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In vitro Antimicrobial Activity of Some Plant Essential Oils against Organisms Associated with Urinary Tract Infection and Roasted Meat

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Abstract

In search for alternative ways to control microbial infections, plant essential oils from clove, fenugreek, garlic, neem, eucalyptus and lemongrass were extracted using Soxhlet apparatus and petroleum ether as a solvent. Phytochemical screening of the oils/extracts revealead the presence of alkaloids, flavonoids, saponins, reducing sugars, steroids, tannins, glycosides and triterpenoids. The oils/extracts were then evaluated for their *in vitro* antimicrobial properties against *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus* and *Candida albicans* of Urinary Tract Infection and meat (suya) origins using disc diffusion and broth dilution techniques. Essential oil from clove was found to have the strongest inhibitory effect being able to inhibit growth of all assayed organisms followed by lemongrass extract, garlic oil, eucalyptus extract and neem oil while essential oil from fenugreek showed no inhibitory effect against all organisms tested. Highest and lowest susceptibility to these oils/extracts was demonstrated by *S. aureus* (mean zone= 11.7mm) and *E. coli* (mean zone= 5.8mm) respectively. Organisms isolated from urine showed more resistance than those isolated from meat. The LC_{50} analysis of the oils/extracts showed relatively high cytotoxic effects against brine shrimps, (<100 ppm). Maximum mortalities took place at a concentration of 1000 ppm whereas least mortalities were at 10 ppm concentration, which means that lethality of the oils/extracts was concentration-dependant.

Key words: Antimicrobial activity, essential oils, UTI, Roasted meat

INTRODUCTION

Essential oils (also called volatile or ethereal oils, because they evaporate when exposed to heat in contrast to fixed oils) are odorous and volatile compounds found only in 10% of the plant kingdom. They are stored in plants in special brittle secretory structures, such as glands, secretory hairs, secretory ducts, secretory cavities or resin ducts (Ahmadi *et al.,* 2002). They can be obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) by expression, fermentation or extraction (Prabuseenivasan *et al.,* 2006). Many investigators have demonstrated the antimicrobial activity of the constituents of some higher plants (Hablemariam *et al.,* 1993) and quite a number of chemical compounds of plant origin have been shown to possess antimicrobial activities (Corthout *et al.,* 1992).

The use of essential oils as functional ingredients in foods, drinks, toiletries, and cosmetics is gaining momentum, both for the growing consumers' interest in the ingredients coming from natural sources, and also because of the increasing concern with harmful synthetic additives (Sacchetti *et al.,* 2005). Due to their bioactive components, essential oils are indeed promising in view of their use as effective antibacterial, antifungal, and anti-oxidant agents.

Urinary tract infections are among the most prevailing infectious diseases with a substantial financial burden on society. Urinary Tract Infections represent at least 40% of all hospital acquired infections and are, in the majority of cases, catheter associated (Ruden *et al.,* 1997).

Meat is a flesh of animals which serves as food. It is obtained from sheep, cattle, goat and swine (Haman, 1977). Meat plays an important role in human diet by contributing both macro and micro nutrients that are required for growth and good health maintenance. The rate of increase in per capita consumption of meat was found to be very high in developed countries when compared with developing nations (Anjaneyulu *et al.,* 2007). Due to its chemical compositions and characteristics, meat is a highly perishable food. This provides an excellent medium for growth of many microorganisms that can cause infection in man and also lead to meat spoilage and economic loss (Hassan *et al.,* 2014). Microorganisms that have been incriminated in food borne illness resulting from consumption of meat and meat products include; *S. aureus, Salmonella spp, listeria monocytogens, Yersinia enterocolitica, Bacillus cereus, E. coli, Clostridium perfringens* as well as yeast and moulds (Saide-Alboronz *et. al.,* 1995).

The aim of this study was to evaluate the sensitivity profile of some bacterial strains isolated from urinary tract infections and from meat (*suya*) to some plant essential oils/extracts with emphasis for their possible future use as alternative strategies to control infectious microorganisms.

MATERIALS AND METHODS

Ethical approval

Ethical approval was obtained from Ethical and Research Committee of Aminu Kano Teaching Hospital (AKTH), Kano.

Sample Collection

Plants, urine and meat samples were collected and used for the study

Plant collection and identification

Clove (Botanical name: *Syzygium aromaticum*, Hausa name: Kanumfari), Eucalyptus (Botanical name: *Eucalyptus camaldulensis*, Hausa name: Turare), Fenugreek (Botanical name: *Trigonella foenum-graecum*, Hausa name: Hulba), Garlic (Botanical name: *Allium sativum*, Hausa name: Tafarnuwa), Lemongrass (Botanical name: *Cymbopogan citratus*, Hausa name: Tsabrenkamshi) and Neem (Botanical name: *Azadirachta indica*, Hausa name: Darbejiya) plant parts were collected, Identified by a taxonomist at the Department of plant Biology, Bayero University, Kano and voucher specimen numbers were provided as Accession Numbers (BUKHAN 0342, BUKHAN 0347, BUKHAN 0384, BUKHAN 0297, BUKHAN 0234, and BUKHAN 0312), from their herbarium. Samples were dried at room temperature and ground into fine powder using mortar and pestle (Mukhtar and Tukur, 1999).

Urine collection

Urine samples (freshly collected clean-catch specimen) were collected from Aminu Kano Teaching Hospital hospitalized (Intensive Care Unit (ICU) patients.

Two skewers of suya meat were obtained randomly from three different suya spots in Gandu, Kano metropolis. The samples were immediately wrapped in sterile aluminum foil to prevent contamination and then transported to Microbiology laboratory of Department of Microbiology Bayero University, Kano for analysis.

Extraction of Plant Materials

The technique adopted by Adepoju *et al.* (2014) was followed with slight modifications. A 250-ml Soxhlet extractor apparatus and petroleum ether as solvent were used for this work. The quantity of the oil yield was determined gravimetrically as the ratio of the weight of the extracted oil to the weight of the plant powder sample used. The obtained oil was kept in a refrigerator before use.

Phytochemical screening of essential oils

The extracts obtained were subjected to some phytochemical screening assays in order to detect the presence of the Alkaloids, Flavonoids, Saponins, , Reducing Sugars, Steroids and Tannins (Harbone, 1984, Sofowora,1984, Ciulci, 1994, Brain and Turner, 1975;).

Urine culture

Urine was mixed by rotating the container. Using a sterile wire loop,

a loopful of urine was inoculated on a plate of cystine lactose electrolyte-deficient (CLED) agar and incubated aerobically at 37^0C overnight (Cheesbrough, 2006).

Meat sample culture for bacteria and yeast

Meat sample (*suya*) was removed from the skewers, and mashed in a sterile laboratory mortar and pestle. One gram (1g) of the mashed *suya* meat was weighed and then aseptically introduced into 9ml of sterile distilled water, properly shaken and a tenfold serial dilution was performed. Loopful of the samples were inoculated aseptically using streak technique on Nutrient agar, MacConkey agar and Mannitol salt agar plates and incubated at 37°C for 24hours (Hassan *et al.,* 2014). The colonies formed were counted to obtain total viable, coliform and staphylococcal counts. Inoculation was also done on Potato Dextrose Agar for the isolations of fungi (yeast). Isolated colonies were purified (by subculturing) to obtain pure cultures which were subsequently identified using standard methods (Buchanan and Gibbons, 1974).

Identification of isolates

The isolated organisms were subjected to various biochemical tests viz: Catalase test Coagulase test Oxidase test, Urease test, Indole test, Methyl Red test, Voges-proskauer test, Citrate utilization test, Gram staining and Motility test (Cheesbrough, 2006, Fawole and Oso, 2007).

Examining fungi in wet preparation

A colony from fungal culture plate was emulsified in sterile distilled water on a slide to make a wet preparation. It was then covered with a cover slip and examined under ×10 and ×40 objectives. Sprouting yeast cell that was tube-like outgrowth from the cells indicated germ tube formation (Cheesbrough, 2006).

Bioassay studies

Bioassay was carried out in order to test the bioactivity or other wise of the essential oils on the test organisms.

Disc preparation for plant essential oils

Filter paper discs were punched from Whatman No. 1 filter paper and sterilized in separate bijou bottles (100 discs per bottle). The different concentration solutions used in the assay were 10 µl (1ml of essential oil), 7.5µl (0.75ml of essential oil and 0.25ml of DMSO), 5.0µl (0.5ml of essential oil and 0.5ml of DMSO) and 2.5µl (0.25ml of essential oil and 0.75ml of DMSO) with each disc capable of adsorbing 0.01 ml of the solution (Serban *et al.,* 2011).

Standardization of inoculum

Using inoculation loop, enough material from an overnight culture of the test organism was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National committee for clinical laboratory standard (NCCLS, 2008).

Sensitivity testing by disc diffusion test

Standardized inocula of the isolates were swabbed on to the surface of prepared and solidified Mueller Hinton agar and Saboraud Dextrose Agar in duplicates for bacteria and fungi respectively. The prepared discs of the extracts and the standard antibiotic discs (Ciprofloxacin for bacteria and fluconazole for fungi) were placed onto the surface of the inoculated media at intervals by means of sterile syringe needle. The plates were incubated at 37°C for 24 h before observation and measurement of zones of inhibition (NCCLS, 2008).

Determination of Lethal Concentration 50 (LC_{50})

Artificial Sea Water- Sodium Chloride (38g) was dissolved in 100ml of distilled water and the pH adjusted to 8.5 using 1N NaOH (Ramachandran *et al.,* 2010). This test was carried out according to procedure described by Krishnaraju *et al.* (2005) in Owokotuma *et al.* (2012).

Brine Shrimp Lethality Test (BSLT)

This test was carried out according to procedure described by Olowa and Nuneza, (2013).

Hatching- About 50 mg of Artemisia salina (Leach) eggs, (Interpet. Ltd. England) was added to about 150 mL solution of sea water in a beaker.

The mixture was allowed to incubate for 48 h in a warm well ventilated room (22-29°C) under a light source. Larvae (nauplii) were collected with a Pasteur pipette after they had been attracted by the light source.

Preparation of samples' test solutions- Stock solution was prepared by emulsifying 20 mg of the essential oils/extracts separately in 0.3ml of dimethylsulphoxide (DMSO) and the volume was made up with 1.7ml of fresh sea water to equal 1000 ppm concentration. After this, serial dilution was done to obtain two additional Concentrations of 100ppm and 10 ppm.

Stationing the Brine Shrimps- Fresh sea water (3.0ml) was transferred into the specimens' vials prepared in triplicates. Then, 0.5ml of each prepared concentrations was introduced into the specimen vials followed by introduction of ten brine shrimps into each specimen vial including the control vial containing 10 nauplii in 5ml sea water. Finally, each specimen vial was topped up with sea water until it reached 5.0ml. All the vials containing the shrimps were left opened for 24 hours. Using probit analysis, the lethality concentration (Lc50) was assessed at 95% confidence intervals. Lc50 of less than 100 ppm was considered as potent (active) Gupta *et al.,* 1996) as mentioned by Meyer *et al.,* 1982. Lc50value of less than 1000 ppm is toxic while Lc50 value of greater than 1000 ppm is non-toxic (inactive). The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number and then multiplied by 100. This is to ensure that the death (mortality of the nauplii) is attributed to the bioactive compounds present in the plant oils/extracts.

RESULTS

Physical properties of the plant essential oils

The physical properties and percentage yields of the essential oils are shown in Table 1. Phytochemical screening for the bioactive components present in the essential oils revealed the presence of numerous secondary metabolites as presented in Table 2. The bacteria were characterized based on their reactions to the various biochemical tests and subsequently identified using Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) as shown in Table 3.

Antimicrobial activity of plant essential oils against UTI and meat isolates

The results of antimicrobial activity of the essential oils against UTI and meat isolates at concentrations of 10µl/disc, 7.5µl/disc, 5.0µl/disc and 2.5µl/disc are shown in Tables 4 and 5 respectively. Clove essential oil showed inhibitory activity against all the tested organisms. Neem essential oil showed a weak inhibitory activity against *P. vulgaris* and *P. aeruginosa* and garlic essential oil showed weak inhibitory activity against *P. aeruginosa* only at 100% concentration, while fenugreek oil showed no inhibitory activity against all tested organisms.

Comparison of antimicrobial sensitivity pattern of organisms of UTI and meat origins

Comparative statistical analysis of antimicrobial sensitivity pattern of organisms of UTI and meat origins was carried out using one-way ANOVA. Comparison of sensitivity pattern of individual species of organisms of UTI and meat origins is presented in the Table 6.

Table 1: Physical properties of the oils/extracts

Plant	State	Colour	Odour	Quantity recovered(g)	% yield
Clove	Liquid	Yellowish	Warm Strong spicy	19.24	16
Fenugreek	Liquid	Dark green	Maple/curry	18.9	15.7
Garlic	Liquid	Pale yellow	Pungent	12.4	10
Neem	Liquid	Golden yellow	Pungent	25.7	21

Table 2: Phytochemical constituents of some plant essential oils/extracts

Plant	Alk	Flav	Sap	Reducing sugars	Steroids	Tannins	Glycosides	Triterpenoids
Clove	_	+	+	_	+	+	+	+
Fenugreek	+	_	+	_	+	_	+	_
Garlic	_	_	+	_	+	+	+	_
Neem	_	_	+	_	+	+	+	_

+= Presence, _= Absence, Alk = alkaloids, Sap = saponins, Fla = flavonoids

Table 3. Biochemical characterization of bacteria associated with urinary tract of patients at AKTH Kano

Gram	Cell morphology	Catalase	Coagulase	Oxidase	Indole	Motility	MR	VP	Urease	Citrate	KIA				Organism
											Slope	Butt	H_2S	Gas	
_	Rods	+	_	_	_	_	_	+	+	+	Y	Y	_	+	*K. pneumoniae*
_	Rods	+	_	_	+	+	+	_	_	_	Y	Y	_	+	*E. coli*
_	Rods	+	_	+	_	+	_	_	_	+	R	R	_	_	*P. aeruginosa*
_	Rods	+	_	_	+	+	+	_	+	_	R	Y	+	+	*P. vulgaris*
+	Cocci	+	+	_	_	_	+	+	+	+	_	_	_	_	*S. aureus*

Key: + = positive, Y= yellow, - = negative, R= red

Table 4: Antimicrobial activity of plant essential oils against UTI pathogens

	Disc potency (µl/disc)/Inhibition zones (mm)															
	Clove				**Fenugreek**				**Garlic**				**Neem**			
Test organisms	**10**	**7.5**	**5.0**	**2.5**	**10**	**7.5**	**5.0**	**2.5**	**10**	**7.5**	**5.0**	**2.5**	**10**	**7.5**	**5.0**	**2.5**
K. pneumoniae	12	11	9	8	0	0	0	0	0	0	0	0	0	0	0	0
E. coli	10	9	8	7	0	0	0	0	0	0	0	0	0	0	0	0
P. aeruginosa	10	9	8	7	0	0	0	0	8	0	0	0	8	0	0	0
P. vulgaris	11	9	8	7	0	0	0	0	0	0	0	0	10	9	7	0
S. aureus	13	13	12	9	0	0	0	0	0	0	0	0	0	0	0	0
C. albicans	22	20	15	12	0	0	0	0	0	0	0	0	0	0	0	0

Table 5: Antimicrobial activity of some plant essential oils against roasted meat isolates

	Disc potency (µl/disc)/Inhibition zones (mm)															
	Clove				**Fenugreek**				**Garlic**				**Neem**			
Test organisms	**10**	**7.5**	**5.0**	**2.5**	**10**	**7.5**	**5.0**	**2.5**	**10**	**7.5**	**5.0**	**2.5**	**10**	**7.5**	**5.0**	**2.5**
K. pneumoniae	15	14	12	10	0	0	0	0	15	12	10	9	15	13	11	8
E. coli	15	14	12	9	0	0	0	0	0	0	0	0	0	0	0	0
P. aeruginosa	15	13	12	9	0	0	0	0	15	13	10	8	0	0	0	0
P. vulgaris	15	14	12	10	0	0	0	0	14	11	9	7	16	14	12	7
S. aureus	18	16	15	13	0	0	0	0	17	16	12	10	18	17	13	8

Table 6: Comparison of Antimicrobial Sensitivity Pattern of Organisms of UTI and Meat Origins

Organisms isolated from UTIs	Organisms isolated from meat	P value	Mean variation
K. pneumoniae	*K. pneumoniae*	< 0.0001	Extremely significant
E. coli	*E. coli*	0.0334	Significant
P. aeruginosa	*P. aeruginosa*	0.0005	Extremely significant
P. vulgaris	*P. vulgaris*	0.0043	Very significant
S. aureus	*S. aureus*	<0.0001	Extremely significant

Brine Shrimp Lethality Test (BSLT)

Statistical analysis of Toxicity test (LC_{50}) of plant essential oils using brine shrimps has shown that, the oils presented relatively low LC_{50} values. Clove oil had the lowest LC_{50} value of 60.203 followed by neem oil with LC_{50} value of 60.203 and garlic oil with LC_{50} value of 80.493.

DISCUSSION

Yields of the essential oils was highest in neem (21%) followed by clove (16%) and fenugreek (15.7%) while garlic oil had the least yield of 10%. Low oil yield from garlic bulb could be due to the solvent used (petroleum ether) in the extraction process or less oil content of garlic bulbs. Gafar *et al.* (2012) reported high yield of 22.5% using n-Hexane as a solvent for extraction of garlic oil.

Phytochemical screening of the oils revealed the presence of alkaloids, flavonoids, saponins, reducing sugars, steroids, tannins, glycosides and triterpenoids. Many plants have been reported to synthesize secondary metabolites as part of their defence system (Phan *et al.,* 2012).

Klebsiella pneumoniae, E. coli, P. aeruginosa, P. vulgaris, S. aureus and *C. albicans* were isolated from urine samples from hospitalized patients in intensive care units. This is in agreement with the work of Manikandan and Amsath (2016) where they isolated same organisms from UTI's. These organisms are those usually implicated in nosocomial UTI and are, in the majority of cases, catheter associated (Richards *et al.,* 1999).

Klebsiella pneumoniae, E. coli, P. aeruginosa, P. vulgaris, S. aureus and *C. albicans* were also isolated and identified from meat (*suya)* which is in line with the work of Yusuf *et al.* (2012) who also reported the presence of these organisms from meat products. The presence of *Enterobacteriaceae* in heat-processed foods indicates inadequate cooking or post-processing contamination (Food Safety Authority of Ireland, 2014). On the whole, the major sources of microbial contamination of suya meat appear to come from butchers and the use of contaminated water and equipment as reported by Hassan *et al.* (2014).

Among the essential oils tested against UTI and roasted meat pathogens, clove oil presented highest activity by inhibiting the growth of all assayed organisms at all tested concentrations while fenugreek oil showed no inhibitory activity at all. Oils of garlic and neem presented no inhibitory activity against UTI isolates but active against proteus and *S. aureus* from meat. Pathogens causing nosocomial infections have been reported to be resistant to most antimicrobial agents (Hsueh *et al.,* 2002). This result is similar to that observed by Khan *et al.* (2009) where they reported that even multi drug resistant strains of bacteria and fungi were sensitive to clove but exhibited strong resistance to other extracts tested.

All organisms demonstrated strong sensitivity to control antibiotics with *P. vulgaris* and *E. coli* being less susceptible. The organisms' sensitivity to clove essential oil could be due to higher number of phytochemicals detected in the oil as it was reported that phytochemicals vary in their antimicrobial potency (Bama *et al.,* 2012).

The work of Barbosa *et al.* (2009) also showed that clove essential oil, among all tested oils presented highest antimicrobial activity against bacteria and fungi isolated from minced meat. Sensitivity test results of organisms isolated from meat to eucalyptus and lemongrass extracts showed that all tested organisms were sensitive to both extracts with lemongrass being more potent. This could be due to higher number of phytochemicals detected in lemongrass extract, tannin in particular was absent from eucalyptus, this phytochemical has been reported to possess antibacterial activity (Scalbert, 1999). This is in line with the work of Hamza *et al.* (2009) and Potdar *et al.* (2015) where they reported that extracts of lemongrass and eucalyptus had a broad spectrum of activity against both Gram positive and Gram negative bacteria and fungi. All tested organisms were highly sensitive to the control (Ciprofloxacin) with *S. aureus* being the most susceptible and *E. coli* the least susceptible.

Although same species of microorganisms were isolated from both urine and meat, a significant difference in their sensitivity patterns to the tested oils was observed using one-way ANOVA statistical analysis. This is in agreement with the work of Singh *et al.* (2011) where they reported that varying sensitivity patterns exist between microorganisms of the same species depending on the strains and origin of the organisms. Organisms isolated from urine showed high degree of resistance to the tested oils/ex except for clove essential oil which was active against all assayed organisms. In contrast, organisms isolated from meat showed high degree of sensitivity to most of the tested oils/extracts except for fenugreek oil which was inactive against all assayed organisms. This finding is in agreement with the results obtained from the work of Khan *et al.* (2009), which reported that microbial strains isolated from nosocomial infection were more resistant than community acquired ones. It was also reported earlier that the resistance to antibiotics as well as mortality is almost two times higher in case of nosocomial infections than in community-acquired infections (Kang *et al.*, 2006).

Lethality assay results evaluated by brine shrimp lethality test. All oils tested showed relatively high cytoxicity with LC_{50} values of 60.203, 80.493, and 64.801 for clove, garlic, and neem respectively. The cytotoxocity of these oils indicates their potentiality for containing bioactive compounds (having LC50 of <100) Meyer *et al.* (1982).

CONCLUSION

The plants contain essential oils which contain various phytochemical constituents, most were found to have inhibitory activity against isolates from UTIs and ready to eat meat (suya). The results from this study showed high toxicity of the oils.

RECOMMENDATIONS

More *in vitro* and *in vivo* tests are required to ascertain their pharmacological features and possible medicinal properties of essential oils of the neem, garlic and fenugreek plants with special consideration to their high toxicity.

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