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RESEARCH ARTICLE

Antimicrobial Effect of Honey and Some Herbal Plant Extracts Against Multidrug Resistance Bacteria Isolated from Patient in Local Riyadh Hospital.

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Abstract

The infectious multidrug resistance bacteria is become a major health problem and limited the option for effective treatment in human population worldwide. New antimicrobial agents are urgently required to overcome this issue. The present study aimed to evaluate antibacterial activities of honey and some herbal plant extracts (*Ginger, Clove and Black cumin)* against *Staphylococcus aureus, Staphylococcus epidemdis*, *Escherichia coli & Klebsiella pneumonia* by using disc diffusion method and broth microdilution assays. Herbal plants and honey samples were purchased from local market at Al-Quwyiyahia, Saudi Arabia. Also, Egyptian honey purchased from local market at Egypt. Herbal plants extracted with water /ethanol solvent and evaluated for antimicrobial activity against bacteria isolated from clinical and water samples. The results indicated that the highest bacterial resistance to antibiotics *Escherichia coli* (57.14 %) followed by *Klebsiella pneumonia* (42.28 %) and *Staphylococcus aureus* (28.57 %). The Egyptian and Saudi laguanza honey were showed strong antibacterial effects and inhibitory effect with concentrations of 50, 20% and 10% followed by Clove and Black cumin ethanolic extracts against tested bacteria. Combination honey with antibiotics or plant extracts mixture (v/v) revealed synergistic effects on tested bacteria. Finally our study proved that the Egyptian and Saudi laguanza honey have strong antibacterial effect depended upon the quality, type and concentration. The marked effect of new formula from combination plant extracts and honey have broad spectrum antimicrobial activity at low concentration 0.0325 mg/ml. but still need further extensive studies should done to spotlight the hidden properties of these plant extracts and honey.

Introduction:-

Antimicrobial agents are the substances known to have therapeutic effect against pathogenic microorganisms either as prevention and treatment [1]. Among foremost health problems, infectious diseases account 41 % of global diseases, the main reason of infectious diseases are natural development of bacterial resistance to various antibiotics due to accumulate of different antibiotic residues inside the same strain, multidrug resistance bacteria causes financial and economic implication treatment failure and spread pathogen bacteria from person to person Although, new generation of antibiotics were produced by pharmacological companies but even drug resistance has increased [2,3]. In addition the side effect of overuse and misuse antibiotic which can harm vital organs like liver, kidney and

pancreas and spleen as well as immune system [4]. Thus situation has forced the attention of scientists towered natural and herbal products in search to develop better quality drugs with improved antibacterial activities as alternatives cure [5]. Honey has been used as a medicine since ancient time in different culture known as 'Folk medicine'. Nowadays honey and other herbal plant medicine have been used as therapeutic agents [6]. The medical professional staff have been rediscovered recently and it is gaining acceptance as an antibacterial agent for the treatment of infectious diseases [7]. Also, honey has been proved to be antimicrobial, antiviral, anti-inflammatory, antitumor and antioxidant [8]. There are no studies that support the systemic use of honey as treatment of multidrug resistant bacteria [9]. Medicinal herbal plants are rich in wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenol and quinines ,which have been used worldwide in herbal medicine to treat several diseases and infection [10]. Several studies over all the world have been showed that plant extracts have antimicrobial activities. Moreover, a large number of plant species have not been studied for potential medicinal value [11]. The present work was aimed to evaluate the antimicrobial activities of honey and some herbal plant extracts against *Staphylococcus aureus, Staphylococcus epidemdis, Escherichia coli & Klebsiella pneumonia*

Material and Methods:-

Source of honey:-

Saudi and Egyptian honey were purchased from local market in Saudi Arabia and Egyptian honey purchased from Ministry of agriculture market, Cairo, Egypt. The honey samples were filtered with manual Seitz filter attached with syringe. The filtered from different samples were aseptically inculcating on nutrient agar then incubate at 37 ^{o}C for 24-48hrs for sterility examination. The sterile samples extracts stored in sterile bottles and kept at refrigerator at 4 ^{o}C until use.

Plant materials and Extractions:-

Herbal plant (*Ginger, Clove & Black cumin)* samples were purchased from perfumer market at Al-Quwayiyaha, Saudi Arabia. The herbal seeds & flowers were extracted according to [12]. *Black cumin* seeds and Clove flowers were washed with distal water and then dried in room temperature and blended with a grinder to obtain powered. *Ginger* powdered was purchased from the previous market. Each plant powder was extract by soaking separately under room temperature with ethanol or water 24-48hrs. For the isolation of pure extracts, the isolated crude extracts were resuspended in a minimum required volume of corresponding solvents and placed on the water bath (80^{o}C) for the evaporation of extra solvents. Plant extracts were filtered with manual Seitz filter attached with syringe. The filtered from different samples were aseptically inculcating on nutrient agar and blood agar then incubate at 37 ^{o}C for 24-48 hrs for sterility examination. The sterile samples extracts stored in sterile bottles and kept at refrigerator at 4^{o} C until use.

Samples collection and bacterial identification:-

Bacterial strain isolated from urine, pus and water samples were obtained from local hospital laboratory at Riyadh, Saudi Arabia and all samples were taken by physician with consideration of all ethics. All procedures were accordance with general ethical principles standards of responsible committee of human (intuitional and national) and with Declaration of Helsinki 1975 as revised 2008 and informed consent was obtained from all patient and physician at local hospital for being included in the study. Suspected colonies were picked up and examined microscopically in Gram stained before being transferred in subculture to be subjected for further identification of pathogens including Gram's staining technique and catalase test to distinguish between *Staphylococci* other Gram positive cocci. The hemolytic patterns and coagulase test with rabbit plasma were used to differentiated between *Staphylococcus species*. Also, Gram negative bacteria were confirmed identification by sub culturing on differential and selective media and tested to oxidase activity, acid production (glucose, lactose and sucrose fermentation), Indole test, Voges-Proskauer test (VP) and hydrogen sulfide production as Table (1). Beside confirmation with biochemical test API-20E (Analytical profile index, BioMerieux) and automated VITEK system.

Evaluation of Antimicrobial Activity of Honey and Plant Extracts:-

Disk diffusion method:-

This method was done according to [13] and slightly modifications [14], bacterial isolates were diluted at concentration of 10^{6} CFU/ml according to reference to McFarland 0.5. Suspension was pure plated with Muller Hinton agar after 1 hour, standard antibiotics disc as well as modified discs of 6 mm were prepared using Whitman filter paper 50 discs were obtained by punching and putting in bottle and sterilizing in hot air oven at 170 ^{O}C for 30 minutes. The discs were impregnating with 20 ul of each separate plant extracts , reference antibiotics and control negative solvent and distal water were aseptically placed with a sterile forceps at the center and peripherally

of seed Muller Hinton agar. The plates were inculcated at 37 ^{o}C for 24 hrs. Examination plates for zone of inhibition were measured and reported. The MBC was determined following the methods described by [15]. Well with no visible growth in MIC assays were subculture using a 10 ul inoculating loop onto a 5% sheep BAP and incubated at 37^{o}C for 24 hrs incubation. The MBC was defined as the lowest concentration of the extract that did not permit any growth.

Broth Micro-dilution Assays:-

Honey and plant extracts were determined by broth microdiluation method as described by [15]. Twofold serial dilutions of plant extracts (0.5,0.25,0.125,0.0625,0.0312–0.0156 mg/ml) in MHB were prepared in sterile polystyrene 96well plates. 100 *ul* of each extract was used for estimation through MICs assays. Well contained medium alone or medium plus plant extracts without bacterial strains and medium plus bacterial strain as control. Also, Antibiotic *(Penicillin G, Erythromycin, Ampicillin, Cephradine ,Clindanycin, Tetracycline & Enrofloxacin*) were diluted unique concentration for each antibiotic , inculcation bacteria size of 1×10^6 CFU/ml of isolated strain with reference McFarland 0.5 standard. After 24 hrs incubation, optical density was measured by spectrophotometer micro- titer plate reader (Biotek) at 450 nm and MIC was determined at total inhibition of growth compared to control positive and untreated medium plus plant extracts. All assays were carried out in triplicate.

Statistical Analysis.

Data were subject to statistical analysis included Means, Standard Division (SD) and T –test at P <0.05 as described by (Iwu *et al* ., 1999). Using SPSS for windows 7 version 15

Results

The results in Table (1) showed that the major bacteria isolated from human patient in local hospital at Riyadh , Saudi Arabia were *S. aureus, S. epidermidis , Ecoli and Klebsiella*. Subculture and identification of bacteria isolates including Gram's staining technique and Catalase test to distinguish between Staphylococci other Gram positive cocci. The hemolytic patterns and coagulase test with rabbit plasma were used to differentiated between *Staphylococcus species*. Also, Gram negative bacteria were confirmed identification by sub culturing on differential and selective media and tested to oxidase activity, acid production (glucose, lactose and sucrose fermentation), Indole test, Voges-Proskauer test (VP) and hydrogen sulfide production and confirmation by API-20E (Analytical profile index, BioMerieux) and automated VITEK system as table (1).

Table (1): Biochemical characteristics of isolated bacteria from human at local hospital

Gram stain	TSI	Man	Mot	In	MR	VP	Cit	Ur	Oxi	Cat	H2S	Coag test	Identification isolated bacteria
Gram–ve bacteria	-	Acid	+	+	+	-	-	-	-	+	-	-	*E. coli*
	+	Acid	-	-	-	+	+	+	-	+	-	-	*K. spp*
Gram+ve bacteria	-	-	-	-	-	-	-	-	-	+	-	+	*S.aureus*
	-	-	-	-	-	-	-	-	-	+	-	-	*S. epidermidis*

+ve : positive reaction -ve : negative reaction

TSI: Triple sugar iron Man: Mantol Mot: Motility In: Indol MR: methyl Red VP: Vagus Proskuour Cit: citrate utilization Ur: Urease Oxi: Oxidase Cat: catalase H2S: Hydrogen sulphide Coa test: Coagulase

Antibacterial effect of honey and Plants extracts:-

Table (2) and Fig(1) revealed that the most isolated bacterial strains were found to be resist to the most used antibiotics *E. coli* was (57.14 %) and *K. pneumonia* (42.28 %) followed by *S. aureus* (28.57%). Egyptian and Saudi Langanesa honey have antimicrobial activity against all microorganisms in different concentrations. *Staphylococcus aureus* was the most affected microbe while *E. coli* and *K. pneumonia* were significantly the least affected microbes comparing with control sample. Also, combination mixture of antibiotics with Egyptian and Langansa honey showed marked antibacterial activity compared with antibiotics alone on isolated bacteria. There was no significant variation in antimicrobial activity of Egyptian and Saudi Langansa honey at different concentration but the other two types of Saudi Tag Al- Nahile and Waadi Al- Nahile honey showed no or limit effect on isolated bacteria. The plant extracts of *Clove and Black cumin* were showed antibacterial activity against multidrug resistance bacterial strains. Ethanol extracts from Clove and Black cumin were showed significant

antibacterial activity against all the test bacterial strains Table (2). The lowest antibacterial effect showed by *Ginger* water and ethanol extract.

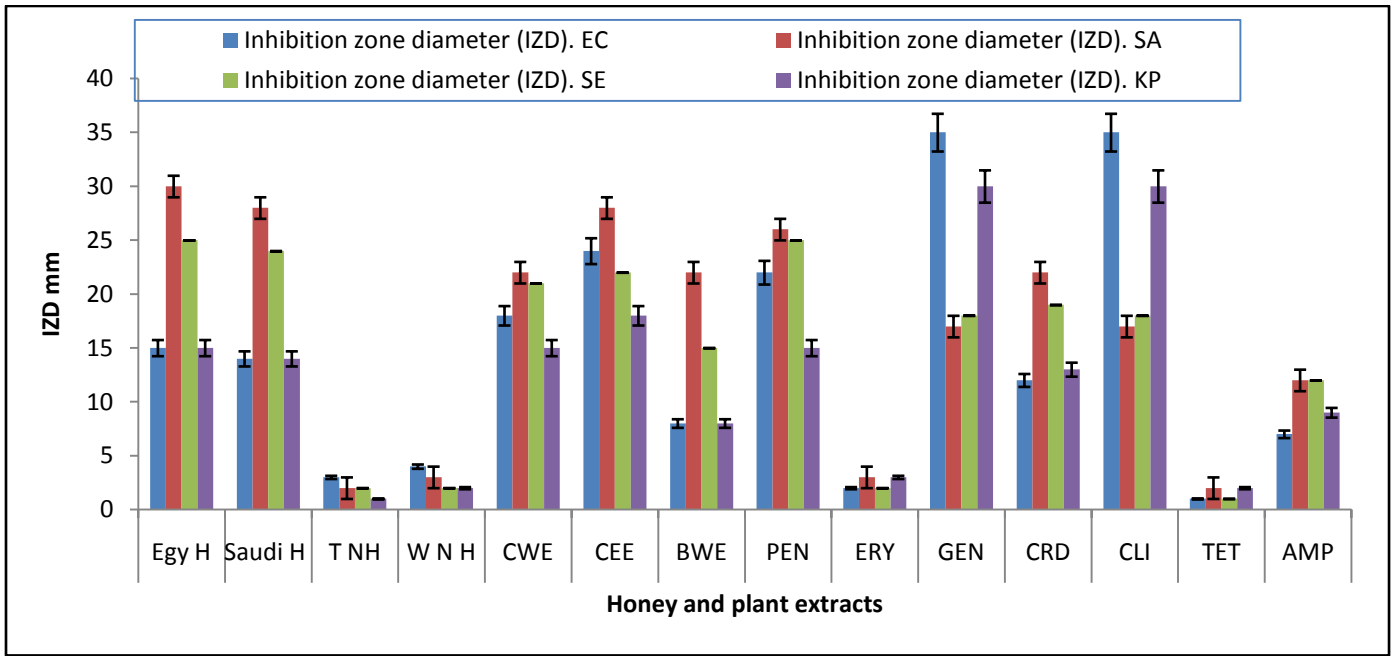
Table (2): Antibacterial effect of honey and plant extracts against isolated bacteria.

Extracts		Inhibition zone diameter (IZD).				MIC mg/ml			
		EC	*SA*	*SE*	*KP*	*EC*	*SA*	*SE*	*KP*
Honey	Egy H	15 ± 0.25	30 ±0.23	25 ±0.65	15 ±0.56	6.25	3.25	3.25	12.5
	Saudi H	14 ± 0.24	28 ±0.45	24 ±0.26	14 ±0.64	6.25	3.25	3.25	12.5
	S T NH	3 ±0.015	2 ±0.23	2 ±0.02	1 ±0.00	25	25	25	25
	SW N H	4 ±0.0	3 ±0.02	2 ±0.25	2 ±0.00	25	25	25	25
Clove	CWE	18 ±0.23	22 ±0.23	21 ±0.53	15±0.56	6.25	3.25	3.25	12.5
	CEE	24 ±0.35	28 ±0.32	22 ±0.56	18 ±0.45	3.25	3.25	3.25	6.25
Black Cumin seed	BWE	8 ±0.56	22 ±0.025	15 ±0.65	8±0.89	6.25	3.25	3.25	25
	BEE	22 ±0.25	26 ±0.25	25 ±0.56	15±0.75	6.25	3.25	3.25	6.25
Ginger	GWE	00 ±0.00®	00 ±0.00®	00 ±0.00®	00±0.00	25	25	25	25
	GEE	00 ±0.00®	00 ±0.00®	00 ±0.00®	00±0.00	25	25	25	25
Antibiotics	PEN	12 ±0.64	22 ±0.26	19 ±0.36	13 ±0.25	6.25*	325*	3.25*	12.5*
	ERY	7 ±0.02	12±0.25	12 ±0.36	9±0.65	12.5*	25	6.25	25*
	GEN	35 ±0.23	17 ±0.53	18 ±0.45	30±0.65	3.25*	12.5	12.5	3.25*
	CRD	12 ±0.56	22 ±0.42	23 ±0.12	13±0.56	25*	25	25	25*
	CLI	14 ±0.65	13 ±0.23	11 ±0.65	10 ±0.65	6.25*	12.5	6.25	12.5*
	TET	7 ±0.56	12 ±0.25	8 ±0.26	6±0.25	25*	12.5	12.5	12.5*
	AMP	14 ±0.45	21 ±0.26	18 ±0.36	13 ±0.75	12.5*	6.25	6.25	12.5*

EC*: E. coli*, SA: *S. aureus*, SE : *S.epidemdis*, KP: *K. pneumonia*, ®: Resistant, S: Susceptible
* ug/ml , Egy H : Egyptian honey, Saudi Lag H: Saudi Langanesa honey, STNH : Saudi Tag Al- Nahile, SWNH: Waadi Al-Nahile honey, CWE: Clove water extract, CEE: Clove ethanol extract , BWE Black seed water extract, BEE : Black seed ethanol extract, GWE : Ginger water extract, GEE : Ginger ethanol extract, PEN: Penicillin G, CRD: Cephradine, ERY: Erythromycin, CLI : Clindanycin, TET: Tetracycline, AMP Ampicillin, GEN: Gentamycin

The results in Table (3) showed clearly that the herbal/ honey mixtures (v/v) have significance antibacterial activity at low concentrations comparing with plant extracts alone or honey alone. Also, combination mixture of antibiotics with Egyptian and/or Langansa honey showed marked antibacterial activity compared with antibiotics alone against all isolated bacteria. The highest antibacterial activity were observed with new formula compared with honey or antibiotic were recorded. The synergistic antibacterial effect between Egyptian and Saudi Langansa honey with antibiotics and/or plant extracts on Gram positive and Gram negative bacteria with inhibition zone diameter ranged from 11 ± 0.26 to 27 ± 0.21 and MIC value 3.25 to 25 mg/ml may be attributed to rich natural honey extract as well as plant extracts rich with contents of active compound alkaloids and flavones.

Fig (1) : Determination of honey and plant extracts antibacterial effect by disc diffusion method



EC : *E. coli* , SA: *S. aureus* SE : *S.epidemdis* , KP: *K. pneumonia* , ®: Resistant, S: Susceptible, * ug/ml , Egy H : Egyptian honey, Saudi Lag H: Saudi Langanesa honey , STNH : Saudi Tag Al- Nahile SWNH: Waadi Al- Nahile, CWE: Clove water extract, CEE: Clove ethanol extract, BWE black seed water extract , BEE : Black seed ethanol extract, GWE : Ginger water extract, GEE : Ginger ethanol extract, PEN: Penicillin G, CRD: Cephradine, ERY: Erythromycin, CLI : Clindanycin, TET: Tetracycline: AMP Ampicillin, GEN: Gentamycin

Table (3):Evaluation antimicrobial activity of honey plant extracts mixture with antibiotics against isolated bacteria by disc diffusion method and MIC assays.

Extracts		Inhibition zone diameter (IZD).				MIC mg/ml			
		EC	*SA*	*SE*	*KP*	*EC*	*SA*	*SE*	*KP*
Egyp H	PEN	18 ±0.29	29 ±0.48	25 ±0.25	15 ±0.48	6.25	3.25	3.25	25
	CLI	16 ±0.25	28 ±0.65	25 ±0.45	15 ±.45	3.25	3.25	3.25	3.25
	TET	14 ±0.22	27 ±0.26	24 ±0.25	14 ±0.25	6.25	3.25	3.25	25
	EN	13 ±0.26	25 ±0.29	25 ±0.36	15 ±0.28	6.25	3.25	3.25	25
	AMP	12±0.45	28±0.65	26±0.25	12±0.21	3.25	3.25	3.25	3.25
Saudi Lag H	PEN	16 ±0.56	27 ±0.21	25 ±0.25	15 ±0.65	6.25	3.25	3.25	25
	CLI	14 ±0.65	25 ±0.25	24 ±0.22	14 ±0.26	3.25	3.25	3.25	3.25
	TET	15 ±025	26 ±0.36	25 ±0.25	15 ±0.29	6.25	3.25	3.25	25
	EN	14 ±0.42	22 ±0.35	23 ±0.23	14 ±0.30	6.25	3.25	3.25	25

	AMP	11±0.36	21±0.45	24±0.26	11±026	3.25	3.25	3.25	3.25
Herbal plant extracts comb	EgyH	22 ±0.36	33 ±0.65	21 ±0.23	12 ±0.26	6.25	3.25	3.25	25
	LagH	16 ±0.45	26 ±0.53	24 ±0.36	14 ±0.25	3.25	3.25	3.25	3.25
Herbal plant extracts comb ANB	PEN	18 ±0.25	34 ±0.58	25 ±0.23	15 ±0.25	6.25	3.25	3.25	25
	CLI	21 ±0.15	29 ±0.45	24 ±0.43	18 ±0.32	3.25	3.25	3.25	3.25
	TET	17 ±0.25	25 ±0.26	25 ±0.43	16 ±0.25	6.25	3.25	3.25	25
	EN	14 ±0.56	26 ±0.26	24 ±0.45	14 ±0.32	6.25	3.25	3.25	25
	AMP	12±0.25	28±0.28	26±0.22	12±0.25	3.25	3.25	3.25	3.25

EC *: E. coli* , SA: *S. aureus* SE : *S.epidemdis* , KP: *K. pneumonia* , ®: Resistant, S: Susceptible, * ug/ml , Egy H : Egyptian honey, Saudi Lag H: Saudi Langanesa honey , STNH : Saudi Tag Al- Nahile SWNH: Waadi Al- Nahile, CWE: Clove water extract, CEE: Clove ethanol extract, BWE black seed water extract , BEE : Black seed ethanol extract, GWE : Ginger water extract, GEE : Ginger ethanol extract, PEN: Penicillin G, CRD: Cephradine, ERY: Erythromycin, CLI : Clindanycin, TET: Tetracycline: AMP Ampicillin, GEN: Gentamycin

Discussion:-

The current issues associate with multiple drug resistance bacteria presents a serious worldwide crisis, requiring constant survey, which continuously challenges scientists population beside diminishing efficacy and increasing toxicity of synthetic drugs thus attract attention of scientists to search in natural & herbal materials to solve this problem [16]. Traditional medicine has been practiced worldwide for centuries, particularly the application of herbal plants for therapeutic purposes. Middle East countries possess a rich source of herbal plants, enough to provide us with alternative remedies [17].

The results in Table (1) demonstrated that the most isolated bacterial strains were found to be resistant to the used antibiotics *E. coli* was (57.14 %) and *K. pneumonia* (42.28 %) followed by *S. aureus* (28.57%). *Staphylococcus* are considered as naturally susceptible to almost antimicrobial agents developed but recent rapid antimicrobial resistance rapidly developed, Staphylococcus are often developed resistant to methicillin resistance to methicillin at 80% among hospital isolates. [18], the most Gram negative *E. coli and K. pneumonia* strains have multidrug resistance to most antibiotics used while Gentamycin (Gen) and Clindamycin recorded inhibitory effect against all isolated bacteria. *E. coli and K. pneumonia* showed susceptibility to antibiotics, Gentamycin inhibition zone diameter were (35 ± 0.23 and 30 ± 0.56 mm) and Clindamycin (CLI) were (14 ± 0.65 and 10 ± 0.56 mm) respectively. Penicillin, Ampicillin and tetracycline were found to be less effective against all tested bacteria isolated from human patient due to increased indiscriminate and frequent use of those antibiotic in this area leading to develop of antibiotic resistance bacteria which necessitate develop and search for novel sources as antimicrobial agents and this finding is agree with [17,18] found that the Gram-negative bacteria more resistant to antibiotics than Gram-positive bacteria such as *E. coli* and *K. pneumonia* exhibited more resistant than *S. aureus* and *S. epidemidis* due to lippolysaccharide (LPS) layer of Gram-negative bacteria in outer membrane have a highly hydrophobic and acts as a strong permeability barrier against hydrophobic molecules and agree with [19,20]. found that the hydrophobic molecules can pass through cell wall of gram-positive bacteria easier than the gram- negative bacteria because cell wall of the gram- positive bacteria contained only peptideglycan.

Table (2) and Fig (1) demonstrate that the antibacterial activity of different concentrations of Egyptian honey & Saudi Langanesa honey have inhibitory effect against all tested bacteria may be due to its complex composition and its ability to generate hydrogen peroxide by the bee-derived enzyme glucose oxidase which is bactericidal effect, this result is agreement with [21], found that the antibacterial activity of honey have been related with oligosaccharides, glycopeptides, phenol, fatty acids, lipids, amylases, ascorbic acid , peroxidases and fructose and low pH these element acting synergistically and may contribute significant to antimicrobial activity [22,23,24]. The results of the broth dilution assays exhibited the real effect of honey or plant extracts at different concentrations [25] . Ginger

ethanolic extracts had no activity against S. aureus and E. coli. on the other hand this result is disagree with [26, 27] who found that the ginger ethanolic extracts inhibit the growth of *S. aureus* and *E. coli*.
Result in table (2 & 3) showed clearly that the Clove extract and Black cumin extract have stronger antibacterial activity may be due to plant extracts compositions include volatile materials that cause membrane disruption to the bacteria and mitochondrial damage, difference between their effects may be due the quantity of the phenol compounds, eugenol.

Tables (3) showed that strong antibacterial activity of combination plant extracts with honey at (v/v) of all concentrations of 50%, 20 %, and 10% were significantly antimicrobial effect on all tested organisms more than plant extract alone or honey alone. The highest antibacterial activity were observed with new formula compared with honey or antibiotic were recorded. The synergistic antibacterial effect between Egyptian and Saudi Langansa honey with antibiotics and/or plant extracts on Gram positive and Gram negative bacteria with inhibition zone diameter ranged from 12 ± 0.00 to 29 ± 0.48 and MIC value 3.25 to 25 mg/ml may be attributed to rich natural honey extract as well as plant extracts rich with contents of active compound alkaloids and flavones and this result is agree with [28,29]. who proved that there is a synergistic effect of antimicrobial activity from the combination of Ginger and honey against isolates from carious teeth and on some clinical isolates. Also, is agree with [30]. who detected the synergistic effect of antimicrobial activity from the combination of garlic and lime against isolates from carious teeth was noted and agree with the study which observed the highly antimicrobial activity for combination of Garlic, Onion, Ginger, Pepper and Coriander seed on different microbes. On the other hand, this result is disagree with [28, 30]. who found that the combination of Garlic and Utazi (*Gongronema latifolium*) may not always yield the desired effect on both Gram positive and Gram negative bacteria and the antagonistic effect is more evident when the combination is used on Gram negative organisms, e.g. *E.coli*.

Our results in Tables (2 &3) showed that the antibacterial activity of honey and plant extracts could be measure with MIC assays in 96 micro plate by spectrophotometer at 450 nm. Beside monitoring ODs of honey and plant extracts compared with ODs of reference antibiotics against the same type of bacteria. The MIC assay is simple, rapid and accurate for bacterial susceptibility test and this finding is agree with [31,32]. showed that the antifungal drug resistance fungi could be determined earlier using a microbroth system based on monitoring of OD. They determined amphotericin B resistance using this system after incubation periods 24 h for fungal growth compared with MIC determination by the CLSI reference method at 24-48 h. Finally our results proved that the Egyptian honey, Saudi Lagunza honey and plant extracts have ability of antibacterial activity against Gram positive and Gram negative bacteria.

Conclusion:-

The present study spot highlight on isolation and identification of multidrug resistance bacteria in human patient at local hospital in Riyadh region Saudi Arabia. On the perspective, plant extract and honey are safe, efficient and a low-cost option for treating multidrug resistant bacteria. Combination plant extracts and honey (v/v) showed strong antibacterial effect on Gram positive and gram negative bacteria which may be reflect active ingredients that inhibit bacterial growth of bacteria. Our results proved that the Egyptian and Saudi honey are natural product that have a strong antibacterial effect but its depend upon the quality, type and concentration. The marked effect of mixture from Ginger, Clove, Black Cumin extracts and honey have a broad spectrum antimicrobial activity. The new formula can be used as antibacterial agents as natural alternative to antibiotics at low concentration 0.0325 mg/ml. Also, the rapidly, accuracy and simplify of reading MIC assays with spectrophotometer minimizing the risk of plant extract toxic effect but still need further extensive studies should done to explore the hidden properties of these plants extract and honey.

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