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ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS OF PLANTS, SPICES AND HERBS AGAINST THE BACTERIA ISOLATED FROM CONTACT LENS

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ABSTRACT

With the emergence of antibiotic era hardly five decades ago people are afflicted with global problem of increasing resistance in almost all pathogens. Due to the increasing infections and elevated rates of synthetic drugs along with their side effects medicinal plants are being used by 80% of population to treat various ailments. So the present study was designed to evaluate antibacterial effect of ethanolic extracts of plants, spices and herbs against the bacteria isolated from contact lens that cause severe infections to human eye. Antibacterial activity of thirty samples of ethanolic extracts of plants, herbs and spices was tested against both gram positive and gram negative bacteria isolated from 60 samples of contact lens that were taken from different sources by disc diffusion method with the measurement of diameter of zone of inhibition around each extract. The results showed the presence of antibacterial activity of almost all the plant, herbs and spices extract with a highest zone of inhibition of 40mm against *S. aureus* by *Bacopa monnieri* and *Emblica officinalis* plant extracts while *Achillea millefolium* extract was resistant against bacteria. Among spices extract turmeric extract showed largest inhibition zone of 35mm against *Micrococcus luteus* and most resistivity was also shown by turmeric extract against Bacillus sp, *P. aeruginosa* and *E. coli*. Herbs extracts showed a maximum zone of inhibition of 25mm against *K. pneumoniae* with mint extract whereas the most sensitivity was observed against *M. luteus*. All the samples exhibited antibacterial activity against *S. aureus, M. luteus,* Bacillus sp. *P. aeruginosa*, *Klebsiella pneumoniae* and *E. coli* except *Achillea millefolium* plant that showed resistivity against all bacterial isolates.

KEYWORDS: Contact lenses, pathogens, infection, herbs, spices, plants, prevention.

INTRODUCTION

Contact lenses are now days used frequently for correcting refractive errors, therapeutic purposes and cosmetic purposes. Besides their few benefits these lenses possess some complications such as asymptomatic keratitis, in filterative keratitis, peripheral ulcers, corneal infections and acute red eye (Willcox *et al*., 2001; Sankaridurg *et al*., 1999). Corneal infection is one of the visions threatening complication caused by wearing contact lenses (Willcox *et al*., 2001). These infections are caused by certain bacteria and fungus such as *Pseudomonas aeruginosa, Staphylococcus aureus,* Bacillus sp., Micrococcus sp., *Fusarium solani, Aspergillus versicolor, Candida albicans, Cladosporium sp* etc (Willcox *et al*., 2001).

Bacterial and fungal contamination of contact lenses is usually caused by neglecting hygienic conditions such as using contaminated lenses wearing contact lenses during eye infection, improper sterilization of contact lenses (Giese and Weissman, 2002). This can be prevented by this contamination by replacing contact lens case regularly; cleaning the lens by scrubbing their interior, allowing the lens to dry between uses (Willcox *et al*., 2001).

Eye makeup has long been used to enhance personal appearance, to improve self-esteem or attract the attention of others. Among the commonest causes leading to eyelid dermatitis, cosmetics would be playing a major role. Cosmetics used as eyeliners, eye shadow, mascara, and eyelash curlers, eye makeup remover etc could all contribute to eyelid Blepharitis. Researchers showed a 67% to 100% bacterial contamination in the samples, notably Staph, Strep and even *E. coli* bacteria. In addition to misuse it, which is using kohl or eyeliner more than one person or may leave these eyeliners exposed to the air accumulate the dust particles laden contaminate with microbes and fungi and transmitted to the eye while using these eyeliners (Al-Aany *et al*., 2009).

Herbs, spices and medicinal plants have been used as traditional healer for almost all medicinal therapy since the prehistoric era. The plants were used as a healer till nineteenth century until the synthetic drugs were discovered (Das A *et al*., 2002; Exarchou *et al*., 2002). World health organization has estimated that 80% of population around the world mainly rely on traditional medicines for curing ailments. Plants, herbs and spices provide remedies for various infectious diseases ranging from minor infections such as skin infections, eye infections to malaria, skin diseases, asthma, and dysentery. A current problem associated with the use of antibiotics has revived the interest in plants with antimicrobial properties (Shiota *et al*., 2004; Abu- Shanab, 2004).

The phytochemicals in plants such as alkaloids, flavonoids, tannins and phenolic compounds possess bioactive significance that play important role in preventing diseases (Duraipandiyan *et al*., 2006).

The use of herbs and medicinal plants is a universal phenomenon. Every culture on earth has relied on the huge variety of natural chemistry found in healing plants for their therapeutic properties. As per World Health Organization (WHO), about 80% of world population use medicinal plants to treat human disease. Spices and herbs have been used for many years by different cultures to enhance the flavor and aroma of foods, in preserving foods and for their medicinal value. These attributes are useful in the development of snack foods and meat products. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components (Alzoreky and Nakahara, 2003; Kumral and Sahin, 2003; Park *et al*., 2009).

Nowadays, due to the misuse of antibiotics, antimicrobial properties of medicinal plants have been evaluated for obtaining safe drugs. Hence, in this study we used five species of plants for medicinal purposes. Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, over use of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of microorganisms? The worldwide emergence of multi drug resistant *Escherichia coli* and many other ß-lactamase producers has become a major therapeutic problem (Khan, A.U.*et al*.2004).

Herbs and spices have been used for thousands of years to enhance the flavor, colour and aroma of food. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value, which forms one of the oldest sciences**.** A large number of plants are used to combat different diseases and possess antimicrobial activity. Several spices particularly garlic, black pepper, clove, ginger, cumin, cardamom, cinnamon and caraway are used extensively in the Indian diet and in Indian medicine. Garlic with its antimicrobial properties is widely used for a number of infectious diseases. Use of spices for medical benefits can be justified as these are easily absorbed by our body and generally do not have any adverse effects (Nielsen PV and Rios R, 2000).

Medicinal plants are an important therapeutic aid for various ailments. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Dahanukar *et al*., 2000, Nair and Chanda, 2006).

In many developing countries traditional medicine is one of the primary health care systems. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection (Satish *et al*., 2008).

The incidence of severe infections in humans caused by pathogenic microorganisms has increased globally and is a key cause of morbidity and mortality in developing countries (Al-Bari *et al*., 2006). The exercise of complementary and alternative medicine is upraised in most of the developing countries in response to World Health Organization directives, culminating in several pre-clinical and clinical studies. These have provided the scientific basis for the efficacy of many plants or plant tissues used in folk medicine to treat infections (Dilhuydy, 2003).

Similarly maximum of crude drugs are obtained from natural sources (Plants) or semi synthetic derivatives of natural products and is commonly used in traditional systems of medicine (Sukanya *et al.*, 2009).

Medicinal plants extracts and active phytochemical contents with known antimicrobial efficiency can be of pronounced implication in therapeutic cures. Plant-based antimicrobial agents may be found in plant leaves, flowers, bark, stems, roots or fruits (Ahmad *et al*., 2012). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.*, 2003).

Medicinal plants are the inexpensive and harmless alternative homes of antimicrobials (Doughari *et al*., 2007). Plants have shown to be a potential source for the new antimicrobial agents. Active compounds of interest present in the medicinal plants have continuously been of great concern to the scientists (Ahmad *et al*., 2010; 2011a; 2011b; 2011c; 2012).

The objective of this research was to evaluate the antibacterial and antifungal activity of crude extracts of some plants, spices and herbs against bacteria and fungi associated with contact lenses.

MATERIALS AND METHOD

Collection and preparation of plant samples

Fresh leaves of different plants and freshly grounded spices were collected from university area, gardens and departmental stores of Lahore city. Leaves were washed thoroughly with tap water. The spices were finely grounded into powdered form with mortar and pestle. The leaves were dried at room temperature for 3-4 days. The dried leaves were then crushed into fine powder in an electric grinder and the powder was stored in polyethylene bag in refrigerator at 4°C for further process (Farhad, 2012).

Extracts preparation

15g of ground dried plant, herbs and spices were added to 150ml of ethanol solvent, shaked, heated below boiling point, stirred for 2 to 3 hour until the concentration of ethanol was reduced to half. The extract was filtered by muslin cloth, then by filter paper (Whatman No. 1) and finally stored in the refrigerator at 4°C for using (Al-Neemy and AL-Jebury, 2006, Babpour *et al*., 2009).

Contact lens sample processing

Sixty samples of contact lenses were taken from different sources. Twenty samples were taken from different shops at local market, twenty were collected from those people who had eye infection wearing lenses and twenty was collected from different students at University of Lahore. The samples were taken in the form of swabs for bacterial and fungal isolation.

Samples were swabbed on nutrient agar, bacterial purification was done by streaking the colonies on the nutrient agar plates (Uraku *et al*., 2012). The inoculums were sub cultured evenly on other selective and differential media's Mannitol Salt Agar (MSA), Eosine Methylene Blue (EMB), Salmonella Shigella agar (SS-agar), Pseudomonas Cetrimide agar, Blood agar and MacConkey agar from pure culture. All the plates were incubated aerobically in an incubator at 37°C for 24 hours by streak plate method. After incubation, plates were examined for growth. These sub-cultured plates were then used in the identification and characterization of the organisms by Gram staining and biochemical characterization. Different biochemical tests such as Catalase test, Coagulase test, Oxidase test, Indole production test, Methyl red test, Voges-Proskauer test, Citrate utilization test, Urease test (IMViC), Hydrogen Sulfide test (H_2S), Triple Sugar Iron (TSI) test (for dextrose, sucrose, and lactose fermentation), Nitrate Reduction test were done for confirmation of isolated bacterial cultures on species level according to protocols described previously (Cheesbrough, 2000; Cappucino and Sherman, 2007).

Determination of antibacterial activity

Antibacterial activity was determined by Kirby-Bauer method of disc diffusion modified by CLSI (Clinical Laboratory Standard Institute) technique. Disc diffusion method for antibacterial susceptibility testing was carried out to assess the presence of the activity of plant extract. The bacterial cultures were adjusted to 0.5 McFarland standards then 20µl of culture was evenly spread on Muller Hinton agar plates. The standard concentration of the plant extract such as 25mg was prepared in 1 ml DMSO. Filter paper discs were placed on media plates with sterile forceps then impregnated 5µl plant extract on each disc. Bacterial plates were incubated at 37°C for 24 hours to observe the antibacterial activity. DMSO was used as negative control while Gentamicin was used as a positive control against bacteria isolates. The zones of inhibition were measured (in mm) according to standard protocol.

RESULTS

Bacterial analysis was conducted on contact lens. Out of 60 samples, 20 samples were collected from Local markets, 20 were collected from people with eye infections and 20 samples were collected from students. 7 out of 60 samples were not contaminated with bacteria while 29 out of 60 samples were without fungal growth.

A total of sixty contact lenses samples were analyzed for bacterial contamination in which 160 (100%) different bacterial isolates were isolated. Most prevalent bacterial isolates were *E. coli* 36 (22.50%), *P. aeruginosa* 29 (18.13%), *K. pneumoniae* 27 (16.88%), *S. aureus* and Bacillus sp. 26 (16.25%) and *M. luteus* 16 (10.00%). All bacterial isolates from contact lenses were listed with percentages in Table 1, Fig 1 and Fig 2.

Tab 1: Prevalence of bacterial isolates from different sources of Lahore

Sr. no	Bacterial isolates	Local market (n=20)	People with eye infection (n=20)	Students (n=20)	Percentage
1	*S. aureus*	11	10	5	26 (16.25%)
2	*M. luteus*	5	6	5	16 (10.00%)
3	*K. pneumoniae*	7	15	5	27 (16.88%)
4	Bacillus sp.	5	13	8	26 (16.25%)
5	*E. coli*	9	20	7	36 (22.50%)
6	*P. aeruginosa*	8	17	4	29 (18.13%)
Percentage		45 (28.13%)	81 (50.63%)	34 (21.25%)	160 (100%)

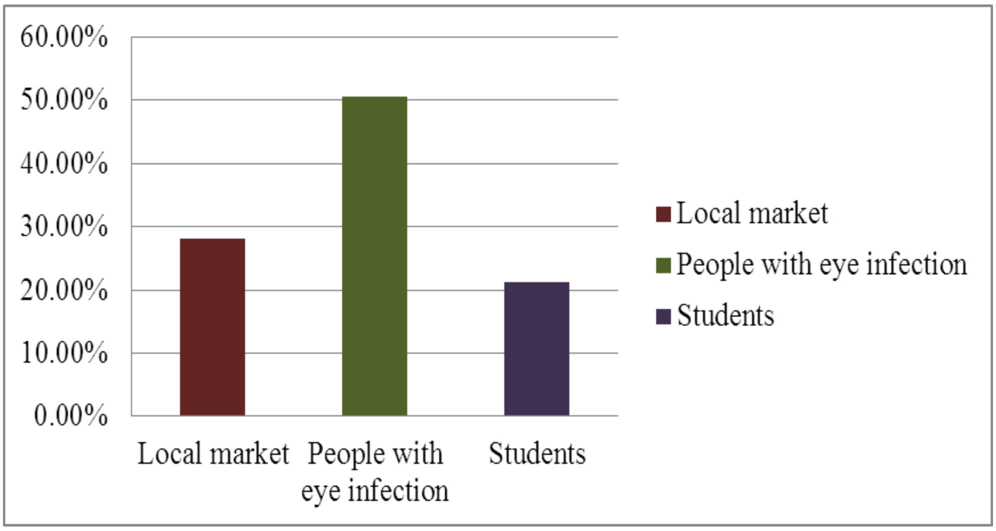

Fig1. Prevalence of bacterial isolates from contact lenses obtained from different sources

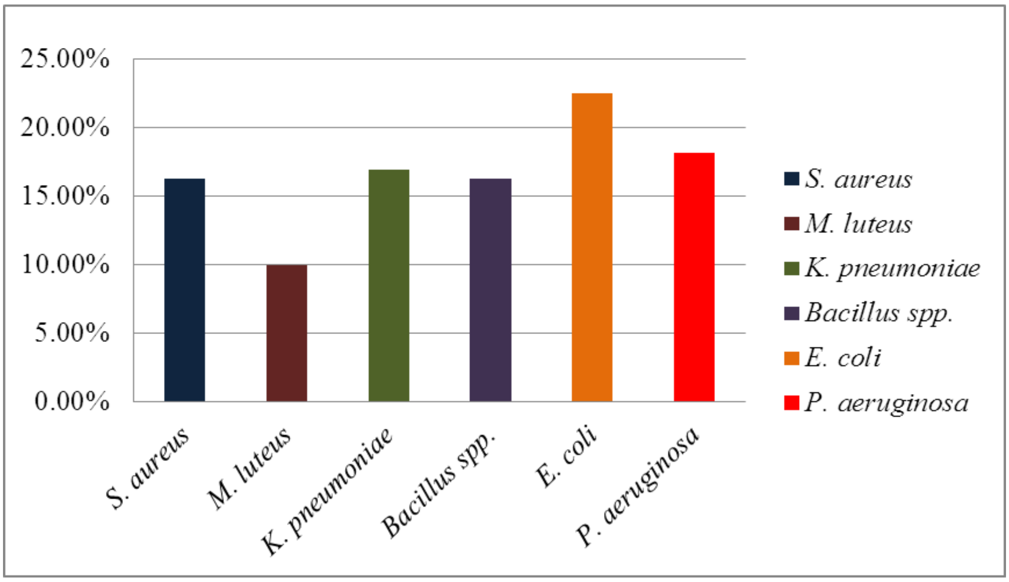

Fig 2: Prevalence of bacterial isolates from contact lenses

The ethanolic extracts of different plants have been tested for their antibacterial activity singly with the concentration of 25mg/1ml DMSO. The ethanolic extracts showed different zones of inhibition for tested microorganisms (Fig 3). The zones of inhibition were measured in mm and the results were compared with standard broad spectrum antibiotic disc such as Gentamicin.

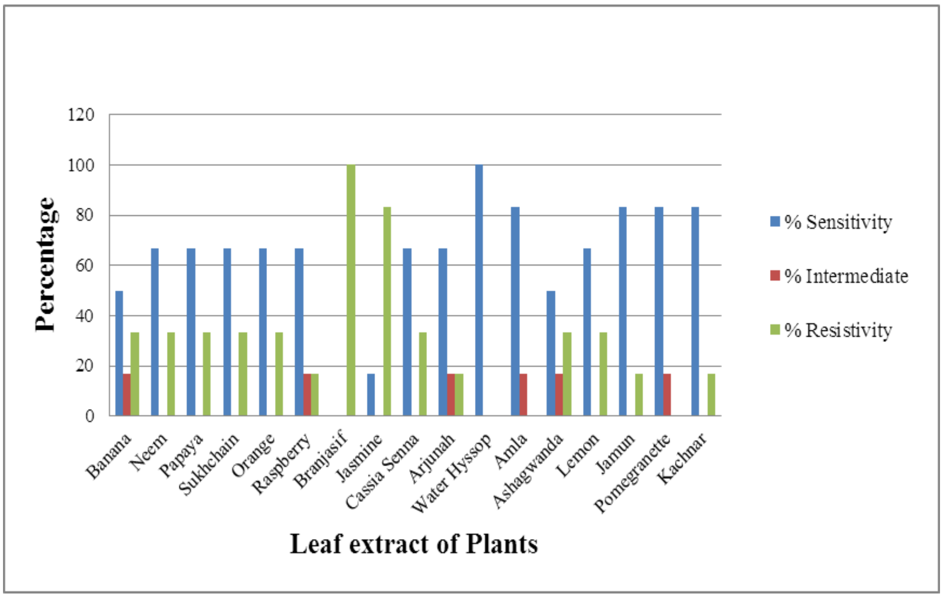

Fig 3: Zones of inhibition of plant extracts against bacterial isolates

Antimicrobial susceptibility testing of ethanolic extracts against bacterial isolates showed that 6 isolated bacterial species showed 100% sensitivity to bacopa (water hyssop) extract, 83.33% sensitivity to emblica (amla), jamun, pomegranette and kachnar extracts, 66.67% sensitivity to neem, papaya, sukhchain, orange, mulberry, cassia and lemon extracts, 50% sensitivity to banana and withania (ashagwanda) extracts, 16.67% sensitivity to jasmine extract while branjasif showed no sensitivity.

All 6 bacterial isolates showed 100% resistance to branjasif, 83.33% resistance to jasmine, 33.33% resistance to banana, neem, papaya, sukhchain, orange, cassia, withania (ashagwanda) and lemon extracts, 16.67% resistance to mulberry, terminalia (arjuna), jamun and kachnar extracts while no resistance was shown by bacopa (water hyssop), emblica (amla) and pomegranette extracts.

All 6 bacterial isolates showed 16.67% intermediate susceptibility to banana, mulberry, terminalia (arjuna), bacopa (amla), withania (ashagwanda) and pomegranette extracts while neem, papaya, sukhchain, orange, branjasif, jasmine, cassia, bacopa (water hyssop), lemon, jamun and kachnar extracts showed no intermediate susceptibility.

Antimicrobial susceptibility testing of bacterial isolates showed that *S. aureus* and *E. coli* showed 88.23% sensitivity, Bacillus sp. showed 64.70% sensitivity, *K. pneumoniae* and *P. aeruginosa* showed 52.95% sensitivity while *M. luteus* showed 35.29% sensitivity against all plant extracts. *M. luteus* showed 23.54% intermediate susceptibility, Bacillus sp. and *B. cereus* showed 5.88% intermediate susceptibility while *S. aureus, K. pneumoniae* and *E. coli* showed no intermediate susceptibility against all plant extracts. *K. pneumoniae* 47.05% resistivity, *M. luteus* and *P. aeruginosa* showed 41.17% resistivity, Bacillus sp. showed 29.42% resistivity while *S. aureus* and *E. coli* showed 11.17% resistivity against all plant extracts.

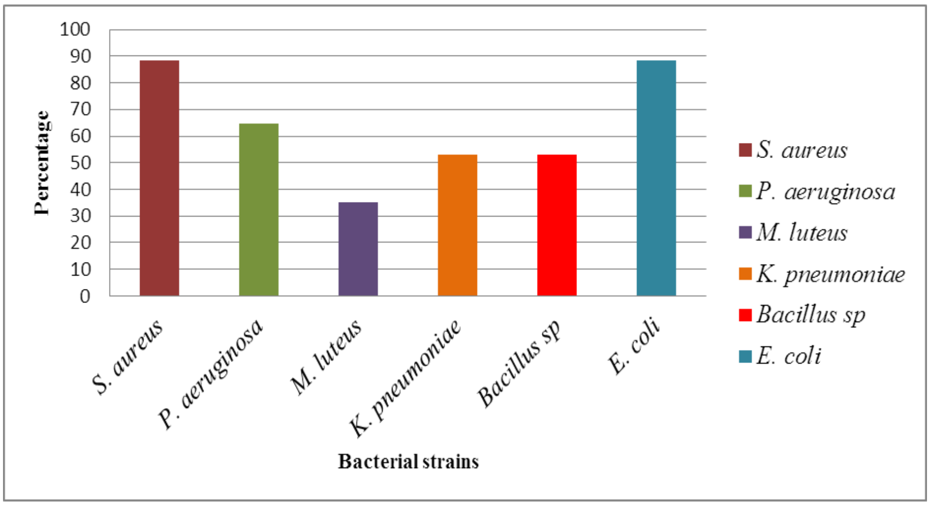

Fig 4: Percentage sensitivity of plant extracts against bacterial isolates

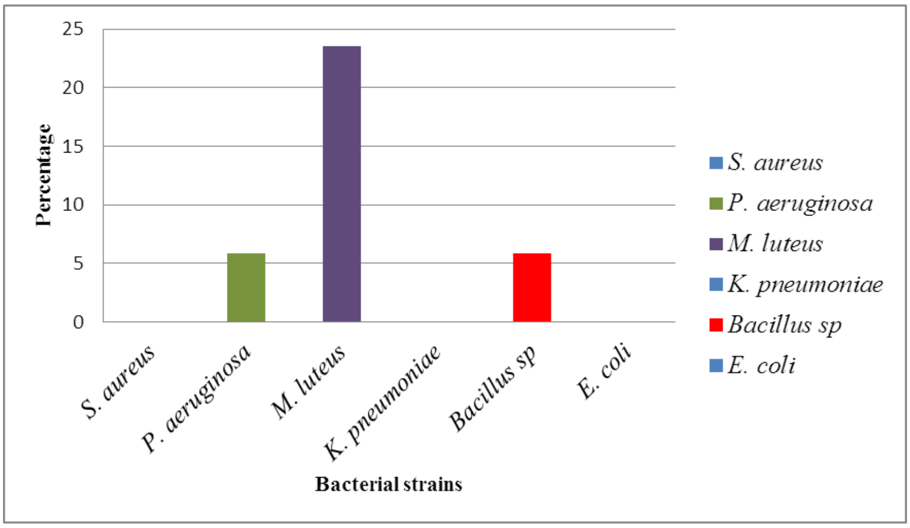

Fig 5: Percentage intermediate of plant extracts against bacterial strains

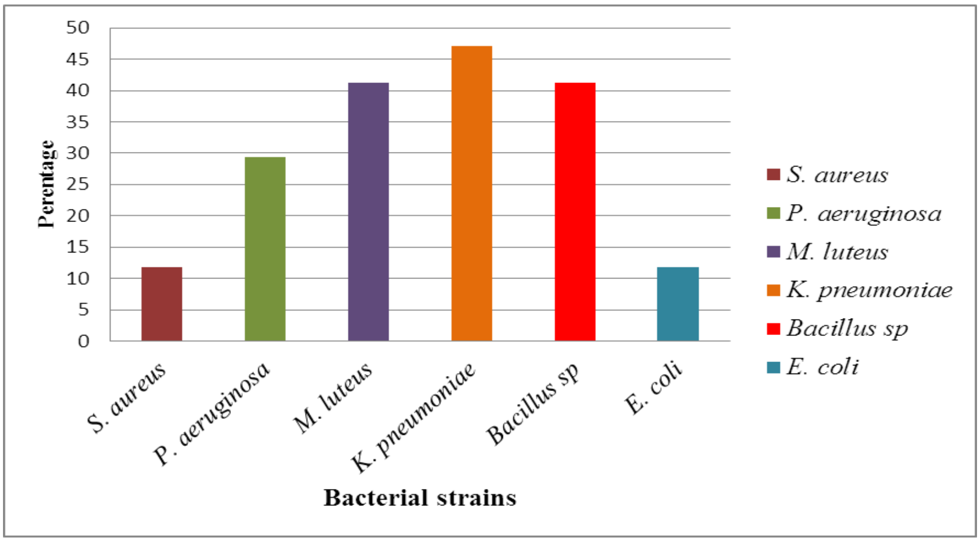

Fig 6: Percentage resistivity of plant extracts against bacterial strains

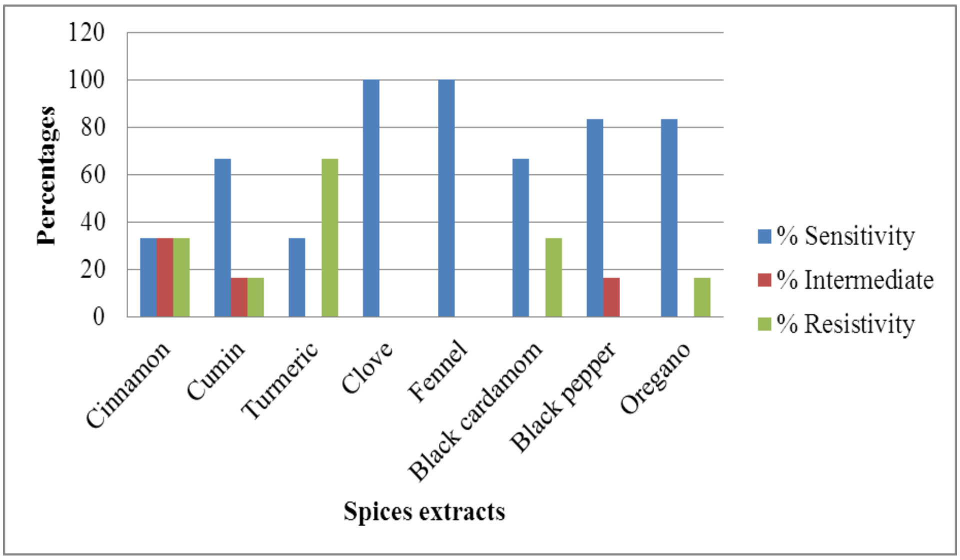

Fig 7: Zone of inhibition of spice extracts against bacterial isolates

Antimicrobial susceptibility testing of ethanolic spices extracts against bacterial isolates showed that 6 isolated bacterial species showed 100% sensitivity to clove and fennel extracts, 83.33% sensitivity to black pepper and oregano, 66.67% sensitivity against black cardamom and cumin extracts while 33.33% sensitivity to cinnamon and turmeric extracts.

All 6 bacterial isolates showed 66.67% resistance to turmeric, 33.33% resistance to cinnamon and black cardamom extracts, 16.67% resistance to oregano and cumin extracts while clove, fennel and black pepper showed no resistivity.

All 6 bacterial isolates showed 33.33% intermediate susceptibility to cinnamon extract while cumin and black pepper showed 16.67% intermediate susceptibility. Clove, fennel and turmeric, black cardamom and oregano showed no intermediate susceptibility.

Antimicrobial susceptibility testing of bacterial isolates showed that *K. pneumoniae* showed 87.5% effectively, *S. aureus*, *M. luteus*, *E. coli* showed 75% effectively, Bacillus sp. showed 62.5% effectively and *P. aeruginosa* showed 50% effectively against all spices extracts. *S. aureus, M. luteus, P. aeruginosa* and *E. coli* showed 12.5% intermediate susceptibility while Bacillus sp. and *K. pneumoniae* showed 0% intermediate susceptibility against all spices extracts. Bacillus sp. and P. *aeruginosa* showed 37.5% resistivity while *S. aureus, M. luteus, K. pneumoniae* and *E. coli* showed 12.5% resistivity against all spice extracts.

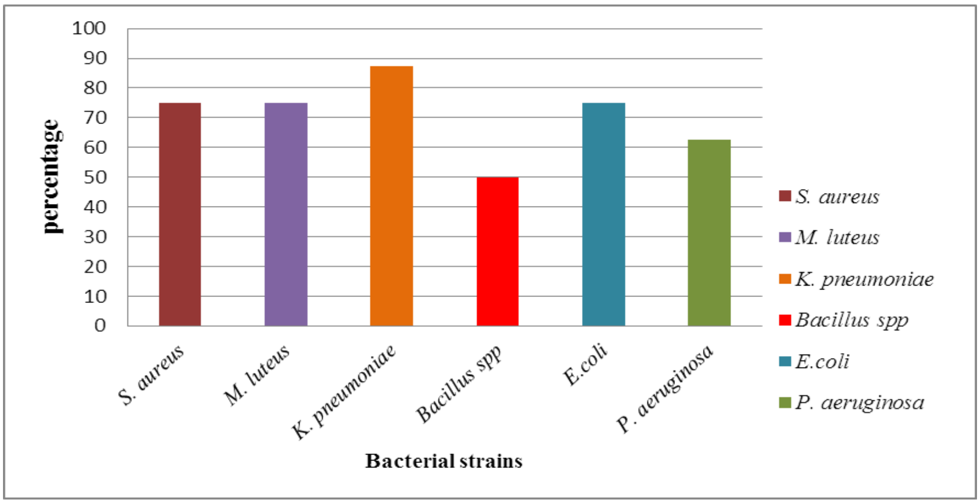

Fig 8: Percentage sensitivity of spices extracts against bacterial strains

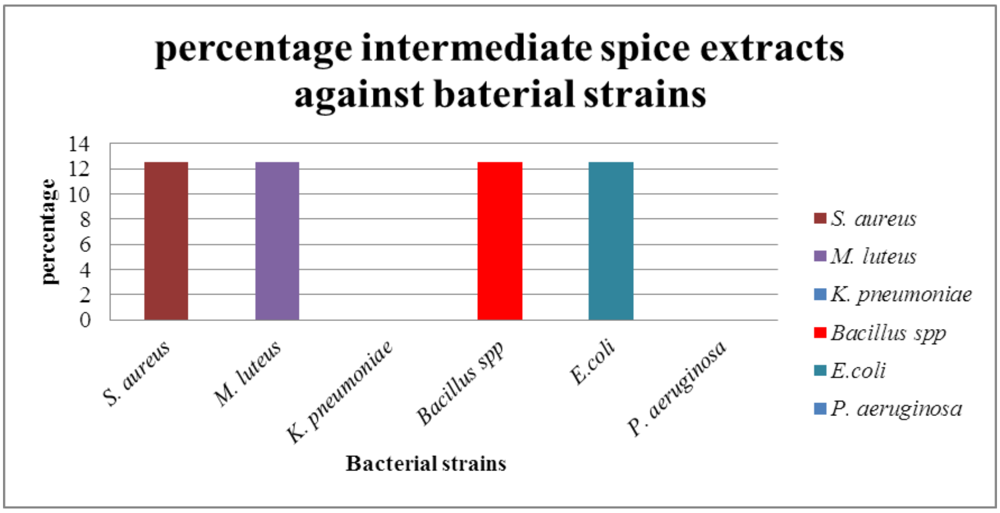

Fig 9: Percentage intermediate susceptibility of spices extracts against bacterial strains

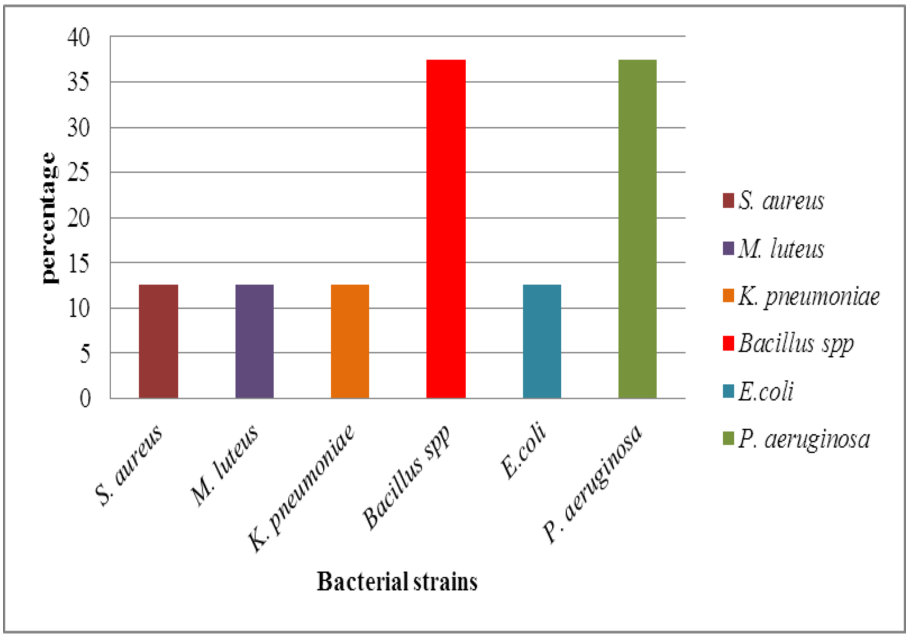

Fig 10: Percentage resistivity of spices extracts against bacterial strains

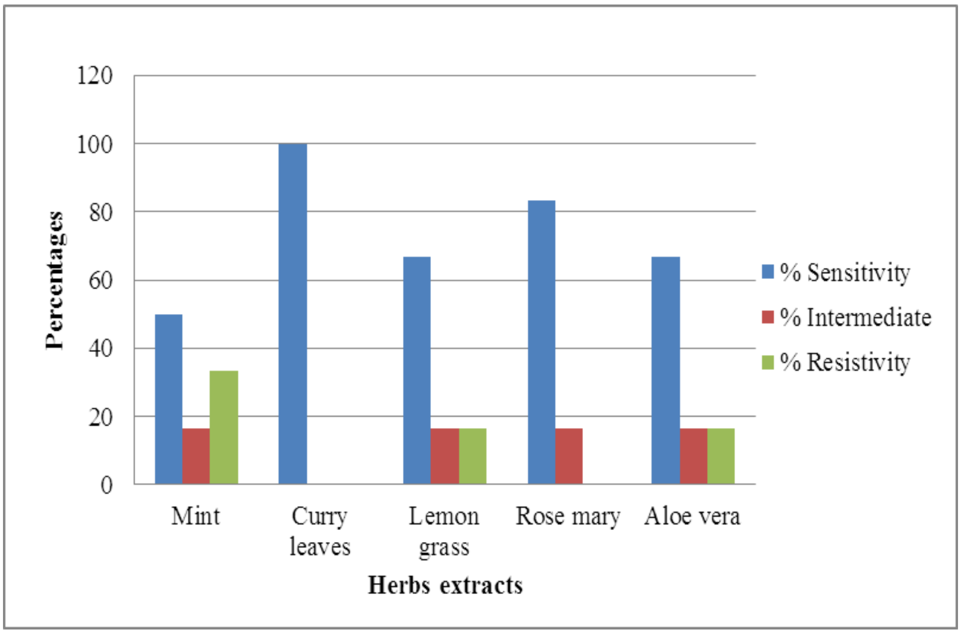

Fig 11: Zones of inhibition of herbs extracts against bacterial isolates

Antimicrobial susceptibility testing of ethanolic plant extracts against bacterial isolates showed that 6 isolated bacterial species showed 100% sensitivity to curry leaves extracts, 83.33% sensitivity to rosemary extracts, 66.67% sensitivity to lemon grass and *Aloe vera* extracts while 50% sensitivity to mint extracts.

All 6 bacterial isolates showed that 33.33% resistance to mint extracts, 16.67% resistance to lemon grass and *Aloe vera* extracts while curry leaves and rosemary extract showed no resistance.

All 6 bacterial isolates showed 16.67% intermediate susceptibility to mint, lemon grass, rosemary and *Aloe vera* extracts while curry leaves showed no intermediate susceptibility.

Antimicrobial susceptibility testing of bacterial isolates showed that *M. luteus* showed 100% sensitivity, *K. pneumoniae* and *E. coli* showed 80% sensitivity, *S. aureus* showed 60% sensitivity, *P. aeruginosa* showed 40% sensitivity while *Bacillus sp.* showed no sensitivity against all plant extracts. *S. aureus* showed 40% intermediate susceptibility, Bacillus sp. and *K. pneumoniae* showed 20% intermediate susceptibility while *M. luteus*, *P. aeruginosa* and *E. coli* showed no intermediate susceptibility against all plant extracts. Bacillus sp. showed 80% resistivity, *P. aeruginosa* showed 60% resistivity, *E. coli* showed 20% resistivity while *S. aureus*, *M. luteus and K. pneumoniae* showed no resistivity against all plant extracts.

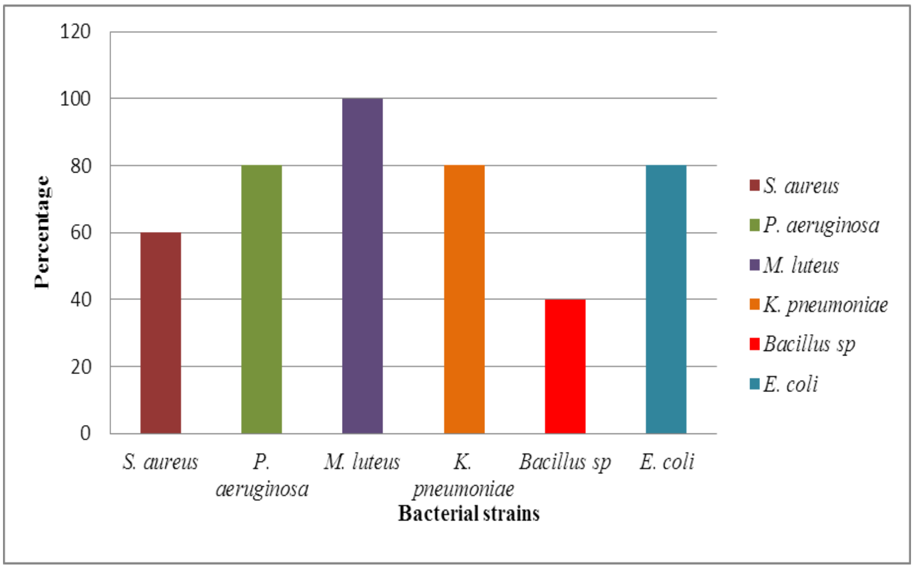

Fig 12: Percentage sensitivity of herb extracts against bacterial strains

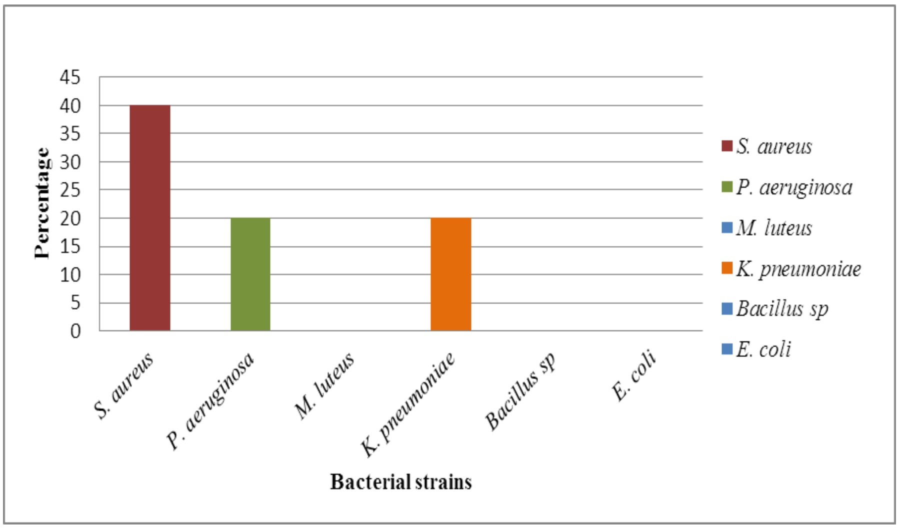

Fig 13: Percentage intermediate susceptibility of herb extracts against bacterial isolates

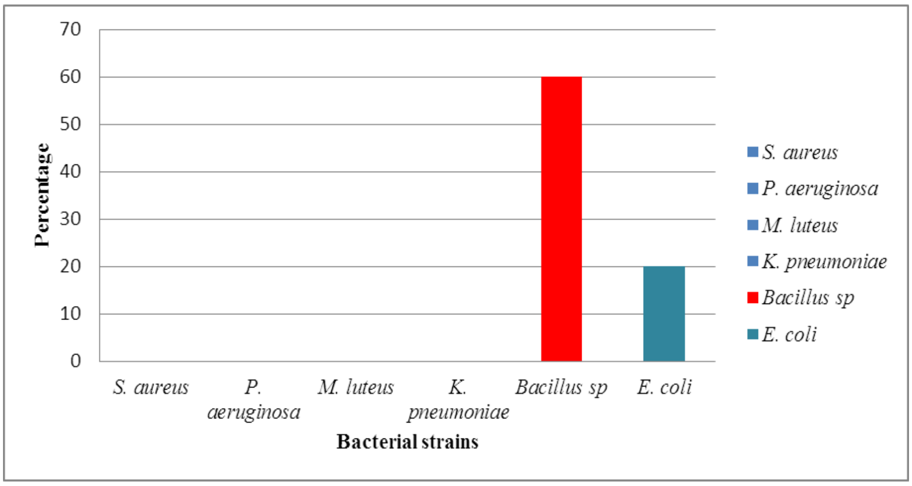

Fig 14: Percentage resistivity of herb extracts against bacterial isolates

DISCUSSION

Approximately 125 million people in the world wear contact lens. Even a minor complication establishes a major health problem in individuals wearing contact lens. One of the reasons for eye infection is the unhygienic handling of contact lens. During this study sixty random samples of lens were subjected to bacteriological study in order to isolate pathogenic bacteria from contact lens. These bacterial isolates were treated with thirty different extracts of plants, spices and herbs. Najia *et al* reported that 65% - 89% of people had infection due to contaminated storage lens cases (Najia *et al.*, 2008).

In this study, bacterial count of 54.52 from various categories of contact lens. Mean bacterial count was found to be greatest in people having eye infection with a bacterial count of 78.90 while least bacterial load of 33.60 was shown by students wearing contact lens. 51.05 of bacterial load were present in the lens sample taken from local market. It was seen that *E. coli* was the most isolated bacteria with a percentage of 36% while *M. luteus* was the least bacteria found in contact lenses. Statistical analysis showed level of bacterial contamination from contact lenses to be significantly different i.e. (P<0.05).

Seventeen leaf extracts of various plants were also observed for antibacterial activity. It was observed that 100% results were exhibited by bacopa against all bacteria with a largest zone of inhibition of 40mm against *S. aureus*. *T. kalaivani et al* reported that ethanolic extract of bacopa showed a potential activity against *K. pneumoniae, E. faecalis. E. coli* and similar results were reported by Ghosh *et al* (2007). In the study conducted by *T. kalaivani* no activity was exhibited against *S. aureus* and *E. coli* but in our study maximum sensitivity was shown against *S. aureus*.

Amla, Jamun, pomegranate and kachnar showed maximum effectiveness i.e. 83.33% against all bacterial isolates. Maximum inhibitory zone was shown by amla against *S. aureus* with inhibition zone of 40mm while *K. pneumoniae*, *E. coli*, Bacillus sp showed inhibitory zones of 35mm, 30mm and 30mm respectively. Intermediate inhibitory zone of 13mm was exhibited by *M. luteus* against amla extract. Jamun, pomegranate and kachnar extracts were sensitive against all bacteria but *M. luteus* showed resitance against jamun extract and *K. pneumoniae* showed resistivity against kachnar extract. Intermediate inhibitory zone of 14mm was exhibited by *P. aeruginosa* against pomegranate extract. Antibacterial activity of amla on both Gram positive and negative bacteria has been reported earlier by Ahmad *et al* (2010).

Khanna and Nag (1973) reported the potential activity of aqueous extracts of emblica against different bacteria including *E. coli, S. aureus, C. albicans, M. tuberculosis*. Antibacterial activity of emblica is mainly due to the presence of flavonoids that are phenolic structures (Ganguly D.K, 2003).

All the previous studies reported maximum activity of emblica extract against *S. aureus*. The ethanolic, methanolic extract of jamun showed activity against pathogenic bacteria like *S. aureus, B. subtilis, P. aeruginosa, K. pneumoniae, E. coli* (Jeethu *et al.*, 2012). 66.67% of sensitivity was exhibited by lemon, terminalia, cassia, mulberry, orange, sukhchain, papaya and neem leaf extracts. Significant activity was shown by mulberry extract against Bacillus sp with an inhibitory zone of 40mm.

Saleem *et al* (2013) examined that leaf extract of mulberry showed maximum activity against *E. coli* with a zone of 26.3mm whereas Yogisha and Raveesha (2009) reported its activity against *S. aureus* with 22mm zone. In our study mulberry extract activity against *S. aureus* and *E. coli* of 17mm and 16mm respectively. A zone of inhibition of 35mm was made against *E. coli, S. enterica*, *K. pneumoniae* by papaya, sukhchain and cassia extracts. No inhibition zone was seen against *K. pneumoniae, P. aeruginosa*, *M. luteus*, Bacillus sp with neem, papaya, sukhchain, orange, mulberry, cassia, terminalia and lemon extracts. Intermediate zones of 14mm and 13mm were exhibited by mulberry and terminalia extracts. The extracts of different parts of terminalia and found that maximum activity was shown by bark extract against *E. coli, P. aeruginosa*, Bacillus sp whereas leaf extract showed no activity against gram positive bacteria. However in present study it was seen that leaf extract showed activity against gram positive bacteria including *S. aureus* and *M. luteus*.

Banana and withania leaf extracts showed 50% sensitivity against all bacteria while jasmine showed 16, 67% sensitivity. Maximum activity was exhibited against *S. aureus* and *K. pneumoniae* with an inhibitory zone of 20mm with banana extract. No inhibition zone was observed against Bacillus sp and *P. aeruginosa* by both extracts. Intermediate inhibition zone of 14mm against *K. pneumoniae* was shown by withania. All the bacterial strains showed resistivity to jasmine extract except for *P. aeruginosa* that was sensitive to this extract. Branjasif extract showed no effect to any of the bacteria. All the strains were resistant to this extract. C. Karaalp *et al* conducted a study to evaluate antibacterial activity of branjasif with various solvents and it was indicated that chloroform extract of branjasif showed activity against *S. epidermidis* and *S, aureus*. The antimicrobial activity is due to the presence of components like flavonoids and tannins (Cowan, 1999). The results of the present study were similar by Souza et al where ethanolic extracts showed no antibacterial activity.

E. coli and *S. aureus* showed the most effectively of 88.223% against all extracts while *K. pneumoniae* showed most resistivity to all extracts. Eight spices including cinnamon, cumin, turmeric, clove, fennel, black cardamom, black pepper and oregano were tested against bacterial isolates from contact lens. It was observed that 100% activity was shown by clove and fennel extracts against all the bacteria. Maximum inhibition zone of 27mm was shown by fennel against *Pseudomonas aeruginosa* while clove showed the largest zone of 25mm against *Klebsiella pneumoniae*.

Lo Cantore *et al* (2004) reported the antibacterial activity of fennel essential oil against these bacteria. Abdelaaty *et al* also reported activity of fennel extracts against both Gram positive and Gram negative bacteria especially against *E. coli* and *P. aeruginosa*. Maximum antibacterial activity of ethanolic extract of clove was shown against *P. aeruginosa* and least against *Staphylococcus aureus* whereas in the present study clove showed largest inhibition zone against *K. pneumoniae*. Black pepper and oregano extracts showed 83.33% effectiveness against all bacterial isolates. Oregano showed an inhibitory zone of 25mm against *K. pneumoniae* while black pepper showed 22mm inhibition zone against same bacteria. An intermediate inhibitory zone of 14mm was shown by *P. aeruginosa* was shown by black pepper extract against *S. aureus* and no zone was observed for *P. aeruginosa* for oregano extract. The inhibitory activity of oregano against *E. coli, Listeria monocytogenes, Micrococcus luteus, Bacillus subtilis, Bacillus cereus, S. aureus, K. pneumoniae,* and *Proteus vulgaricus*. In other studies antibacterial activity of oregano was exhibited against MRSA Naik and Tariq, 2006). Antibacterial activity of oregano is also reported against *E. coli* strain *E. coli* O157:H7 by Moreira *et al*., 2005). S.K. Shiva reported that black pepper extract showed more activity against gram positive bacteria than gram negative bacteria but present study showed almost similar results for both types of bacteria.

66.67% of sensitivity was shown by black cardamom and cumin against *S. aureus, K. pneumoniae, E. coli* where black cardamom showed a zone of inhibition of 20mm against *S. aureus* and *E. coli* and no zone was shown by *P. aeruginosa* and *M. luteus*. Gilani *et al* 2010 reported black cardamom extracts activity against *B. subtilis, S. aureus, P. aeruginosa* and *S. cerevisiae*. Cumin extract indicated maximum activity against *S. aureus, M. luteus and P. aeruginosa* with 30mm inhibition zone while *bacillus sp.* showed no zone of inhibition and an intermediate zone of 13mm was shown against *E. coli*. Aqueous extract of cumin is reported to have antimicrobial activity against *P. aeruginosa* and *E. coli* with an inhibitory zone of 10mm and 18mm respectively (Stefanini *et al*., 2003) while methanolic extracts have been reported to inhibit the growth of *S. aureus* up to 11mm and *E. coli* up to 10mm (Das *et al*., 2012).

In present study ethanolic extract of cumin showed activity against both Gram positive and Gram negative bacteria. Cinnamon and turmeric extracts exhibited 33.33% of sensitivity against *S. aureus* and *E. coli* with an inhibitory zone of 15mm and 18mm respectively whereas greatest sensitivity was shown against *M. luteus* by turmeric extract with a zone of inhibition of 35mm and 20mm zone was observed against *S. aureus*. No inhibitory zone was observed against *K. pneumoniae*, Bacillus sp. and *P. aeruginosa* by both extracts. An intermediate inhibitory zone of 14mm and 13mm was shown against *M. luteus* and *P. aeruginosa* by cinnamon extract while 17mm of intermediate zone was exhibited by *K. pneumoniae* by turmeric extract.

Indu *et al* 2006 reported that cinnamon extract exhibited antibacterial activity against both *E. coli* and *B. subtilis* whereas Gur *et al* 2006 examined turmeric extract activity against both these bacteria. Turmeric is effective against *S. aureus, E. coli* and *B. subtilis* due to the presence of cucuminoid which is a phenolic compound reported by Chandara *et al* (2005). Better results were shown by ethanolic extracts as compared to aqueous extracts which also agrees with the present study (Cowan, 1999). It was seen that among all the bacterial isolates *K. pneumoniae* showed the most sensitivity i.e.87.5% against all spice extracts and *P. aeruginosa* showed least sensitivity of 50% against the extracts. Bacillus sp showed most resistivity.

Five different herbs i.e. mint, curry leaves, lemon grass, rosemary and *Aloe vera* were tested for their antibacterial activity against all six bacteria isolated from contact lens and it was observed that curry leaves extract showed 100% sensitivity against all bacteria with s maximum inhibitory zone against *S. aureus*. Rosemary extract exhibited 83.33% sensitivity to all isolates except for *S. aureus* where it exhibited an intermediate inhibitory zone of 13mm. *Aloe vera* and lemon grass showed 66.67% sensitivity against *S. aureus, S. enterica, M. luteus, K. pneumoniae* and *E. coli* with inhibitory zone of 25mm against Bacillus sp. of *Aloe vera* extract and 22mm against *M. luteus* of lemon grass extract. Intermediate inhibitory zone was shown by lemon grass extract against *S. aureus* i.e. 13mm while 14mm inhibition zone was exhibited by *Aloe vera* extract against *K. pneumoniae*. No inhibitory zone was shown against *P. aeruginosa* by *aloe vera* extract. *M. luteus* showed 100% sensitivity against all extracts while Bacillus spp. showed 60% sensitivity against all extracts.

Fleming reported that a significant activity of mint is due to the presence of components such as limonene, menthofuran, menthyl acetate, menthone and menthol. The present study of mint is correlated with the observation of Bupesh *et al* 2007 where he examined the antibacterial activity of mint leaf extract against *S. aureus, P. aeruginosa* and *K. pneumoniae sp*. Curry leaf extract showed most susceptibility against *S. aureus* that plays an important role in skin diseases (Barsi and Fan, 2005).

It was reported that the roots of curry leaves exhibit antimicrobial properties that is due to the presence of carbazole alkaloids. Similar to this study another study was conducted with methanol extract of curry leaves that was reported to show effectiveness against all these bacterial isolates (Malwal and sarin, 2011).

Lemon grass exhibited antimicrobial activity against these bacteria. Studies of other researchers indicated that extract of lemon grass are more effective against Gram positive bacteria than Gram negative bacteria but the present study shows good effectiveness against Gram negative bacteria as well (Hinelumathy C.K, 2011; Bassole I.H.N, 2003).

Different researchers suggested antibacterial activity of lemon grass essential oils and concluded that Enterococcus was most sensitive whereas *P. aeruginosa* was most resistant (Olivera *et al.*, 2000). Certain reports on rosemary extracts suggested that the presence of phenolic compounds in rosemary is usually responsible for its antibacterial activity (Fernandez *et al*., 2005).

Essential oil of rosemary effects the growth of yeasts and bacteria. Most sensitive bacteria to rosemary extract are *S. aureus* but in present study *S. aureus* forms an intermediate inhibitory zone with rosemary extract. *Aloe vera* extracts showed sensitivity against *S. aureus* with inhibitory zone of 18mm in a study conducted by Agarry *et al* 2005 but in present study 21mm inhibitory zone was formed against *S. aureus*.

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