Studies on the Effect of Some Natural Products on Bacteria Implicated in Respiratory Tract Infection

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Abstract: Respiratory tract infections are the most common infectious disease. The increasing of resistance of some pathogenic bacteria that are associated with respiratory tract infection is another concern. There is growing interest in using natural antibacterial compounds, such plant extracts, honey bee and royal jelly. This study focused on determining the antibacterial activity of ethanolic extracts of some medicinal plant and Honey bee and Royal jelly on respiratory tract infection. Twenty five percentage *Staphylococcus aureus* were isolated from sputum samples collected from 71 patients having Respiratory tract infections, 34% *Streptococcus pneumoniae*, 30% *Klebsiella pneumoniae and 11% Pseudomonas aeruginosa*. The sensitivity patterns of the isolated bacteria to tested antibiotics were prepared as follow: for *Staphylococcus aureus* is highly susceptible for Levofloxacillin and Imipenem with inhibition zone (30mm), *Streptococcus pneumonia*e highly susceptible for Levofloxacin (42mm), *Klebsiella pneumonia*e highly susceptible for Meropenem (40mm), followed by Imipenem (34mm) and *Pseudomonas aeruginosa* highly susceptible for Levofloxacin (36mm).Four plant extracts (Caraway, Anise, Clove, and black cumin), honey bee and royal jelly were reported to have an inhibitory effect against tested bacterial strains. The highest activity against *S.aureus* was exhibited byhoneybee (40mm), followed by Black cumin (26), Royal jelly (26mm), caraway (22mm), while Cinnamon (20mm) show the lowest activity. For *S.pneumoniae* it was exhibited byCaraway (28mm) and Clove (20mm). For *K.pneumoniae* it was Anise (28mm), Caraway (26mm) and honey Bee (20mm). *P.aeruginosa* was resistant to all tested plant extracts and honey bee and royal jelly. Results also proved that ethanolic extract of Cinnamon, Garlic, Ginger, Cumin, Cardamom, fennel, Turnips, onion, Red chili pepper and Peppermint were found to be inactive against all bacterial strains tested.

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1. Introduction:

Respiratory tract is the part of the human system that plays a vital role in breathing processes. In human, the respiratory system can be subdivided into an Upper respiratory tract and a Lower respiratory tract based on anatomical features ***Perkin (2003).*** The respiratory tract is constantly exposed to microbes due to the extensive surface area, for instance, the lungs have an exposed internal surface area of approximately 500m^2 ***Underwood (1992).*** However, respiratory infections are a common and important cause of morbidity and mortality worldwide. ***Ndip et al. (2008)***.

The most common bacteria implicated as causative agents of respiratory tract infection was included but not limited to *Pseudomonas* spp., *Streptococcus* spp., *Proteus* spp., *Klebsiella* spp., *Staphylococcus* spp., *Enterobacter* spp., *Acinetobacter* spp., and *Haemophilus influenza* ***(Gazi et al., 2004).*** Up to 15% of acute pharyngitis cases may be caused by bacteria, commonly Group A streptococcus in *Streptococcal pharyngitis* ("Strep Throat"). Generally, patients with strep throat start with a sore throat as their first symptom and usually do not have runny nose or cough or sneezing. Pain and pressure of the ear caused by a middle ear infection (Otitis media). Here is a number of acute and chronic infection that can affect the lower respiratory tract. The two most common infections are bronchitis and pneumonia. Lower respiratory tract infection can also be applied to other types of infection including lung abscess, acute bronchitis, and emphysema. Symptoms include shortness of breath, weakness, high fever, coughing and fatigue. ***Robert et al. (2004).***

Antibiotic is a substance or compound that kills or inhibits the growth of bacteria. Antibiotics belong to the broader group of antimicrobial compounds, used to treat infections caused by microorganisms, including fungi and protozoa. (***Davey 2000).*** Antibiotics are the mainstay of bacterial treatment, the goal of these drugs is to kill the invading bacteria without harming the host ***Archer and Ronald (2001)***.

Over time, many antibiotics have lost effectiveness against common bacterial infections because of increasing drug resistance (***Perez et al., 1990; Barie, 1998; Domin, 1998).*** There have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant **(*Aibinu et al., 2003).***The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs (***Pretorius et al., 2003)*** Because of widespread micro-organism resistance to chemicals, using natural antimicrobial products has been increased due to fewer adverse effects and ease of utilization **(*Moreillion et al., 2005).***

Medicinal plants have always been considered as a source for healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural (***Kalemba and Kunicka, 2003).*** In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years**.** Researchers are increasingly turning their attention to natural products and looking for new leads to develop better drugs against cancer, viral and microbial infections ***(Ibrahim, 1997; Towers et al., 2001 a***nd ***Koshy et al., 2009).*** The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains. The indiscriminate use of antibiotics has resulted in many bacterial pathogens rapidly becoming resistant to a number of originally discovered antimicrobial drugs. There is, thus, a continuous search for new antibiotics, and medicinal plants may offer a new source of antibacterial agents. (***Kudi et al., 1999; Palombo and Semple, 2001).***

The antibacterial capacity of honey, first reported in 1980, is currently being revised. Two main theories have been proposed to explain this capacity one is that it is due to the action of the hydrogen peroxide in honey that is produced by glucose oxidase in the presence of light and heat (***Dustmann, 1979),*** and the other is that it is the non peroxide activity, which is independent of both light and heat, that inhibits microbial growth (***Roth, et al., 1986).*** This non peroxide activity, which remains unaltered even during long storage times, depends on the flower source of the nectar used and so not all honeys possess this activity (***Molan and Russell 1988).*** The major components of honey are sugars, which themselves possess antibacterial activity due to the osmotic effect they have (***Molan, et al., (1992),*** although studies carried out to test this antimicrobial activity use concentrations at which the sugars are not osmotically active. It is also well known that honey contains lysozyme, a powerful antimicrobial agent (***Bogdanov, 1997).*** Royal jelly, a glandular bee-milk like substance has biological and Pharmaceutical properties and health tonic (***Jianke and Shenglu, 2003)***.Royal jelly is also a food source that is exclusively given to queen bee larva, which are genetically identical to other female bees in the hive but are phenotypically different due to differentiation caused by the queen's royal jelly diet (***Crane, Eva 1999 Berrevoets, et al., 2009).***

The study was carried out in Mansoura university hospitals, Egypt over period of 12 month. on a number of 71 patients (40 males and 31 females) all of them are suffering from respiratory tract diseases (Acute bronchitis, chronic obstructive pulmonary disease, Pneumonia, Chronic suppurative lung disease, bronchial asthma) their ages ranged from 15 years to 75 years, Data entry was completed using Microsoft excel. SPSS Program and medical programs for statical analysis (SPSS Inc., Chicago, IL, USA) was used for data analysis.

2. Materials and methods:

Seventy one patients were subjected to the history (Sex, Age, smoking habit) and through clinical examination each patient is subjected to complete physical examination including symptoms and signs indicating the development of bacterial infection.

Sample collection:

Sputum samples were collected in sterile plastic container under complete aseptic conditions, the best sample is the early morning sputum. The patient should expectorate from deep down in the lung. If patient failed to expectorate, sputum may be induced by expectorant or by inhalation of heated saline hypertonic first morning sputum sample was collected directly into a sterile wide mouthed container and transported to laboratory according to standard protocol (***Cheesbrough, 2000).***

Preparation of sputum samples:

Add to the sputum about 5 ml of sterile physiological saline to obtain as pure a culture as possible of a respiratory pathogen it is necessary to reduce the number of commensal inoculated.

Isolation and identification of bacterial strains:

Isolation was performed using horse blood agar media (Oxoid), each Plate was inoculated by spreading aloofly of prepared sputum sample.

Incubation of culture plates were placed in an inverted position for 24to 48 hours at 37°C.

All the bacterial isolates were identified using morphological, microscopy and biochemical tests following standard procedures described by ***Cowan and Steel (1974) and Cheesbrough (2000)***, by Gram stain, Growth on MacConkey (Oxoid), Mannitol salt agar media (Oxoid), Catalase test, Coagulase test, Motility test, Urease test, Indole Production test, Methyle red test, Voges-Proskauer test,Citrate utilization test and Oxidase test.

Antibiotic susceptibility testing

Antibiotic susceptibility tests were performed using the disc diffusion method.Fourteen different antibiotics (Levofloxacin, Azithromycin, Meropenem, Ciprofloxacin, Gentamycin, Imipenem, Cefotaxime, Cloxacillin, Amoxicillin\clavulanic acid, Ceftazidime, spiramycin, Piperacillin, Ampicillin\sulbactam, Ampicillin) were tested for determination of their antibiotic effects on the isolated strains.

Antimicrobial activity of medicinal plant extract:

Fourteen type of medicinal plants including Cinnamon *(cinnamomum zeylanicum)*, Clove (*Syzygium aromaticum*), Black cumin (*Nigella sativa*), garlic (*Allium sativum),* ginger *(Zingiber officinalis),* Cumin *(Cuminum cyminum),* Cardamom *(Elettaria cardamomum),* Fennel (*Foeniculum vulgare*), Turnip (*Brassica rapa var. rapa*), Onion (*Allium cepa*), Red chill (*Capsicum frutescens*), Peppermint (*Mentha piperita*), Anise (*Pimpinella anisum*) and Caraway (*Carum carvi*) were purchased from local retail markets in Mansoura city. All plants were first cleaned by tap water in order to remove any debris or dirt, and later using sterile distilled water. They were dried in laminar flow biological safety cabinet. Concerning to Garlic was skinned manually then placed in a hot air oven for drying at temperature 65˚ C for 42 h ***(Ekwenyeand Elglam, 2005)***, thechili fruits were dried at 60°C in an air oven and peppermint leaves were dried at dark room temperature for 15 day ***(Essawi and Srour, 2000)***. The dried plants were crushed immediately before assay using an electric grinder.

Organic plant extracts were prepared by soaking 50 gm of the dried powder separately in 200ml of analytical organic solvents (ethanol 95%), using a conical flask plugged with cotton wool. The mixture was kept at 20˚C over night under continuous shaking at 130 rpm. The mixures were then filtered through whatman filter paper (No.2). The filtrates were evaporated using vacuum rotary evaporator. Stock solutions of crude ethanolic extracts were prepared by diluting the dried extracts with 10% Dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 10 mg/ml.***(Hoque et al., 2008).***

An agar well diffusion method was employed for determination of antibacterial activities. 0.1ml of bacterial suspension was spread onto the surface of nutrient agar medium. Wells of 8 mm diameter were cut from the agar with sterile cork borer. The base of each well was sealed with 50 ul of sterilized molten nutrient agar. The wells were filled by adding 300 ul of the different plant extracts, while DMSO was used as control. The plates were incubated for 24 h at 37C. After incubation, the inhibition zones around each cup were measured with a caliper and recorded.

Antimicrobial activity of Honey Bee and Royal Jelly:

Honey Bee and Royal Jelly samples were purchased from local retail markets in Mansoura city. Honey Bee Samples were stored at 23-25°C in dark place while Royal Jelly stored at 0°C. The samples honey and royal Jelly were used without dilution. An agar well diffusion method was employed for determination of its antibacterial activities. The wells were filled by adding 300µl of the honey bee and royal jelly which had been warming in a 35-40°C water bath. The plates were incubated for 24 h at 37C. After incubation, the inhibition zones around each cup were measured with a caliper and recorded.

Statistical analysis

All data were run on IBM compatible personal computer using the software Statical Package for Social Scientist (SPSS) Program and medical programs for statical analysis (SPSS Inc. Chicago, IL, USA).The trend x^2 test for statistical comparisons between the groups and a $P<0.05$ was considered as statistically significant.

3. Results:

Seventy one patients their ages ranged from 15years to 75 years, 40 male and 31females all of them are suffering from respiratory tract diseases (12 chronic bronchitis, 22 Pneumonia, 21 chronic obstructive pulmonary disease, 16 chronic suppurative lung disease).

A number of 71 bacterial isolates have been isolated from sputum samples (Tab.1), were identified according to Gram stain into two divisions which proved to be:

1- Forty two isolates were Gram positive cocci:

Members of this group were initially identified by Catalase test and divided into two groups: one includes 18isolates which gave positive catalase test (Active bubbling), positive Coagulase test, ferment Mannitol sugar on Mannitol salt agar media, and gave β hemolysis on blood agar media that show they were ***Staphylococcus aureus***, while the other include 24isolates which gave negative Catalase test, negative Coagulase test, alpha hemolysis on blood agar media, Positive Bile solubility test, positive Optochin

susceptibility test that show they were ***Streptococcus pneumoniae.***

2-Twenty nine isolates were Gram negative stain: Members of this group were initially identified by their growth on MacConkey agar media, and divided into two groups: one includes 21 isolates which their growth on MacConkey agar media with pink color (Lactose fermentation), negative Motility test, positive Urease test, negative Indole production test, negative methyl red test, positive Voges-Proskauer test, and positive Citrate utilization test that show they were ***Klebsiella pneumoniae*****,** while the other groupincludes 8isolates which their growth on MacConkey agar media with no change in color, the isolates gave positive Oxidase test, positive Catalase test, negative Urease, negative motility test, and growth on 42˚C, β hemolysis on blood agar, colonies express exo-pigment pyocyanin (blue green color) on Nutrient agar media and they also have a characteristic fruity smell that show they were ***Pseudomonas aeruginosa.***

Bacterial infection of male were *Staphylococcus aureus (26.8%), Streptococcus pneumoniae* (31.7%)*, Klebsiella pneumoniae (24.4%) and Pseudomonas aeruginosa (17.1%),* while in females were *Staphylococcus aureus (23.33%), Streptococcus pneumoniae (36%), Klebsiella pneumoniae (36*%) and *Pseudomonas aeruginosa* (3.3%).(Tab. 2).

There was a significant relationship between the occurrence of the isolates and the gender of patients (P* =0.02).

According to age the bacteria isolated in Age ≤35 were *Staphylococcus aureus* (25%), *Streptococcus pneumoniae* (17%), *Klebsiella pneumoniae* (50%) and *Pseudomonas aeruginosa* (8%), While in age ≥35 were *Staphylococcus aureus (25.4%), Streptococcus pneumonia* (37.3%), *Klebsiella pneumoniae (25.4%) and Pseudomonas aeruginosa* (11.9 %). (Tab. 3).

There was a significant relationship between the occurrence of the isolates and the age of patients (P* =0.01).

Bacteria isolated from nonsmoking patients were *Staphylococcus aureus (28.9%), Streptococcus pneumoniae (31.58%), Klebsiellapneumoniae* (31.58 %) *and Pseudomonas aeruginosa* (7.89%) while smoking patients were *Staphylococcus aureus* (21.3%), *Streptococcus pneumoniae* (36.4%), *K*lebsiella *pneumoniae* (27.3%) and *Pseudomonas aeruginosa* (15.2%). (Tab. 4).

There was no significant relationship between the occurrence of the isolates and the smoking habit of patients (P* =0.2743).

Bacteria isolated from patients with chronic bronchitis were *Staphylococcus aureus (*41.66 %), *Streptococcus pneumoniae* (25%), *Klebsiella pneumoniae (25%)* and *Pseudomonas aeruginosa* (8.33%), While pneumonia were *Staphylococcus aureus* (13.6%), *Streptococcus pneumoniae* (50%), *Klebsiella pneumoniae* (27.3%), *and Pseudomonas aeruginosa* (9%), *while Chronic obstructive pulmonary disease were Staphylococcus aureus* (28.6%), *Streptococcus pneumonia*e (14.3 %), *Klebsiella pneumoniae* (47.6%) and *Pseudomonas aeruginosa* (9.5 %) and Chronic suppurative lung disease *Staphylococcus aureus* (25.35%), *Streptococcus pneumoniae* (33.8%), *Klebsiella pneumoniae* (29.57%) and *Pseudomonas aeruginosa* (11.27%).(Tab.5)

Screening of antibiotic sensitivity of tested bacteria:

The result of disc diffusion test (Tab.6) indicated that *Staphylococcus aureus* is highly susceptible for Levofloxacillin, Imipenem with inhibition zone (30mm), followed by ciprofloxacillin (26mm), Meropenem (22mm), Azithromycin and Amoxicillin/clavulanic Acid(21mm), Cloxacillin (20mm), while show intermediate sensitivity to Cefotaxime(21mm), spiramycin (18mm) giving rise to zone diameter, respectively and resistant to the other four examined antibiotics**.** Concerning to *Streptococcus pneumonia*e highly susceptible for Levofloxacin (42mm) followed by Ciprofloxacin (40mm), Meropenem (36mm), Azithromycin (34mm), Imipenem (28mm), and Gentamycin (20mm) respectivelyand resistant to the other eight examined antibiotics. Concerning to *Klebsiella pneumonia*e highly susceptible for Meropenem (40mm), followed by Imipenem (34mm), Cefotaxime (32mm), Azithromycin (26mm) and Ciprofloxacin (30mm), Levofloxacin (26mm), Piperacillin (22mm), Gentamycin (18mm), while show intermediate sensitivity to Ceftazidime (20mm), respectively and resistant to the other five examined antibiotics. Concerning to *pseudomonas aeruginosa* highly susceptible for Levofloxacin (36mm), followed by Imipenem (34mm), Ceftazidime (32mm), Ciprofloxacin (20mm) and Gentamycin (30mm) respectively, while show intermediate sensitivity to Amoxicillin/clavulanic Acid (26mm), and resistant to the other six examined antibiotics.

Screening of antibacterial activities of medicinal plant extracts:

The results of the well diffusion test (Tab.7) indicated that ethanolic extracts of medicinal plants 10 mg/ml concentration as fellow:

The ethanolic extracts of clove showed antibacterial activities against *Staphylococcus aureus* with inhibition zone (20mm), *Streptococcus pneumonia*e with inhibition zone (20mm). Black cumin seed inhibit the growth of *Staphylococcus aureus* (26mm).Anise seed inhibit the growth of *Klebsiella pneumoniae* (28mm). Caraway seed inhibit

the growth of *Staphylococcus aureus* (22mm), *Streptococcus pneumonia* (28mm) *Klebsiella pneumonia* (26mm). Garlic, fennel, Ginger, Cumin, Cardamom, Radish, Fenugreek, Turnips, onion, Red Chili pepper and Peppermint were inactive against all bacterial strains tested.

Caraway extract proved to be the most inhibitor against almost tested bacteria, followed by clove extract. It can be seen that the most susceptible species to this plant extracts was *Staphylococcus aureus*.

Screening of antibacterial activities of Honey Bee and Royal Jelly:

The result of well diffusion test (Tab.8) indicated that honey bee showed antibacterial activities against *Staphylococcus aureus* with inhibition zone (40mm) and *Klebsiella pneumoniae* with inhibition zone (20mm),and royal jelly showed antibacterial activities against *Staphylococcus aureus* with inhibition zone (26mm).

Table (1): Bacteria that are isolated from sputum culture of all cases:

Bacteria	NO OF cases	% OF cases
S. aureus	18	25%
S. pneumoniae	24	34%
K. pneumoniae	21	30%
P. aeruginosa	8	11%
Total	71	100%

Table (2): Relation between sex and bacteria isolated:

Sex		Bacteria isolated				Total Of cases
		S. aureus	***S.pneumoniae***	***K. pneumoniae***	***P. aeruginosa***	
Male	**count**	11	13	10	7	41
	%	26.8	31.7	24.4	17.1	57.7
Female	**count**	7	11	11	1	30
	%	23.33	36	36	3.3	42.7
Total	**count**	18	24	21	8	71
	%	25	34	30	11	100

χ^2 = 0,262,Odd ratio=0.2857, Z Statistics= 2.412 and P*= 0.0295% Confidence, α 5%
P* (Correlation between bacterial isolates and sex)

Table (3): Relation between age group and bacteria isolated

Age		Bacteria isolated				Total Of cases
		S. aureus	***S. pneumoniae***	***K. pneumoniae***	***P. aeruginosa***	
Age≤35	count	3	2	6	1	12
	%	25	17	50	8	17
Age≥35	count	15	22	15	7	59
	%	25.4	37.3	25.4	11.9	83
total	NO	18	24	21	8	71
	%	25.35	33.8	29.57	11.27	100

χ^2 = 0,334, Odd ratio=0.286, Z Statistics= 2.41 and P*= 0.0195% Confidence, α 5%
P* (Correlation between bacterial isolates and age group)

Table (4): Relation between bacterial isolates and smoking habit:

smoking habit		Bacteria isolated				Total of cases
		S. aureus	***S. pneumoniae***	***K. pneumoniae***	***P aeruginosa***	
Non-Smoking	**count**	11	12	12	3	38
	%	28.9	31.578	31.578	7.89	53.52
Smoking	**count**	7	12	9	5	33
	%	21.3	36.4	27.3	15.2	46.48
Total	**NO**	18	24	21	8	71
	%	25.35	33.8	29.57	11.27	100

χ^2 = 0,689, Odd ratio=1.5354, Z Statistics= 1.093 and P*= 0.274395% Confidence, α 5%
P* (Correlation between bacterial isolates and smoking habit)

Table (5): Relation between bacteria and diagnosis of all cases:

Diagnosis	*S. aureus*		*S. pneumoniae*		*K. pneumoniae*		*P. aeruginosa*		No of cases	% of cases
	NO	%	No	%	No	%	NO	%		
Chronic bronchitis	5	41.66	3	25	3	25	1	8.33	12	16.9
pneumonia	3	13.6	11	50	6	27.3	2	9	22	30.99
COPD	6	28.6	3	14.3	10	47.6	2	9.5	21	29.58
CSLD	4	25	7	43.75	2	12.5	3	18.75	16	22.54
Total	18	25.35	24	33.8	21	29.57	8	11.27	71	100

Chronic obstructive pulmonary disease: COPD
Chronic suppurative lung disease: CSLD (Lung abscess, Bronchiectasis)

Table (6): Antibiotic Sensitivity test of Bacteria isolatedexpressed by its Diameter of inhibition zone:

Antibiotics	***S. aureus***	***S. pneumoniae***	***K. pneumoniae***	***P. aeruginosa***
Levofloxacin **(LEV)**	30(S)	42(S)	26(S)	36(S)
Azithromycin **(AZM)**	21(S)	34(S)	30(S)	0(R)
Meropenem (MEM)	22(S)	36(S)	40(S)	0(R)
Ciprofloxacin **(CIP)**	26(S)	40(S)	30(S)	30(S)
Gentamycin **(GM)**	20(S)	20(S)	18(S)	30(S)
Imipenem **(IPM)**	30(S)	28(S)	34(S)	34(S)
Cefotaxime **(CTX)**	21(I)	0(R)	32(S)	0(R)
Cloxacillin **(OB)**	20(S)	0(R)	0(R)	0(R)
Amoxicillin\clavulanic Acid(AMC)	21(S)	0(R)	0(R)	26(S)
Ceftazidime **(CAZ)**	0 (R)	0(R)	20(S)	32(S)
Spiramycin **(SP)**	18(I)	0(R)	0(R)	0(R)
Piperacillin **(PIP)**	0(R)	0(R)	22(S)	0(R)
Ampecillin\sulbactam**(SAM)**	0(R)	0(R)	0(R)	0(R)
Ampicillin **(AMP)**	0(R)	0(R)	0(R)	0(R)

S: Sensitive; I: Intermediate; R: Resistant

Table (7): Antibacterial activity of Plant extract against Bacterialstrains using well diffusion test expressed by its Diameter of inhibition zone:

Bacteria / Tested plants	***S. aureus*** **(DIZ) mm**	***S. pneumoniae*** **(DIZ) mm**	***K. pneumoniae*** **(DIZ) mm**	***P.aeruginosa*** **(DIZ) mm**
Cinnamon	--	--	--	--
Clove	18	20		--
Black cumin	26	--	--	--
Garlic	--	--	--	--
fennel	--	--	--	--
Ginger	--	--	--	--
Cumin	--	--	--	--
Cardamom	--	--	--	--
Radish	--	--	--	--
Turnips	--	--	--	--
Red chilli pepper	--	--	--	--
peppermint	--	--	--	--
Anise	--	--	28	--
Caraway	22	28	26	--
DMSO10%	--	--	--	--

Values calculated as means of duplicates DMSO: Dimethyl Sulfoxide
Concentration of all of the plant extracts were 10mg/ml.(-) no inhibition zone

Table (8): Antibacterial activity of honey Bee and Royal jelly against Bacterialstrains using well diffusion test expressed by its Diameter of inhibition zone

Bacterialstains	*S. aureus (DIZ)/mm*	*S. pneumoniae (DIZ)/mm*	*K. pneumoniae (DIZ)/mm*	*P. aeruginosa (DIZ)/mm*
Honey Bee	40	0	20	0
Royal jelly	26	0	0	0

4. Discussion:

According to the present study, common Bacteria causing respiratory tract infections were *Streptococcus pneumoniae,* followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *P*seudomonas *aeruginosa.* Nearly similar results were reported by ***Aroma Oberoi et al. (2006), Shanavas et al. (2015)*** found that the most common pathogen isolated from sputum sample were *Klebsiella Pneumoniae*, *Streptococci, Staphylococcus aureus and Pseudomonas species, and* ***Taura, et al. (2013)*** who found that *common Bacteria causing respiratory tract infections are Streptococcus pneumoniae 25.6%, Followed by Klebsiella Pneumoniae 20.9%.*

This study found that Men 58% reported respiratory tract infections more frequently than women 42%. This result agree with other studies conducted by ***Matthew (2007)*** who reported that most Respiratory Tract Infections (RTIs) is more severe in males than in females (***Panda et al., 2012)*** whose reported that, 63.4% were from males while 36.6% were from females, However, these results contradicts the data obtained by ***El- Mahmood et al. (2010),*** in which in a similar study, found that 49.1% were from males while 50.9% from females.

In this study the commonest Bacteria in infected male is *Streptococcus pneumoniae* 31.7% Followed by *Staphylococcus aureus 26.8, Klebsiella Pneumoniae 24.4% and Pseudomonas aeruginosa 17.1%.*While in females are *S.pneumoniae*, *K.pneumoniae 36*% followed by *Staphylococcus aureus 23.33%* and *P.aeruginosa* 3.3%.

In this study the occurrence of bacterial pathogens varies with age, in that, age group more than 35 year 83% more infected than below 35 years17%. **Goto *Kumagai (2008)*** *and* ***Go to Iwasaki (2011)*** were reported that the majority number (57.7%) of the patients with respiratory tract infection were aged 70 years or older. The commonest Bacteria isolated in Age ≤35 is *Klebsiella pneumoniae* 50%, followed by *Staphylococcus aureus* 25%, *Streptococcus pneumoniae* 17% and *Pseudomonas aeruginosa* 8%, While The commonest Bacteria isolated in age ≥35 is *Streptococcus pneumoniae*, followed by *Staphylococcus aureus, Klebsiella Pneumoniae 25.4% and Pseudomonas aeruginosa* 11.9 %. ***Panda et al. (2012)*** reported that higher occurrence of *K. pneumoniae* among patients ranging from 51-60 and 60-70 years.

This study proved that *Streptococcus pneumoniae and Klebsiella pneumoniae were the commonest Bacteria isolated from nonsmoking patients,* followed by *Staphylococcus aureus 28.9% and Pseudomonas aeruginosa* 7.89% while in smoking patients were *Streptococcus pneumoniae* 36.4% followed by *Klebsiella Pneumoniae* 27.3%, *Staphylococcus aureus* 21.3%, and *Pseudomonas aeruginosa* 15.2%.

The commonestbacteria frequently isolated from patients with chronic bronchitis was *Staphylococcus aureus (*41.66 %).This result agree with ***Goto et al. (2005)*** who find that*S. aureus* (25.4%) were the commonest frequently isolated from the patients with chronic bronchitis. On the other hand ***Go to Iwasaki (2007)*** found that *Streptococcus pneumoniae* (27.1%), frequently common bacteria isolated from the patients with chronic bronchitis. While in this study pneumonia were *Streptococcuspneumonia*e (50%) This result agree with ***Goto et al. (2005)*** who investigated that the commonest bacteria frequently isolated from the patients with bacterial pneumonia were *Streptococcus pneumoniae* (23.4%), on the other hand, ***Goto Iwasaki (2007)*** find out that the commonest bacteria frequently isolated from the patients with bacterial pneumonia were *Staphylococcus aureus* (21.9%). Commonest bacteria frequently isolated from patients with chronic obstructive pulmonary disease *were Klebsiella Pneumoniae* (47.6%), *Staphylococcus aureus* (28.6%), *Streptococcus pneumonia*e (14.3 %) and*Pseudomonas aeruginosa* (9.5 %). ***Zalacain, et al. (1999)*** demonstrated that *Haemophilus influenzae, Streptococcus viridans, Streptococcu spneumoniae and Moraxella catarrhalis* were the most frequent pathogensin patients with stable chronic obstructive pulmonary disease (COPD).

All the isolates displayed variable sensitivity to the antibiotics tested as detailed as shown in (Tab.6) *Staphylococcus aureus* was highly susceptible for Levofloxacillin, Imipenem, followed by ciprofloxacillin, Meropenem, Azithromycin and Amoxicillin/clavulanic Acid, Cloxacillin and Gentamycin, while show intermediate sensitivity to Cefotaxime, spiramycin giving rise to zone diameter, respectively, and resistant to Ceftazidime,

Piperacillin, Ampecillin/sulbactam and Ampicillin. ***Taura, et al. (2013)*** found out that the sensitivity patterns of the isolated bacteria were Ceftazidine, Ciprofloxacin, Ofloxacin, Gentamicin and Chloramphenicol shows activity on all the pathogens isolated. **Kalyani Murthy, (2014)** found out that the isolated strains of *Staphylococcus aureus* showed resistance to Methicillin, Oxacillin, Vancomycin and sensitivity to Gentamycin.

Our result proved that *Streptococcus pneumoniae*washighly susceptible for Levofloxacin followed by Ciprofloxacin, Meropenem, Azithromycin, Imipenem, and Gentamycin respectively *S.pneumonia*e resistant to Cefotaxime, Cloxacillin, Amoxicillin\clavulanic Acid, Ceftazidime, Spiramycin, Piperacillin, Ampecillin\sulbactam, Ampicillin. ***Rosa del Campo, etal. (2005)*** investigated that *Streptococcus pneumonia* was resistance to penicillin, 73%; to Cefotaxime, 33%; to erythromycin, 42%; to tetracycline, 58%; to chloramphenicol, 48%; and to trimethoprim-sulfamethoxazole, 67%. Resistance to fluoroquinolones was not detected. ***Iroha et al. (2013)*** found that *Streptococcus* spp. were susceptible to ciprofloxacin, ceftazidime and amikacin but resistant to amoxicillin/clavulanic acid, and ampicillin.

In this study *Klebsiella pneumonia*e was highly susceptible for Meropenem,followed by Imipenem, Cefotaxime, Azithromycin and Ciprofloxacin, Levofloxacin, Piperacillin, Gentamycin, while show intermediate sensitivity to Ceftazidime, and resistant to Cloxacillin, Amoxicillin\clavulanic Acid, Spiramycin, Ampecillin\sulbactam, Ampicillin, ***Goto Iwasaki (2011)*** found that imipenem had the most potent activity and inhibited the growth of all *Klebsiella pneumoniae* strains, ***Shanavas et al. (2015)*** investigated that *Klebsiella Pneumoniae* was found to be most sensitive to Imipenem, Amikacin and Gentamicin.

In this study *Pseudomons aeruginosa* highly susceptible for Levofloxacin, followed by Imipenem, Ceftazidime, Ciprofloxacin and Gentamycin respectively, while show intermediate sensitivity to Amoxicillin\clavulanic Acid,and resistant to Azithromycin, Meropenem, Cefotaxime, Cloxacillin, Spiramycin, Piperacillin, Ampecillin/sulbactam and Ampicillin. This result agree with the finding of (***CHANDER et al. (2013)*** found out that the most effective antimicrobial drugs of *P. aeruginosa* were Imipenem and ciprofloxacin. on the other hand ***Piyush et al. (2011)*** investigated that ceftazidime show more resistance and Imipenam was found to be the most sensitive drug against *P. aeruginosa* in Lower respiratory tract infection patients.

Investigation on the crude ethanol extracts of clove, Black cumin, Anise, Caraway seed showed different degrees of growth inhibition, by using the well diffusion test as shown in (Tab.7) Caraway extract proved to be the most inhibitor against almost tested bacteria, followed by clove extract, Anise and Black cumin seed. Ethanolic extract of Cinnamon, Garlic, Ginger, Cumin, Cardamom, fennel, Turnips, onion, Red Chili pepper and Peppermint were inactive against all bacterial strains tested.

In this study Clove was detected to exhibit an inhibitory effect against *Staphylococcus aureus* with inhibition zone (18mm), *Streptococcus pneumonia*e (20mm).Similar result was reported by ***Aureli et al. (1992), Conner, (1993)*** who found that Clove had strong and consistent inhibitory effect against several pathogens. ***Tayel and El-Tras, (2009)*** reported that ethanolic extract of clove inhibited the growth of *Pseudomonas aeruginosa and Staphylococcus aureus.*

This study found that Black cumin seed extract show antimicrobial effect against growth of *Staphylococcus aureus*. It is nearly similar to those of ***Tayel and Etras (2009)*** who mentioned that Black cumin was active against gram positive bacteria and

gram negative.On the other hand ***Ibtisam Mohammed (2011)*** found out that *Escherichia coli and Pseudomonas aeruginosa* and *Staphylococcus aureus* were resistant to Black cumin extract.

Ethanolic extract of Caraway seed inhibit the growth of *Staphylococcus aureus*, *Streptococcus pneumonia*, *and Klebsiella pneumoniae*. Similar result reported by ***Sadowska, (1998), Toxopeus, (1992)*** Caraway essential oil performs medium antimicrobial activity. Hence it inhibits growth of many bacteria *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Vibrio cholerae, Mycobacterium tuberculosis* and ***Katarzyna, et al. (2013)***found that Caraway essential oil exhibited medium antimicrobial activity and carvone can be recognized as a one of the active component.

Cardamom and turnip showed no antimicrobial effect against test strains. These finding aren't accordance with finding of other investigations, ***Gieslene et al. (2008)*** found the alcoholic extracts of roots of turnips were potent antimicrobial activity against *pseudomonas aeruginosa*. ***Hêro and Akrayi (2012)*** found out that Cardamom has inhibitory effect against *Staphylococcus aureus*. Regarding Turnip ***Katayoon et al. (2011)*** was found out that the highest antimicrobial activity was observed by methanolic extract on *pseudomonas aeroginosa* was sensitive to this extract.

Though garlic and it's component identified as allicin has been recognized and studied most extensively for their antimicrobial activities, ***Shingh***

and Shukla (1984), ***Feldberg et al. (1988)***, no effect were observed to all bacteria tested in this study. *while* ***Ankri and Mirelman*** **(1999), *Iram Gull, et al. (2012)*** found out that maximum inhibitory effect against *Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

Ginger showed no antimicrobial effect against test strains. Similar result was reported by ***Ibtisam Mohammed (2011)*** who found that ethanolic extract of ginger didn't inhibit *Staphylococcus aureus* and *Pseudomonas aeruginosa*. On the other hand these results compare with the findings of ***Tayel and El-tras (2009)*** who reported the antimicrobial activity of Ginger against *S. aureus*.

Ethanolic extract of Anise seed inhibit the growth of *Klebsiella pneumoniae*. These findings are accordance with ***AL Maofari, et al. (2013)*** who found the essential oils of the *Pimpinella anisum* extracts showed various antibacterial activities against gram-negative and gram-positive organisms.

Fennel showed no antimicrobial effect against test strains. These finding aren't accordance with findings of other investigations, ***Mandeel et al., (2003), Tayel andEl-Tras (2009)*** who find that fennel had inhibitory effect against bacterial growth.

Red chili (pepper) and onion showed no antimicrobial effect against test strains. These findings are accordance with findings of ***Agoglu et al. (2007), Panutat and vatanyoopaisarn (2005).*** On the other hand, ***Dorantes et al. (2000)*** reported that chili extracts are inhibitory to some microorganisms.

In this study honey bee showed antibacterial activities against *Staphylococcus aureus* and *Klebsiella pneumoniae*, while Royal jelly showed antibacterial activities against *Staphylococcus aureus*onlyas shown in(Tab.8).These finding are accordance with findings of ***Mierzejewski (2014)*** who found that Honeybee and royal jelly displayed a varying degree of bacterial inhibition Zones, ***Miorin et al.(2003)*** foundthathoney samples had antibacterial activity against *S. aureus*, and ***Hayam et al (2011)*** investigated thatundiluted honey samples also inhibited the growth of *Staphylococcus Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*,

Four plants (Caraway, Anise, Clove, and black cumin) and honey bee and royal jelly were shown to have an inhibitory effect against *S. aureus*, *S. pneumoniae* and *Klebsiella pneumoniae* which are an important pathogen in respiratory infection. The most effective activity against *Staphylococcus aureus* was exhibited by honey bee (40mm), followed by Black cumin (26), Royal jelly (26mm), caraway (22mm),while Cinnamon(20mm)show the lowest activity. The most effective activity against *Streptococcus pneumoniae* was exhibited by Caraway (28mm) and Clove (20mm). The most effective activity against *Klebsiella pneumoniae* was Anise (28mm), Caraway (26mm) and honey Bee (20mm).*Pseudomonas aeruginosa* was resistant to all tested plant extracts, honey bee and royal jelly.

Caraway and honey bee were found to be the most effective natural product against test strains.

In the present study, Gram positive bacteria *(Staphylococcus aureus*) were found to be more susceptible to Clove, black cumin, Caraway, honey bee and Royal jelly samples.

The results of the present study are quite encouraging as some plant extract exhibited antimicrobial activity against most of the pathogens, but the antimicrobial activity varies widely, depending on the type of plant extracts and microorganism.

They might be considered as possible alternatives to antibiotics for treatment of multidrug-resistant strains infections. Even though, the results obtained in this study indicated that some of the antibiotics used to treat respiratory tract infections in this community are still effective, but still there is danger of drug resistance which need to be tackled.

This study opens up the possibility for the search of new antimicrobials as an alternative to the antibiotics.

It is hope that this study positively participate in solving the problem of antibiotic resistance.

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