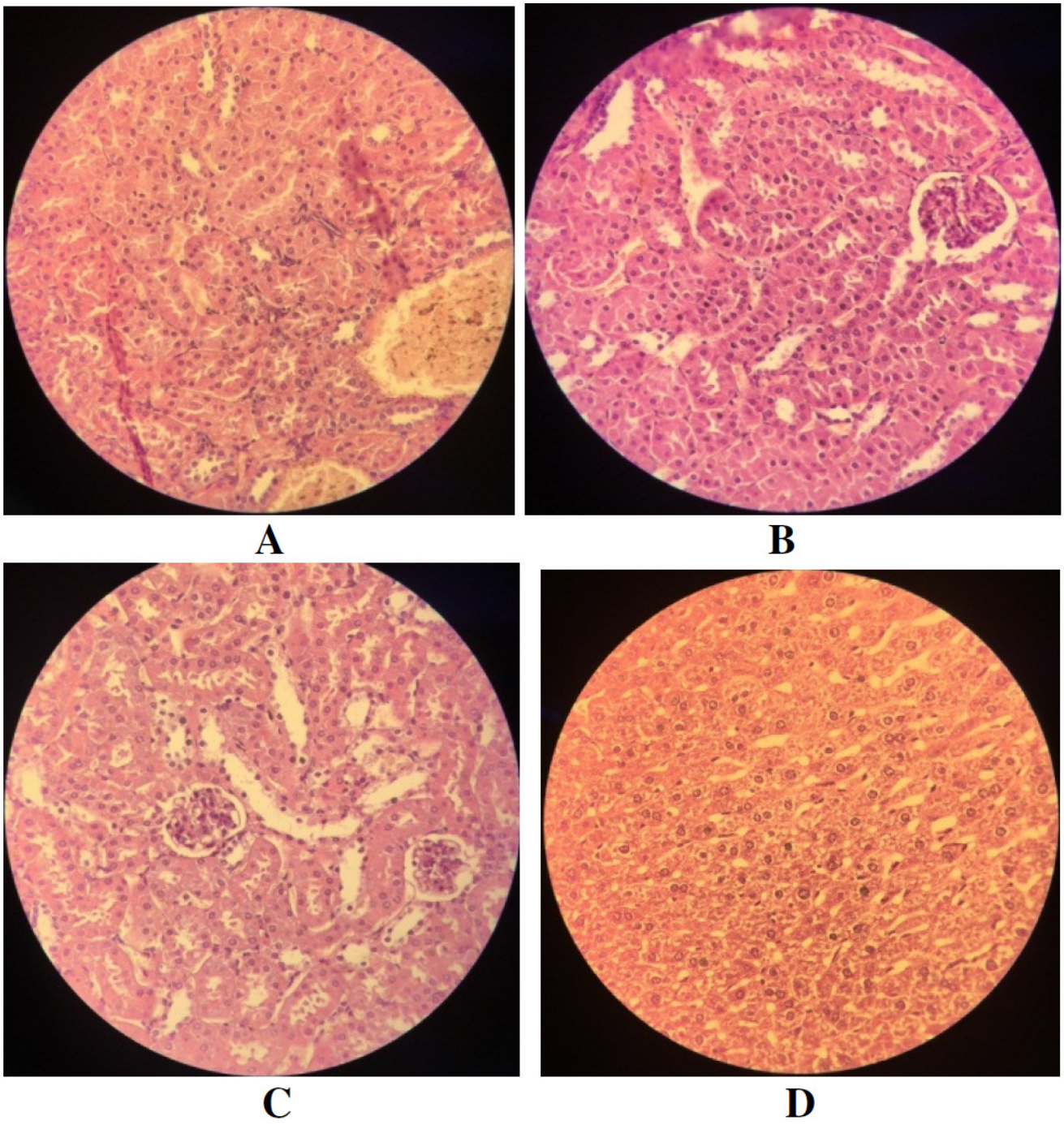


Fig. 5 : Histological features of Kidney tissues of the stick cherry extract treated with animals Microscopic examination the Kidney showed no minor histological changes versus the control animals With the light microscope, few hapatocytes had condensed or fragmented nuclei at three concentration (Fig. A. control, B. treated with 125, C. treated with 250, D. treated with 500).

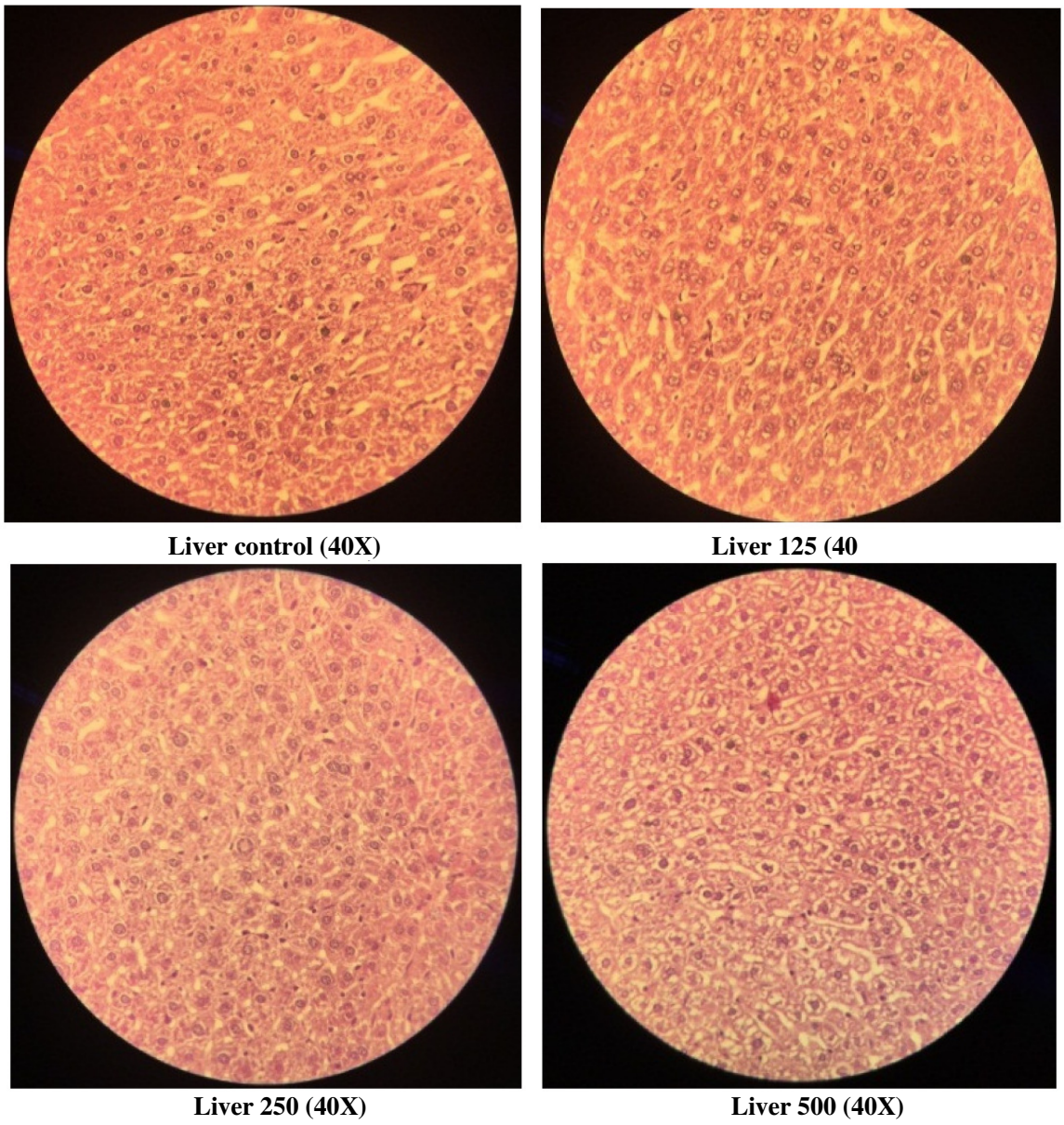


Fig. 6 : Histological features of Liver tissues of the stick cherry extract treated with animals Microscopic examination the Liver showed no minor histological changes versus the control animals With the light microscope, few hapatocytes had condensed or fragmented nuclei at three concentration (Fig. A control, B. treated with 125, C. treated with 250, D. treated with 500)

Conclusion

The antibiotic sensitivity by using different pathogenic bacteria were showed different activity, only trimethoprim influence on the different strains of microorganism except *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It appears that extracts with highest antimicrobial activity against bacteria with chloroform solvent This may mean that the activity is not related to the differences in cell wall structure. Because there is such a wide range of MICs for different strains of the same bacterial species with different solvent. It was observed different bioactive compound with various functional properties by investigation of FTIR technique and GC-Mass chromatography such as different phenols compound and sugars so the antimicrobial activity was attributed to these compound. The potency of many of the extracts on the test bacteria was apparently may be due the presence of a general metabolic toxin or due possibly to another mechanism of action. It may be interesting to investigate the mode of action of the extracts against bacteria and resistant clinical strains.

References

Alviano, D.S. and Alviano, C.S. (2009). Plant extracts: search for new alternatives to treat microbial diseases. Curr. Pharm. Biotechnol. **10**: 106-121.

Arti, S.; Pramod, K.V. and Dixit, V.P. (2003). Effect of Semecarpus anacardium fruits on reproductive function of male albino rats, Asian J Androl; 5: 121-124.

Bar, H.; Bhui, D.K.; Sahoo, G.P.; Sarkar, P.S.; De, P. and Misra (2009). Green synthesis of silver nanoparticles using latex of *"Jatropha curcas*", Colloids and Surfaces A, 339 (1–3): 134–139.

Bidlack, W.R.; Omaye, S.T.; Meskin, M.S. and Topham, D.K.W. (2000). Phytochemicals as Bioactive Agents. CRC press, Boca Raton, FL.

Borges, A.; Abreu, A.C.; Ferreira, C.; Saavedra, M.J.; Simões, L.C. and Simões, M. (2015). Antibacterial activity and mode of action of selected glucosinolate hydrolysis products against bacterial pathogens J. Food Sci. Technol. 52(8): 4737-4748. Cross Ref View Record in Scopus Google Scholar.

Chong, K.T. and Pagano, P.J. (1997). In vitro combination of PNV-140690, a Human Immunodeficiency Virus type 1 protease inhibitor with Ritonavir against Ritonavir-sensitive and Resistant Clinical Isolates. Antimicrob. Agents Chemo. 41(ll): 2367-2377.

Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J.P.E.; Tognolini, M.; Borges, G. and Crozier, A. (2013). Dietary (Poly) phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases, Antioxid. Redox Signaling, 18(14): 1818–1892.

Małolepsza, U. and Urbanek, H. (2000). Plant flavonoids as biochemical active compounds (in Polish). Wiad. Botan. 44: 27–37.

Eze, E.A.; Oruche, N.E.; Onuora, V.C. and Eze, C.N. (2013). Antibacterial screening of crude ethanolic leaf extracts of four medicinal plant, Journal of Asian Scientific Research, 3(5):431-439.

Felipe, R.; Terezinha, D. and Reoli, P. (2009). Comparison of three experimental designs employed in gentamicin microbiological assay through agar diffusion., Braz. J. Pharm. Sci., 45(3) São Paulo July/Sept.

Guillermo, C.; Francisco, L.; Maria, P.G.; Carine, S. and Víctor, L. (2016). Bioactive and Functional properties of sour cherry Juice (*Prunus cerasus*). Food Funct. 7: 4675-4682.

Hayaloglu, A.A. and Demir, N. (2015). Physiochemical characteristics, antioxidant activity, organic acid and sugar contents of 12 sweet cherry (*Prunus avium* L.) cultivars grown in Turkey. J. Food Sci., 80(3): 564-570.

Jan, S.; Małgorzata, L.; Aleksander, G. and Przemysław, B. (2016). Cultivar effect on the sweet cherry antioxidant and some chemical attributes. Folia Hort. 28/19: 5-102.

Kalaimagal, C. and Umamaheswari, G. (2014). Antibacterial Activity from Aerial Parts of Double Flower Variety of *Tabernaemontana divaricata*. 3(10): 1833-1837.

Kandakumar, S.; Sathya, V. and Manju, V. (2014). International Journal of Chem Tech Research, 6(11): 4770-4776.

Kim, D.O.; Heo, H.J. and Kim, Y.J. (2005). Sweet and sour cherry phenolics and their protective effects on neuronal cells. J Agric Food Chem. 53(26): 9921-7.

Kirakosyan, A.; Seymour, E.M. and Wolforth, J. (2015). Tissue bioavailability of anthocyanins from whole tart cherry in healthy rats. Food Chem. 171: 26-31.

Mahady, G.B. (2005). Medicinal plants for the prevention and treatment of bacterial infections. Curr. Pharm. Des., 11: 2405-2427.

Marino, M.; Bersani, C. and Comi, G. (2001). Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. Int J Food Microbiol. 67(3): 187-95.

Mary, S.; Hridhya, K.V. and Kulandhaivel, M. (2018). Evaluation of Antimicrobial Activity and Minimum Inhibitory Concentration of Ethanolic Extract of Three Medicinal Plants against Bacteria causing Skin Infection. Journal of Pure and Applied Microbiology, 12(1): 375-379.

Mary, S.; Hridhya, K.V. and Kulandhaivel, M. (2018). Evaluation of Antimicrobial Activity and Minimum Inhibitory Concentration of Ethanolic Extract of Three Medicinal Plants against Bacteria causing Skin Infection. Journal of Pure and Applied Microbiology, 12(1): 375-379.

Marzoqi, A.; Hameed, I. and Salah, I. (2015). Analysis of bioactive chemical components of two medicinal plant (*Coriandrum sativum* and *Melia azedarach*) leaves using gas chromatography-Mass Spectrometry (GC-MS). African Journal of Biotechnology. 14(40):2812-2830.

Maysoon, M. and Najeeb, M.S. (2019). Synthesis of silver nanoparticles using stick sweet cherry extract Research Journal of BiotechnologyPrunus SP and evaluation of their biological activities and effect on biochemical marker. 14(I): 241-248.

Maysoon, M.; Najeeb, M.S.; Ghassan, M.S.; Arieg, A.; Wahab, A.M. and Abbas, A.M. (2014). Biological Effects of Stick Cherry, Soybean Seed and Licorice Root Extracts on Concentration of Serum Hormone Levels in Male Mice. Journal of Al-Nahrain University Science, 17(4): 18-26.

Maysoon, M. and Najeeb, M.S. (2018). Effect of stick sweet cherry extracts (Prunus (SP) on some biochemical markers in albino mice and biological activities in different types of bacteria. Iraqi Journal of Medical Science. 16(2): 114-124.

McCune, L.M.; Kubota, C.; Stendell-Hollis, N.R. and Thomson, C.A. (2011). Cherries and health: a review. Crit Rev Food Sci. Nutr., 51: 1–12.

Michael, T.; Madigan, J.M.M. and Parker, J. (2000). Brock Biology of Microorganisms 9th edition. Prentice Hall Inc. New Jersey USA. 69.

Monika, C.; Pawel, S.; Magdalena, S.; Dorota Semik, S. and Tomasz, T. (2017). Characterization of Antimicrobial Properties of Extracts of Selected Medicinal Plants, Polish Journal of Microbiology, 66(4): 463–472.

Onwa, C.N.; Bbira, M. and Magaji, S. (2016). Antibacterial activities of *Plectranthus cyaneus* leaf extracts, against five bacterial wound pathogens in Uganda.

Prashant, T.; Bimlesh, K.; Mandeep, K.; Gurpreet, K. and Harleen, K. (2011). Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia, 1(1): 98-106.

Randy, L.L. (2014). Acute Kidney Injury From Cherry Concentrate in a Patient With CKD. AJKD, 63(3): 503–505.

Remmelt, V.; Catherine, W.; William, B.; Elodie, S.; Carole, M.; Claude, P.; Julie, L.; Dalal, W.; Said, E.; Fabien, D.; Elisa, M.P.; Michel, P.; Nathalie, J.; Eric, M.; Séverine, S. and Stéphanie, D. (2018). Beneficial effects of cherry consumption as a dietary intervention for metabolic, hepatic and vascular complications in type 2 diabetic rats. Cardiovasc Diabetol 1.

Russo, A.; Longo, R. and Vanella, A. (2002). Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. Fitoterapia 1: 21-29.

Saleem, M.M.N.M.; Mohammad, A.A.W. and Al-Amiery, A.A.H. (2010). In vivo study of cherry stick effect on concentration of serum total cholesterol, triglyceride and total protein in white albino male mice. J Fac Med Baghdad. 52(3): 340-3.

Satyavani, K.; Ramanathan, T. and Gurudeeban, S. (2011). Digest Journal of Nanomaterials and Biostructures, 6(3): 1019-1024.

Seymour, E.M. and Ou, B. (2011). Phytochemical and diverse antioxidant profile of whole tart cherries (*Prunus cerasus*). FASEB J, **25**: 773.14

Shikuo, L.; Yuhua, S.; Anjian, X.; Xuerong, Y. (2007). Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. Green Chem., 9: 852-858.

Sivakumari, V. and Shanthi, G. (2009). Antibiotic Susceptibility of Common Bacterial Pathogens Isolated From Diabetic Pus, Advanced Biotech., 10-13. Special Bacteria Pathogens Journal. Vol 2, (No1& 2): 01-09.

Szliszka, E.; Czuba, Z.P.; Domino, M.; Mazur, B.; Zydowicz, G. and Krol, W. (2009). Ethanolic extract of propolis (EEP) enhances the apoptosis- inducing potential of TRAIL in cancer cells. Molecules, 14: 738-754**.**

Tenover, F.C. (2006). Mechanisms of antimicrobial resistance in bacteria. Am J Med. 119(6): S10.

Teodora, E.C.; Carmen, S.; Zaharie, M. and Elena, M. (2014). Minor Volatile Compounds in Traditional Homemade Fruit Brandiesfrom Transylvania-Romania, as Determined by GC-MS Analysis. Not Bot Horti Agrobo, 42(2): 530-537.

Thammarat, P.; Kulsing, C.; Wongravee, K. and Leepipatpiboon, N. (2018). Identification of Volatile Compounds and Selection of Discriminate Markers for Elephant Dung Coffee Using Static Headspace Gas Chromatography-Mass Spectrometry and Chemometrics. Molecules. 23(8): pii: E1910.

Vimbela, G.V.; Ngo, S.M.; Fraze, C.; Yang, Lo. and Stout, D.A. (2017). Antibacterial properties and toxicity from metallic nanomaterials International Journal of Nanomedicine, 12: 3941-3965.

Whelton, S.P.; Hyre, A.D.; Pedersen, B. (2005). Effect of dietary fiber intake on blood pressure: a meta-analysis of randomized, controlled clinical trials. J Hypertens., 23(3): 475-81.