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Bacterial contamination of some medicinal plant materials sold at Omdurman local market, Khartoum state, Central Sudan

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Abstract

Plants have long been used as herbal medicines in many countries. However, microbial contamination of these medicines may affect human health. In the present study fifteen medicinal plants namely, *Acacia nilotica ssp. nilotica, Trigonella foenum-greacum*, *Nigella sativa*, *Hyphaene thebaica*, *Nauclea latifola*, *Cyperus rotundus*, *Cymbopogon schoenanthus* spp, *proximus*, *Artemisia herba-alb*, *Cassia acutifolia*, *Solenostemma argel*, *Tamarindus indica*, *Ziziphus spina-christi, Lepidium sativum*, *Foeniculum vulgare* and *Coriandrum sativum* were evaluated for bacterial contamination. The identified bacterial isolates consists of fifteen bacterial species, the most dominant bacteria were *Leuconostoc pseudomes enteriodes, Sphingomonas paucimobilis* and *Staphylococcus lentus*. The highest total bacterial load was $53x10^5$ cfu/g found in *Coriandrum sativum* exceeded maximum acceptable contamination limits set by the WHO (i.e 10^5 to ≤ 10^7 cfu/g. The levels of contamination varied greatly between the commercially available plant samples investigated.

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Introduction

The use of medicinal plants is continually expanding worldwide. Despite of the modern advances achieved in the field of synthetic chemistry, the most efficient drugs available had derived directly or indirectly from herbs**.** The local uses of plants in healthcare are much higher than used modern synthetic drugs (Saeed *et al*., 2004).

Herbal drugs are subjected to contamination by microorganisms from soil, air and water which may present potentially pathogenic microorganisms to human. The presence of microbial contaminant in non-sterile medicinal plant materials can reduce or even inactivate the therapeutic activity of the medicinal plants and has the potential to adversely affect patients taking the plants. The global and national markets for medicinal plants have been growing rapidly and significant economic gains are being realized with global sales of herbs (Araujo and Bauab, 2010). The microbial load of some medicinal plants sold in some local markets in Africa were investigated and the findings reiterate the need for constant quality assessment of herbal materials in the market in order to ensure that medicinal plant materials are suitable for human health (Mac Donald *et al.*, 2010). According to some reports, the consumers may possibly fall into illness because of taking herbs incriminated with pathogenic microorganisms (Khattak, 2012) and sometimes the presence of antibiotic resistant microbial isolates in the medicinal plants will lead to transfer of antibiotic resistance strains to consumers (Esimone *et al.*, 2007). Different studies that have been performed on herbal medicinal plants revealed the presence of bacterial pathogens with multiple drug resistance (Oluyege and Adelabu, 2010). The previous research on medicinal plant contamination by microbes has assessed fungal species and the aflatoxins that they produce (EL-Kamali *et al.*, 2016). Using those contaminated herbal medicines may lead to infection of other health related risks.

Therefore, this warrants urgent training of herbalists and management scale-up for quality and safety of medicinal plants (Abdalla *et al*., 2016). This study, however, focuses on bacterial contamination by enumerating the types and levels of bacteria found on selected plants purchased in Omdurman market, Khartoum State, Central Sudan, to the best of our knowledge, not been studied before.

Materials and methods

Plant Materials

A total of fifteen different medicinal plant parts *Acacia nilotica* ssp. *nilotica, Trigonella foenum-greacum*, *Nigella sativa*, *Hyphaene thebaica*, *Nauclea latifola*, *Cyperus rotundus*, *Cymbopogon schoenanthus* spp*. proximus*, *Artemisia herba alb*, *Cassia acutifolia*, *Solenostemma* argel, *Tamarindus indica*, *Ziziphus spina-christi, Lepidium sativum*, *Foeniculum vulgare* and *Coriandrum sativum* (Table 1) were purchased from a local market in Omdurman, Khartoum State, central Sudan (during the period September–October, 2016). The Plant parts were collected aseptically with gloves in to sterile pouches. The plants were identified at the Botany Department, Faculty of Science and Technology, Omdurman Islamic University.

Determination of the presence of Bacterial Contamination

The media

Nutrient agar, Baired–Parker agar, Xylose–Lysine deoxychcolate agar and Macon key agar were used to enumerate the total bacteria population.

Sample preparation

The dried plant samples were cleaned to remove adhering polyethylene and other unwanted materials. The samples were stored at 4ºC till further analysis.

Procedure for Determination

The determination of bacterial contamination was established for bacterial load, total bacterial

count and specific pathogen in the studied medicinal Plant samples. For the calculation of bacterial contents, the plant materials were tested to determine the number of microorganisms per gram samples. The standard plate count method was used for detecting and determining the number of microorganism in plant material. One gram of each of the studied plant parts were infused in 9ml of distilled water. Serial dilutions were made; viability assessed using Pour Plate method.

The plates were incubated at 37**º**C for 24h. The plates were placed on a colony counter and the number of Colony Forming Units was taken.

Then all isolates were identified by using colonies morphological characters: form, pigment, color and size, standard biochemical tests (Antonia *et al.*, 2010) and using Vitek2 Compact System (Biomere resistentux, USA).

Results and discussion

A total of fifteen bacterial strains were isolated from fifteen selected medicinal plant parts and then were identified. The distribution of the microbial load from the studied plant parts is shown in Table 2. The dominant bacteria in most of the tested samples belong to *Leuconostoc pseudomes enteriodes, Sphingomonas paucimobilis* and *Staphylococcus lentus.*

Table 1: Total Bacterial Count of Selected Medicinal Plant materials obtained From Omdurman Local Market/Sudan

Plant sample	Count of bacteria	Predominant bacterial species isolated
Cymbopogon schoenanthus spp. *proximus*	52x10 ^{3}	*Kocuria rosea, Enterococus cesorum, Sphingomonas paucimobilis* two isolates*, Klebsiella pneumonia* spp *ozaenae and Pantoea* spp.
Trigonella foenum-graecum	40x10 ^{3}	*Leuconostoc psedumesenteriodes, Aeromonas salmonicida* and *Sphingomonas paucimobilis* two isolates.
Artemisia herba-alba	50x10^{2}	*Leuconostoc psedumesenteriodes* two isolates*, Aeromonas salmonicida* and *Sphingomonas paucimobilis,* two isolates and three isolates
Lepidium satinum	12x10^{2}	*Staphylococus lentus, Sphingomonas paucimobilis* two isolates*, Klebsiella pneumonia* spp *ozaenae* and *Pantoea* spp.
Nigella sativa.	44x10^{3}	*Enterococus cesorum, Sphingomonas paucimobilis* two isolates*, Leuconostoc psedumesenteriodes,* and *Pantoea* spp.
Solenostemma argel	26x10 ^{3}	*Staphylococus lentus,* two isolates and *Leuconostoc psedumesenteriodes*
Coriandrum sativum	53x10 ^{5}	*Kocuria rosea, Klebsiella pneumonia* spp *ozaenae* and *Pantoea* spp.
Foeniculum vulgare	88x10 ^{3}	*Staphylococus lentus* three isolates*, Elizabethkingia meningoseptica* and *Leuconostoc psedumesenteriodes* and two isolates.
Cassia acutifolia	44x10 ^{2}	*Staphylococus lentus,* three isolates*, Leuconostoc psedumesenteriodes* two isolates*, Kocuria rosea. Sphingomonas paucimobilis.*
Hyphaene thebaica	67x10 ^{3}	*Leuconostoc psedumesenteriodes* two isolates*, Aeromonas salmonicida* and *Sphingomonas paucimobilis* two isolates.
Cyperus rotundus	11X10^{5}	*Staphylococus lentus* three isolates*, Elizabethkingia meningoseptica, Enterococus cesorum* and *Sphingomonas paucimobilis,* two isolates
Acacia nilotica ssp.nilotica	25x10 ^{1}	*Staphylococus lentus* three isolates*, Leuconostoc psedumesenteriodes* and

Plant sample	Count of bacteria	Predominant bacterial species isolated
Naucla latifola	30x10 [1]	*Klebsiella pneumonia* spp *ozaenae.* *Staphylococus lentus* three isolates*,* *Leuconostoc psedumesenteriodes* and *Sphingomonas paucimobilis* three isolates*.*
Tamarindus indica	20x10 [3]	*Staphylococus lentus, Leuconostoc psedumesenteriodes* two isolates and *Elizabethkingia meningoseptica.*
Ziziphus spina-christi	10x10 [4]	*Staphylococus lentus,* three isolates*, Leuconostoc psedumesenteriodes, Klebsiella pneumonia* spp *ozaenae Sphingomonas paucimobilis* two isolates three isolates and *Aeromonas salmonicida,* two isolates

Morphological examinations showed that several colonies have the same shape, color or size. The colonies were then observed chemically through Gram staining and different biochemical tests. Gram staining showed that 46.7% of the bacteria were Gram negative and 53.3% were Gram positive (Table 3).

Bacterial species identification was conducted using Vitek 2 Compact System which was based on the difference of biochemical tests measuring carbon utilization, inhibition, resistant and energy activation. Gram positive bacteria were*, Staphylococcus lentus, Leuconostoc pseudomes enteriodes, Entercoccus. cecorum* and *Kocuria rosea.* Gram negative were Pantoea spp, *Sphingomonas paucimobilis, Klebsiella pneumonia* spp *rosea, Aeromonas salmonicida* and *Elethbethkingia meningoseptica.* The results of Vitek 2 Compact System biochemical test reaction are presented in (Table 4). Medicinal plant materials sold in marketplaces are always assumed to be safe and efficient, however, they can be potentially toxic because of poor hygienic practices in plant processing or storage (Sowza *et al*., 2011).

It is evident that policies and regulations need to be develop and implemented in order to address possible contamination by opportunistic pathogens.

Table 2. Morphological and Microscopical characteristics of Colonial bacteria isolated on nutrient agar.

Colonial morphology	Motility	Gram stain	Spore	Proble identify
Small, pale cream to pale pink	-	s+	-	*Kocuria rosea*
small, white colonies	-	r+	-	*Enterococcus cecorum*
yellow-white–pigmented	+	r-	-	*Sphingomonas paucimobilis*
Smooth white to cream colonies	-	s+	-	*Leuconostoc pseudomesenteroides*
Smooth Brown	-	r-	-	*Aromonas salmonicida*
Shiny and smooth, golden yellow	-	s+	-	*Staphylococcus lentus*
Mucoid shiny White or gray	-	r-	-	*Klebsiella pneumonia ssp ozaenae*
Smooth and pale pink	+	r-	-	*Pantoea* ssp
Rouph Yellow to orange	-	-r	-	*Elizabethkingia meningoseptica*

r: rod ; s :spherical

Table 3. Biochemical tests of isolated bacteria in nutrient agar.

Bacteria	ca	Ox	Co	Ind	Mr	Vog	Cit	glu	Suc	Mal	Lac	Man
Kocuria rosea	+	+	-	+	-	-	-	+	-	-	-	-
Enterococcus cecorum	-		-	-	-	+		-	+		+	+
										+		
Sphingomonas paucimobilis	+	+		+	-	+	+	-	+	-		+
Leuconostoc pseudomesenteroides	-		-		+	-		+	+	+	+	+
Aromonas salmonicida	+	+	-	-	+	-	-	+	-	-	-	-
Staphylococcus lentus	+	+	-		+	-		+	+	+	+	
Klebsiella pneumonia ssp *ozaenae*	+	-	-	-	+	-	-	+	+	+		+
Pantoea ssp							+	+	+	+	+	+
Elizabethkingia meningoseptica	+	+		+	-	+	-	-	-	-	-	+

Ca;catalase, ox; oxidase, co; coagulase, ind; indole, mr; methyl red, vog; vogas prosquer, cit citrate, glu; glucose, auc; sucrose, mal. maltose, lac. lactose, man; manose.

Table 4. Biochemical tests used for identification of (G +ve) bacterial strains.

Tests	Isolates						
	S.1	S.l (2)	S.l (3)	L.p	L.ps (2)	E.c	K.r
AMY	+	+	+	-	+	+	-
PIPLC	-	-	-	-	-	-	-
DXYL	-	-	-	-	+	-	-
ADH1	+	+	+	-	-	-	-
BGAL	+	-	-	+	+	+	-
AGLU	+	+	+	-	-	+	-
APPA	-	-	-	-	-	-	-
CDEK	+	-	-	-	-	+	-
AsPA	-	-	-	-	-	-	-
BGAR	-	-	-	+	-	-	-
AMAN	-	-	-	-	-	-	-
PHOS	-	-	-	-	-	-	-
LeuA	-	-	-	+		-	-
ProA	-	-	-	+	-	-	-
BGURr	-	-	-	-	-	-	-
AGAL	+	+	-	+	+	+	-
PyrA	+	+	+	-	-	-	-
BGUR	-	-	+	-	-	-	-
AlaA	-	-	-	+	-	-	+
TyrA	-	+	+	-	-	-	-
DSOR	+	+	+	-	-	-	-
URE	-	-	+	-	-	-	-
POLYB	-	-	-	-	+	-	-
dGAL	+	-	-	+	+	-	-
dRIB	+	+	+	-	+	+	-
ILATK	+	-	+	-	-	-	-
LAC	+	-	+	+	-	-	-
NAG	+	+	+	-	-	+	-
dMAL	+	+	+	+	-	+	-
BACI	+	+	+	-	-	-	-
NOVO	-	-	-	-	-	-	-
NC6.5	+	+	+	-	-	+	-
dMAN	+	+	+	-	-	+	-
dMNE	+	+	+	+	+	-	-
MBdG	+	+	+	-	+	+	-
PUL	+	-	-	-	-	-	-
dRAF	+	-	-	+	-	+	-
0129R	-	-	-	-	-	+	-
SAL	+	+	+	-	-	+	-
SAC	+	+	+	+	+	+	-
dTRE	+	+	+	+	-	+	-
ADH2s	-	-	-	-	-	-	-
OPTO	+	+	+	+	-	+	-

Table 5. Biochemical tests used to identification of (G -ve) bacterial strains.

Tests	Isolates							
	S.p	S.p(2)	S.p(3)	A.S	A.S(2)	E.m	P.SSP	K.pn
APPA	+	+	+	-	-	+	-	-
ADO	-	-	-	-	-	-	-	-
PyrA	-	-	-	-	-	+	+	+
IARL	-	-	-	-	-	-	-	-
dCEL	+	+	+	-	-	-	+	+
BGAL	+	+	+	-	-	+	+	+
H_2S	-	-	-	-	-	+	-	-
BNAG	-	-	-	-	-	+	-	-
AGLTp	-	-	-	-	-	+	-	-
Dglu	+	+	+	-	+	-	+	+
GGT	-	-	-	-	-	-	+	+
OFF	-	-	-	-	-	-	+	-
BGLU	+	+	+	-		+	+	+
dMAL	-	-	-	-	-	-	+	+
dMAN	+	+	+	-	-	-	+	+
dMNE	+	+	+	-	+	-	+	+
BXYL	+	+	+	-	-	-	-	+
BALaP	-	-	-	-	-	-	-	-
ProA	-	-	-	-	-	+	-	-
LIP	-	-	+	-	-	-	-	-
PLE	-	-	-	-	-	-	-	+
TyrA	+	+	+	-	-	+	+	+
URE	-	-	-	-	-	-	+	+
dSOR	-	-	-	-	-	-	-	+
SAC	+	+	+	-	-	-	+	+
dTAG	+	+	+	-	+	-	-	-
dTRE	+	+	+	-	+	-	+	+
CIT	+	-	+	-	-	-	+	-
MNT	-	-	-	-	-	-	+	-
5KG	-	-	-	-	-	-	-	-
ILATK	+	-	+	-	-	-	+	+
AGULU	-	+	-	-	-	+	-	+
SUCT	-	-	-	-	-	-	+	-
NAGA	-	-	-	-	-	-	-	-
AGAL	-	+	-	-	-	-	-	+
PHOS	-	-	-	-	-	+	+	-
GLYA	-	+	-	-	-	-	-	+
ODC	-	-	-	-	-	-	-	-
LDC	-	-	-	-	-	-	-	-
IHISa	-	-	-	-	-	-	-	-
CMT	+	+	+	+	-	-	-	-
BGUR	-	-	-	-	-	-	-	-
O129R	-	-	-	-	-	-	+	-
GGAA	-	-	-	-	-	-	-	-
IMLTa	-	+	-	-	-	-	-	-
ELLM	-	-	-	-	-	-	+	-
ILATa	-	-	-	-	-	-	-	-

Isolates bacteria: *S. l: Staphylococcus lentus, S.L* (2)*: Staphylococcus lentus* 1 and 2 isolates, *S.l (3): Staphylococcus lentus,* three isolates*, L. ps: Leuconostoc pseudomesenteriodes, L.ps* (2)*: leuconostoc pseudomesenteriodes* two isolates*, E.c: Enterococcus cecorum, K.r: Kocuria rosea. P: Spingomons paucimobilis, S.p (2): Spingomonsa paucimobilis* two isolates*, S.P (3): Spingomonsa p aucimobilis,* three isolates*, A.s: Aeromonas salmonicida, A.s (2): Aeromonas salmonicida* two isolates*, E.m: Elizabethkingia meningoseptica, P.* spp *Pantoea* spp*, K.pn: Klebiella pneumonia* ssp *ozaenae.*

Biochemical tests

AMY: D-Amy Gdalin, PIPLC: Phosph Atidylinositolphopholipase c, dXYL: D-xylose, ADH1: Arginine Di hydrolase1, BGAL: Beta Galactosidase, AGLU: Alpha Glucosidase, APPA: Ala-phe-pro Arylamidase, CDEX: cyclodextrine, As PA: l Aspartate Arylamydase, BGAR: Beta–Galactopyranosidase Resorufine, AMAN: Alphamanoaidase, PHOS: Phosphatase, Leu A: leucine Arylamidase, ProA: L-Proline Arylamidase, BGURr: Beta glucoRonidase, AGAL: Alpha

Galactosidase, PyrA: L-pyrrolydonyl Arylamidase, BGUR: Beta Glucoronidase, Ala A: Alanine arylamidase, TyrA: Tyro Arylamidase, dsor: D-sorbitol, URE: Urease, POLYB: Polymixin B-resistant, DGAL: D-Galactose, dRIB: d-Ribose, ILATK: L-lactate alklinistatin, Lac: Lactose, NAG: N-Acetyle N-Glucosamine, dMAL: D-Maltose, BACI: Bacitracin resistant, NOVO: Novobiocin resistant, Nc6.5: Growth in NC6.5, dMAN: D-M annitol, dMNE: D-Mannose, MBdG: Methyle –B-d-Gluco-pyrinoside, PUL: Pullulane, dRAf: D-Rafinosse, O129: resistant. (com-vibrio), SAL: Salicin, SAC: Sucrose, dTRE: D-Trehalose, ADH_2 S: Arganine Dihydrolase-2- (thioglcolate Sigma), OPTO: Optochin resistant. APPA: Ala-phe-pro Arylamidase, ADO: Adnitol, PyrA: L-pyrrolydonyl Arylamidase, IAR L: L-Apiratol, dCEL: d-cellobiose. BGAL: Beta Galactosidase, H_2S: H_2S Production, BNAG: Beta- N-Acetyle N-Glucosamine, AGLTP: Gluamyl Arylamidase PNA,dGlu d-Glucosidase, GGT: GAMA-Glutamyl -transferase, OFF: fermentation/ Glucoe, BGLU: Beta-Glucosidase, dMAL: D-maltose, dMAN: D-M annitol, dMNE: D-Mannose, BXYL: Beta-xylose, BAL ap: Beta Alanine arylamidase PNA, ProA: L-Proline Arylamidase, lip: Lipase, PLE: Palatinose, Tyr A: Tyro Arylamidase, URE: Urease, dsor: D-sorbitol, SAC: Sucrose, TAG: D-tagatose, dTRE: D-Trehalose, Cit: Citrate, MNT: Malonate: 5KG: 5-Keto-D-Gluconate: ILATK: L-lactate alklinistatin, AGLU: Alpha Glucosidase, SUCT: Succinate alklinisation, NAGA: Alpha- N-acetyle-Glactosaminodase, AGAL: Alph—Glactosaminodase, PHOS: Phosphatase, GLYA: Glycin arylamidase, ODC: Ornithine decarboxylase, LDC: Lysine decarboxylase: LHISa: L-Histidine assimilation, CMT: Coumarate, BGUR: Beta Glucoronidase, O129: resistant. (com-vibrio), GGAA: GLU-GLY-Arganine arylamidase, IMLTA: L-malate assimilation, ELLM: Ellman, ILATa: -L- lactate assimilation.

Conclusion

Microbial contamination can lead to impaired performance of the medicinal plant materials due to disruption of the modification of physical characteristics and this lead to inactivation of the active ingredients in the materials.

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