Antibacterial activities of the combined extracts of *Eucalyptus grandis*, *Moringa oleifera*, *Punica granatum* and *Syzgium aromaticum* against pathogenic organisms and formulation of sanitizer

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***ABSTRACT: Plants have been known for their ability to synthesize a wide variety of chemical compounds long before recorded history. These bioactive compounds defend plants against attack from predators such as insects, fungi and herbivorous animals. In traditional medicine, plants have been used to prevent and cure infectious conditions and diseases caused by pathogenic microorganism. Therefore this study aimed to investigate the combined effect of Eucalyptus grandis* (Eu, E), *Moringa oleifera* (Mo, M), *Punica granatum (*Po, P) *and Syzygium aromaticum (*Cl, C) *crude plant extracts against pathogenic organisms and using the extract with the highest synergistic or additive effect against the selected bacteria strains to formulate sanitizer as a preventive measure against the spread of disease resulting from these bacteria. This study was conducted against four bacteria strains, Klebsiella pneumonia* (KP), *Proteus mirabilis* (PM), *Staphylococcus aureus (SA) and Staphylococcus epidermidis (SE) using disc diffusion method.* **The interaction between the two and three combination extracts revealed that they were synergistic, additive and antagonistic against the four selected strains of bacteria. The highest additive effect was observed in KP and PM when there was a combination of PCE while a synergistic effect was observed in KP when there was a combination of Po & Mo. Staphylococcus epidermidis showed significant inhibitory effect to all the single extract used but displayed antagonistic effect to all the two and three combination extracts. Combination of Eu & Cl and the single extract of pomegranate showed indifferent effects against Staphylococcus aureus. The formulated sanitizers, however, were effective against the hand microbiome in volunteer samples with no side effects on human tissue.**

Keywords: Plant extracts, Disc diffusion, Bacteria, Antimicrobial testing, Sanitizer.

INTRODUCTION

Natural products derived from plant have proven to be the richest source of medicinal compounds long before recorded history. These products are substances produced by living organisms found in nature or by chemical synthesis (Samuelson, 1999). Natural products have had major impact in the treatment of various diseases including high blood pressure, heart disorders, asthma, pains and cancer (Manuchair, 2002). Almost all the anti-cancer drugs are naturally produced either from microbes or plants for example Vinblastine (Gilani *et al*., 1999; Gopal, 2006).In traditional medicine, plants have been used to prevent and cure infectious conditions and diseases caused by pathogenic microorganism.Microorganisms are ubiquitous in nature and are beneficial to life. They live almost in all habitats and are able to grow on any surfacebe it home or workplace (Filho *et al*., 1985; Kramer *et al*., 2016). Although most bacteria are harmless, some of them are pathogenic especially in people with weakened immune system. These pathogens spread in our environment among people with direct or indirect contact on hands as well as inanimate objects (Mathai *et al*., 2010).

According to Larson (2001), bacteria may stay on the hand for as long as a month depending on the physical condition of human hand (Larson, 2001). Fingernails have greater capacity to harbor varieties of microorganisms. Sullivan and Ellen (2003) reported that artificial nails habor pathogenic bacteria such as *Pseudomonas aeruginosa*, *Serratiam arcescens* than natural nails (Sullivan and Ellen, 2003). Other microorganisms found on the nails are *Staphylococcus Acinetobacter, Enterobacter, Klebsiella, Aeromonas, Trichophyton, Epidermophyton, Acremonium, Scopulariopsis, Cladosporium, Candida* and *Rhodotorula*. These hands are needed to protect from bacterial pathogens in order to avoid transmission of genes and nosocomial infections. Hand sanitizer is a liquid used to decrease infectious agents on the hands. Alcohol based sanitizer are preferable to hand washing and effective in killing microorganisms than ordinary soap and water especially in healthcare setting (Boyce and Pittet 2002; Bolon, 2016).Over the years, plants rich in wide variety of secondary metabolites including alkaloids, terpenoids, phenylpropanoids, tannin, saponin and flavonoids have been found to have a powerful potential for antimicrobial activities. *Eucalyptus grandis* is commonly known as flooded gum or rose gum in Queensland (Boland *et al*., 2006). It belongs to the family of Myrtaceae and indigenous to Australia. To the best of my knowledge, little or no report have been made on it crude extract. However, Soyingbe *et al* have reported the antimicrobial activity of its essential oil against antibiotic resistance organisms affecting both Gram positive and Gram negative organisms (Soyingbe *et al*., 2013). *Moringa oleifera* is the most widely cultivated species of the genus *Moringa* and it belongs to the family Moringaceae. It exhibit characteristics such as antihelmintic (Bondya *et al*., 2002), antibiotic, detoxifier, antipyretic (Singh and Kumar, 1999), acrid, bitter (Oliveira, 1999) outstanding immune builder and also used in many developing countries to treat malnutrition and malaria. The plant has also been reported to possess antimicrobial activity against wide array of pathogens including *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* (Crapper *et al*., 1973; Nikkon *et al*., 2003; Rahman *et al*., 2009). *Punica granatum* commonly known as pomegranate is one of the first domesticated fruits that have been cultivated in different countries throughout the world for medicinal purpose (Morton, 1987). It is an ancient fruit with distinctive characteristics such as antioxidant, anti-cancer, anti-inflammatory, bactericidal, and fungicidal properties.

Several studies have investigated the antibacterial activity of different part of pomegranate against a diverse range of microorganisms and have found out that it inhibit the growth of certain pathogenic Clostridia species, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Bhadbhade *et al*., 2006; Menezes *et al*., 2006; Amy *et al*., 2013; Dilshad *et al*., 2016).*Syzygium aromaticum* which is also known as clove is one of the most valuable sweet-flavored spice found in the family of Myrtaceae. It has been used for centuries as food preservative and for many medicinal purposes. Several studies have reported the antibacterial activity of clove against a diverse range of both gram negative and gram positive bacteria (Cai and Wu, 1996; Burst and Reinders, 2003; Fu *et al*., 2007; Demirpek *et al*., 2009; Gupta *et al*., 2014) and has also been found to exhibit characteristics which include anti-oxidant, anti-inflammatory (Kim *et al*., 1998), anti-mutagenic (Miyazawa *et al*., 2003), anti-viral (Hessein *et al*., 2000) and etc. Considering that the emergence of drug resistant bacteria is one of the most serious threats constraining successful treatment of microbial infections this study have therefore aimed to investigate the combined effect of *Eucalyptus grandis, Moringa oleifera, Punica granatum and Syzygium aromaticum* crude plant extracts against pathogenic organisms and using the extract with the highest synergistic or additive effect against the selected bacteria strains to formulate sanitizer as a preventive measure against the spread of disease resulting from these bacteria.

MATERIALS AND METHODS

Collection of samples

The fresh leaves of *Eucalyptus grandis*, *Moringa oleifera*, pericarp of *Punica granatum* and dried bud of *Syzygium aromaticum* were collected from Botanical garden Noida sector 37, India.

Methanolic extraction of crude drug from plant materials

Five grams of each dry plant materials (eucalyptus and moringa leaves, with pericarp of pomegranate and dried bud of clove) was weighed and were added separately to 50 ml of methanol solution in the ration of 9:1 (9 parts of methanol: 1 part of water). The mixture was placed in water bath and heated at 60°C for 1 hour. The mixture content was then filtered using Whatman No. 2 filter paper and the crude drug extract obtained was stored for further experimentation (Joshi *et al*., 2008; Mithun *et al*., 2015).

Preliminary screening of the extract for antimicrobial activity

Preparation of culture media

The culture media used were prepared according to the manufacturers' instruction and were sterilized by moist heat in an autoclave at 121°C for 15minutes. They were allowed to cool and then poured into sterile petri dishes and allowed to solidify.

Nutrient agar: Peptone, beef extract, and Sodium Chloride were in sterile distilled water and dissolved properly and pH of the Medium was adjusted to 6.8±0.2 then Agar-Agar was added into the Medium. The Medium was sterilized by autoclaving at 121°C for 15minutes. The medium was allowed to cool before pouring into petri dishes.

Bacterial strains

Klebsiella pneumonia (Gram –ve), *Proteus mirabilis* (Gram –ve), *Staphylococcus aureus* (Gram +ve) and *Staphylococcus epidermidis* (Gram +ve) pathogens were selected for evaluation of antimicrobial activity of the extract.

Antibiotics

The antibiotics-standard ciprofloxacin (10mg/ml), erythromycin (10mg/ml) and resteclin (100mg/ml) were used.

Antimicrobial screening test of the extracts

The antimicrobial activity of crude extracts (clove, moringa, eucalyptus and pomegranate) were tested singly and in combination using four bacteria strains. Two strains of Gram negative bacteria (*Klebsiella pneumonia, Proteus mirabilis)* and two strains of Gram positive bacteria *(Staphylococcus aureus* and *Staphylococcus epidermidis)*. The method used was similar to Kirby-Bauer disc diffusion method of the antibiotic sensitivity test according to CLSI guidelines (2014).

For the antimicrobial activity of singly extract, four petri plates where used for the four microorganisms and each test plate was divided into six sessions, four for the extracts and two for controls. The table is shown below;

Table 1: Shows individual extract plate division

Pomegranate (Po)	Clove (Cl)	Eucalyptus (Eu)	Moringa (Mo)	Positive control	Negative control

When two extracts where combined, four petri plates were prepared for the four microorganisms and each test plate was divided into six sessions. Combined extracts solution were prepared in 1:1 according to the table below. At this stage control plate was prepared separately in order to get the better estimate of the extract.

Table 2: Show two combinations

Cl & Eu	Mo & Po	Eu & Po	Cl & Mo	Cl & Po	Eu& Mo

For the last combination same number of plate aforesaid was prepared for the four microorganisms and each plate was divided into four sessions and labeled as follows and the extracts were mixed in 1:1:1

Table 3: Shows three combinations

PCE	CME	EPM	PMC

The plates were inoculated with 50ul bacterial suspension spread uniformly with the help of glass spreader in an aseptic condition and incubated at 37°C for 24 h. Disc were prepared using Whatman filter paper no 2, separately loaded with the methanolic extract of the prepared samples and were placed onto the center of the separate sessions of the inoculated plates. The plates were incubated at 37°C for 24 hours. Next day, the diameter of zone of inhibition was then measured to determine the effectiveness of the extracts against microorganism in terms of zone of inhibition. The higher the zone of inhibition, the more effective is the combined extract (Kokare, 2008). Combination with maximum activity

was then selected for formulation.

Formulation of Sanitizer

The combined extract of *Eucalyptus grandis* leaves, pericarp of *Punica granatum* and dried bud of *Syzygium aromaticum* that shows maximum activity was the major constituent of the sanitizer. Acrypol 956 was added to deionized water in a beaker with constant stirring. After uniform mixing, all the extracts were added to Isopropylalcohol, poured into the beaker that contain acrypol956 and mixed for 60 minutes. Glycerin was added to the mixture to adjust the pH of the formulation to pH 7-7.5. Finally 0.5% of perfume was mixed with slow stirring to obtain uniform product.

Table 4: Composition of alcohol-based herbal hand sanitizer formulation

No. of Items	Ingredient	Quantity taken	Purpose
1	Deionized water	11.5 ml	Diluent
2	Acrypol956	0.15 g	Gelling agent
3	Isopropylalcohol (IPA)	18 ml	Vehicle
4	Clove	1 ml	Antibacterial
5	Eucalyptus	1 ml	Antibacterial
6	Pomegranate	1 ml	Antibacterial
7	Glycerin	0.6 ml	Humectant

Test for effectiveness of the herbal hand sanitizer

The efficacy of the formulated herbal hand sanitizer was aseptically performed on volunteer by using a rinse wash method. Three palm washes were taken for a single volunteer on three consecutive days. On the first day, sterile water was used to rinse palm for 10 seconds and the hand wash sample was collected on a sterile petri plate under sterile condition. 50ul of the hand wash sample was plated on an agar plate and incubated at 37°C for 24 hours. On the second day and third day, the herbal hand sanitizer and synthetic hand sanitizer which serve as control were used respectively.

RESULTS

Preliminary screening of the extract

The initial antimicrobial testing of the 4 selected plant materials were carried out singly and in combination in order to check it effectiveness against the selected bacteria strains. After incubation the results observed in terms of zone of inhibition formed on the test plate were used to determine the synergetic, additive and antagonist effect of the extracts. All the singly extracts did not produce zone of inhibition on all the four selected bacteria strain. Clove produced the highest zone of inhibition on *Klebsiella pneumonia* at 12.5mm followed by eucalyptusand Pomegranate at 10.5mm. Moringa did not show any activity on *Klebsiella pneumonia*. Eucalyptus produced the highest inhibitory activity on *Proteus mirabilis* with inhibition zone diameter of 8.6mm while the lowest inhibition was produced by Clove with an inhibition zone diameter of 7.5mm.In the case of *Staphylococcus aureus*, extract of clove, eucalyptus and moringa did not produce any activity against the organism. However, inhibition zone diameter of 20.5mm was produced by pomegranate. Pomegranate showed highest inhibitory activity to *Staphylococcus epidermidis* at 21.5mm while lowest inhibition was produced by moringa with an inhibition diameter of 10.7mm. The result of the sensitivity test of the singly extracts are presented in the table below.

Table 4: Shows antibacterial activity of individual extract of the selected plant materials based on zone of inhibition in diameter (mm)

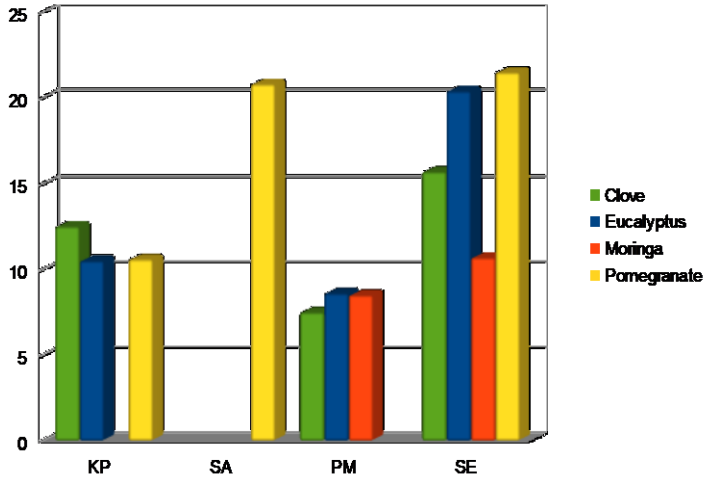
Bacteria Strains	Cl	Eu	Mo	Po
Klebsiella pneumonia	12.5	10.5	--	10.5
Proteus mirabilis	7.5	8.6	8.5	--
Staphylococcus aureus	--	--	--	20.5
Staphylococcus epidermidis	15.7	20.4	10.7	21.5

Key: Cl: clove Eu: eucalyptus Mo: moringa Po: pomegranate

Figure 1: Effectiveness of single extracts based on zone of inhibition in diameter

Two combination extracts

The interaction between the two and three combination extracts revealed that they were synergistic, additive and antagonistic against the four selected strains of bacteria. Combined extract of Mo & Po produced the maximum inhibitory activity on *Klebsiella pneumonia* at 18.6mm while



the lowest inhibition was produced by Eu & Po at 14.3mm. The combined extract of Po & Cl produced the highest inhibitory activity on P*roteus mirabilis* with inhibition zone diameter of 16.6mm while the lowest inhibition was produced by Eu & Mo with an inhibition zone diameter of 9.4mm. The inhibition zone diameter produced in the case of *Staphylococcus aureus* varied, it ranges from 17mm-21mm.The combined extract of Eu & Cl produced the highest inhibitory activity at 20.5mm and lowest at 17.1 by Eu& Mo and Po & Mo. Combined extract of Po & Moshowed highest inhibitory activity against *Staphylococcus epidermidis* at 18.5mm while lowest inhibition was produced by Cl & Mo with an inhibition diameter of 12.5mm.

Table 5: Zone of Inhibition (diameter in mm)

Bacteria Strains	Cl & Eu	Mo & Po	Eu & Mo	Cl & Po	Eu & Po	Cl & Mo
KP	17.4	18.6	16.5	17.4	14.3	16.5
PM	10.5	10.5	9.4	16.6	14.6	13.5
SA	20.5	17.1	17.1	18.2	17.5	19.4
SE	14.3	18.5	14.5	17.2	17.2	12.5

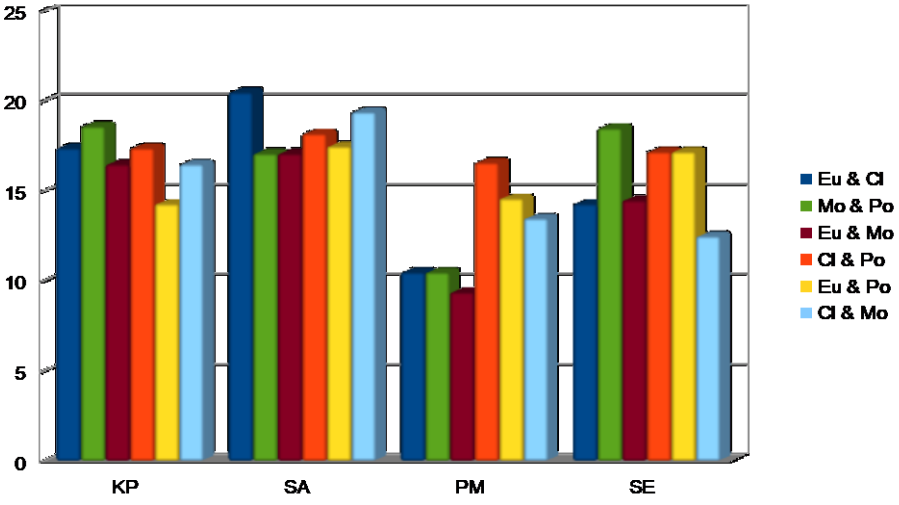


Figure 2: Effectiveness of two combination extracts based on zone of inhibition in diameter

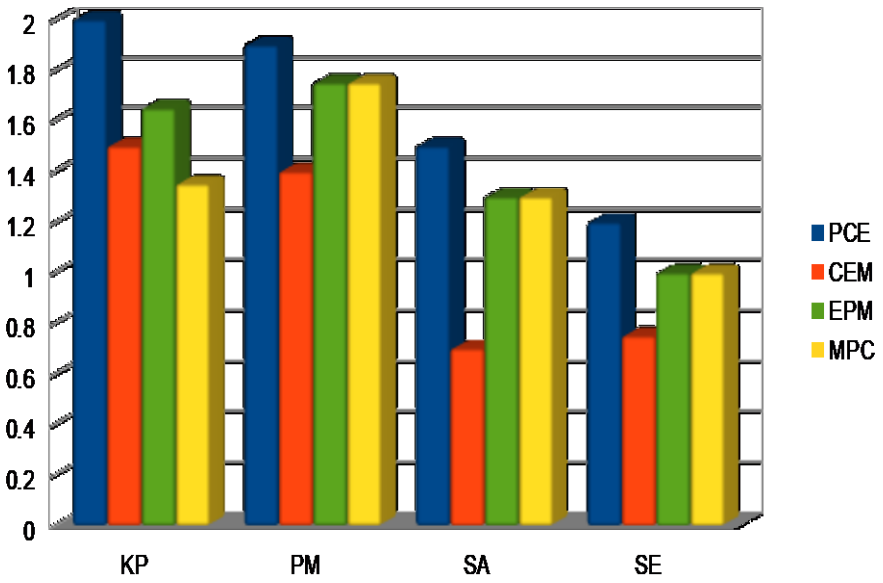
All the three combination extracts showed significant inhibitory activities against all the four selected bacteria strains. Combined extract of PCE

produced the maximum inhibitory activity on *Klebsiella pneumonia* at 20.5mm while the lowest inhibition was produced by MPC at 13.5mm. Also, combined extract of PCE produced the highest inhibitory activity on P*roteus mirabilis* with inhibition zone diameter of 19.5mm and the lowest inhibition was produced by CEM with an inhibition zone diameter of 14.4mm. The inhibition zone diameter produced in the case of *Staphylococcus aureus* varied, it ranges from 7.5mm-12.5mm.The combined extract of PCE produced the highest inhibitory activity at 12.4mm and lowest at 7.5mm by CEM Combined extract of PCEshowed highest inhibitory activity against *Staphylococcus epidermidis* at 15.6mm while lowest inhibition was produced by CEM with an inhibition diameter of 9.4mm.

Table 6: Shows antibacterial activity of the three combination extracts against pathogenic bacteria based on zone of diameter in mm

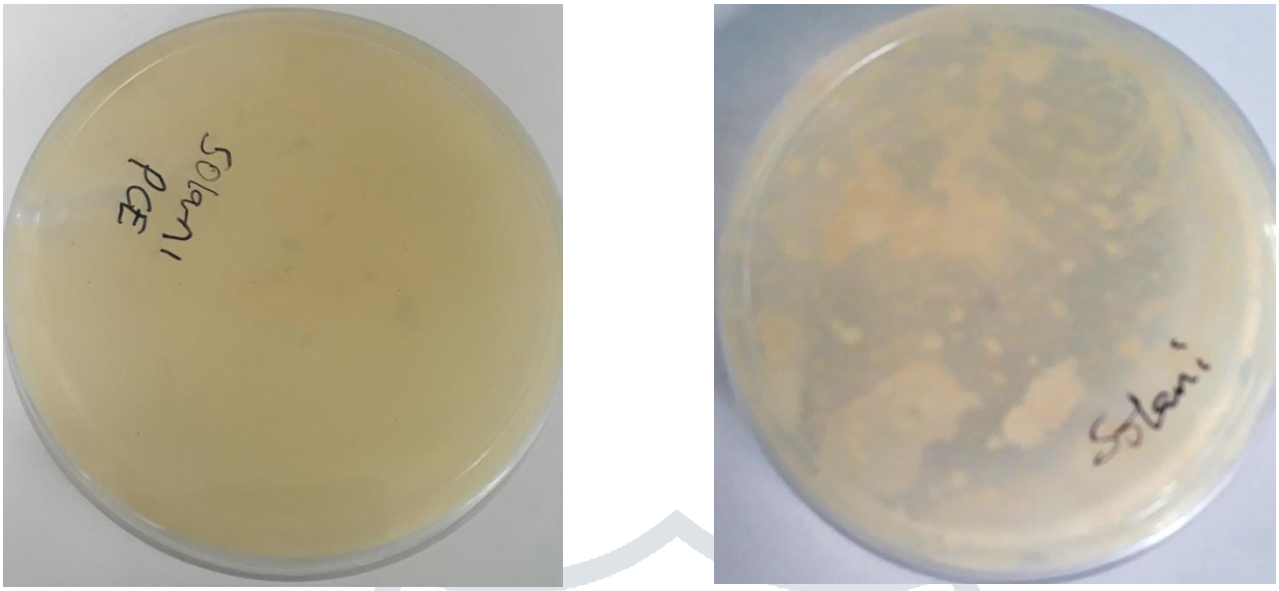
Bacteria Strains	PCE	CEM	EPM	MCP
KP	20.5	15.4	16.5	13.5
PM	19.5	14.4	18.5	17.5
SA	12.4	7.5	11.3	10.3
SE	15.6	9.4	12.5	13.5

Figure 3: Effectiveness of three combination extracts based on zone of inhibition



Effectiveness of formulated sanitizer

When the combination of PCE was used to formulate herbal hand sanitizer, there was growth reduction in bacterial flora obtained after the use of herbal hand sanitizer and synthetic hand sanitizer. Sterile water showed four different types of bacteria growth and the herbal hand sanitizer showed a single bacteria growth while the commercially available hand sanitizer showed three bacteria growth. Highest reduction was observed in the herbal hand sanitizer. The percentage of reduction shown by herbal hand sanitizer was 75% while it was found to be lesser in the case of synthetic hand sanitizer which shows 25% reduction rate.



Herbal hand sanitizer plate **Commercially available sanitizer plate**

Figure 4: Antimicrobial activity of formulated sanitizer against hand microbiome

DISCUSSION

To the best of my knowledge little or no report has been made on the combined effect of *Eucalyptus grandis, Moringa oleifera, Punica granatum and Syzygium aromaticum* crude plant extracts against pathogenic organisms. According to literature, none of the plant derived chemicals with antimicrobial properties have been used clinically as antibiotics despite it abundance (Gibbons, 2004). In this study effects of single and combined extracts of 4 selected plant materials were verified. Several studies have reported the antibacterial activity of clove against a diverse range of both gram negative and gram positive bacteria (Cai and Wu, 1996; Burst and Reinders, 2003; Fu *et al*., 2007; Demirpek*et al*., 2009; Gupta *et al*., 2014) and this activity has been attributed to the presence of phytochemical constituents such as eugenol, eugenyl acetate, β-caryophyllene, 2-heptanone, (Chaieb *et al*., 2007), gallic acid, oleanolic acid, ellagicacid,phenylpropanoides (Cai and Wu, 1996) acetyl-eugenol, methyl salicylate, iso-eugenol, methyl-eugenol, (Yang *et al*., 2003). These phytochemicals are capable of reacting with cell membrane phospholipids, change their permeability and also denature proteins (Cai and Wu, 1996; Burst and Reinders; 2003 Chaieb *et al*., 2007; Gupta *et al*., 2014). Shailesh (2015) reported that methanolic extract of clove exhibit significant inhibitory activity against Gram-negative and Gram-positive bacteria. This finding support the present study where methanolic extract of clove showed inhibitory activity to Gram-negative bacteria; *Klebsiella pneumonia, Proteus mirabilis* (KP & PM) and Gram-positive bacteria *Staphylococcus epidermidis* (SE) but resistance to *Staphylococcus aureus* (SA) with no zone of inhibition. However another study reported that SA showed inhibitory activity to ethanolic and methanolic extract in terms of zone of inhibition which is in contrast to this finding (Aneja and Joshi, 2010). This difference might be due to the use of clove oil or pure extract instead of crude extract.

Sensitivity to the extracts differ significantly among the test organisms. Pomegranate extract was the most effective extract that showed best inhibitory activity against *Staphlococcus aureus*. It zone of inhibition is larger in size for SA & SE compare to erythromycin, one of the antibiotic used as positive control. Significant sensitivity was observed in KP and no activity against PM. Similar to this finding, several studies have demonstrated the antimicrobial activity of different part of pomegranate against highly pathogenic and antibiotic resistance organisms including Staphylococcus, Streptococcus, Klebsiella, *E.coli* and Proteus species (Bhadbhade *et al*., 2006; Menezes *et al*., 2006; Amy *et al*., 2013; Janani and Estherlydia, 2013; Dilshad *et al*., 2016; Ashraf *et al*., 2017). According to Menezez *et al* antimicrobial property of pomegranate has been attributed to the presence of primary constituents that include ellagitannin and punicalagin (Menezez *et al*., 2006). It may also be indicative of the presence of phytochemicals such as phenol, saponin and tannin. This is in agreement with previous reports by Singh *et al* who revealed that presence of hydrolysable tannins and polyphenolics most especially punicalagin and gallagic acid in the pomegranate extract contribute to it antimicrobial activity (Singh *et al*., 2002). Furthermore, Janani and Estherlydia, suggested that antimicrobial activity of pomegranate may be attributed to polyphenol structures reason because, polyphenols may affect the bacterial cell wall, inhibit enzymes and denature proteins (Janani and Estherlydia, 2013). Moreover, Puupponen-Pimia *et al* have reported the mutagenic property of phenol against mutant strain E.coli that was strongly affected by this compound (Puupponen-Pimia *et al*., 2001).

Result of the present study shows that methanolic leave extract of *Eucalyptus grandis* at concentration of 100mg/ml produced inhibitory activity against *Staphylococcus epidermidis, Klebsiella pneumonia* and *Proteus mirabilis.* Previous study by Soyingbe *et al* found that essential oil of *Eucalyptus grandis* showed significant inhibitory effect against both Gram positive and Gram negative bacteria and also inhibited the growth of antibiotic resistance organisms such as *Staphylococcus aureus*, and *Klebsiella pneumonia* at a concentration dependent manner (Soyingbe *et al*., 2013), but in contrast to this finding, *Eucalyptus grandis showed no activity against staphylococcus aureus.* Flavesone, grandinol, leptospermone, euglobals G8-G12, Isoleptospermone and euglobal-11c present in the leaves of *Eucalyptus grandis* may be responsible for its antimicrobial activity. Also out of the four selected plant materials, saponins are highly present in the methanolic extract of *Eucalyptus grandis* which may be responsible for its antimicrobial activity.

Despite the fact that individual extract of *Moringa oleifera* showed weak antimicrobial activity against the selected bacteria strains this study

however shows different effects that resulted from the combination of the crude plant extracts. The interaction between the two and three combination extracts revealed that they were synergistic, additive and antagonistic against the four selected strains of bacteria.

Susceptibility pattern of the combined extracts

Few studies have reported the effect of combining antibiotics with essential oils (Fadila and Tajelmolk, 2015), antibiotics with crude plant extracts (Ofokansi *et al*., 2012) and also combination of essential oils (Cristiana and Ancuta, 2016) but this is the first time a report would be made on the effect of combining *Eucalyptus grandis, Moringa oleifera, Punica granatum* and S*yzygium aromaticum.* The interaction study shows that extract of Po & Mo and PCE combination have a promising antimicrobial activity against gram negative bacteria *Klebsiella pneumonia* since synergistic and additive effects was observed respectively. Antagonistic effects were observed in the combination of Eu & Po, Eu & Cl, Po & Cl while extracts of Eu & Mo, Cl & Mo produced moderate additive effect. Combination of CME, EPM and MPC displayed an antagonist effect against *Klebsiella pneumonia.*

A synergistic effect was observed in PM when there was a combination of Po & Cl. PCE, MPC and EPM display an increase zone of inhibition compared to the combination of two extracts which implies additive effect. Eu & Cl, Eu & Mo with Po & Mo manifest similar behaviors against *Proteus mirabilis*. They produce zones of inhibition that doesn't differ significantly from their individual extracts. The CME combination produced an indifference activity with the extract of Eu & Po against *Proteus mirabilis.*

Staphylococcus epidermidis showed significant inhibitory effect to all the single extract used but displayed antagonistic effect to all the two and three combination extracts. Pomegranate extract was the most effective extract that showed a strong inhibitory activity against *Staphylococcus epidermidis.* The presence of sterols, tannin, glycosides, phenols, saponin, flavonoids and reducing sugar in the plant extract could be responsible for the antimicrobial activity exhibited by the selected plant materials but according to Duke *et al*., 2003; Esimone *et al*., 2006; Ghaleb and Mohammed, 2008, a reduction in the activity of the combination extracts may be linked to incompatibility of the phytochemicals present in the extract or competition between the extracts for the active site.

Combination of Eu & Cl and the single extract of pomegranate show indifferent effects against *Staphylococcus aureus* while significant antibacterial activities were observed in the remaining two combination extracts. All the three combination extract showed lower inhibitory activities which denote antagonistic effects. According to Duke *et al* this may implies that the crude extracts have different phytochemical constituents with different mechanism of actions (Duke *et al*., 2003). Esimone *et al* equally stated that attack of two phytochemicals on different active site of bacteria could either lead to additive or a synergistic effect (Esimone *et al*., 2006). Closely in accordance with Esimone *et al*, Ghaleb and Mohammed reported that numerous compounds within the crude extracts may interfere with the action of one another (Ghaleb and Mohammed, 2008).

Activity of Ciprofloxacin was observed to be more than those of the test agents used singly and also in combinations while erythromycin was found to be lesser than the activities of those combined extracts. This is similar to a study by Ofokansi *et al* who tested for the antibacterial activities of the combined extract of *Phyllanthus muellerianus* with ciprofloxacin, in which majority of the test organism were susceptible to ciprofloxacin (Ofokansi *et al*., 2012). It is possible that if the minimum inhibitory concentration of these combined extracts were investigated it could produce antimicrobial activity comparable to that of the standard antibiotics against the selected organism.

Hence a new way can be found to come back antibiotic resistant of pathogenic organism and provide safe and healthy living through germ free hand, although the removal is not 100% but a major number can be reduced.

Effectiveness of formulated sanitizer on selected bacteria strains

When the combination of PCE was used to formulate herbal hand sanitizer, there was growth reduction in bacterial flora. Percentage of reduction shown by the herbal hand sanitizer was equivalent to 75% because it inhibited three out of the four bacteria found on the hand of the volunteer. The result obtained was then compared to sterile water control and commercially available hand sanitizer (25%) which inhibited a single bacterial out of the initial four types of bacteria found on the volunteer's rinse water. Therefore natural bioactive constituents present in pomegranate, clove and eucalyptus extracts showed superior inhibition against hand microbiome than the chemicals present in commercially available hand sanitizer.

CONCLUSION

The results of this study were encouraging, it indicates that all pairwise combinations of the four selected medicinal plants have a great potential for treatment of infectious diseases and reduction of drug resistance. Considering that PCE has the highest inhibitory effect from all the tested formulation against two bacteria, it was used for the production of the herbal hand sanitizer. The formulated herbal hand sanitizer therefore showed 75% growth reduction rate as against commercially available synthetic sanitizer that showed 25% reduction of the bacterial flora. Hence the herbal hand sanitizer can be adopted for use.

The study therefore recommends that active ingredients should be extracted and the molecular basis for the various interactions (synergetic, additive, indifferent and antagonistic) should be studied because it may serve as a lead compound for the development of drugs and formulation of new product of economic value.

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