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Original Research Article

Bacterial screening associated with respiratory infection and the synergistic effect of ginger and garlic tea extract conducted at assulfan herbal research center

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ABSTRACT

Respiratory tract infection are the most common infectious diseases in human due to its common mode of transmission and leading a heavy burden to Public Health. The study was aimed to isolate the bacterial pathogens that are associated with respiratory infection and using synergistic effect to evaluate the antibacterial activity of ginger and garlic tea extract of potential isolates. The present study revealed the presence of 41 isolates of respiratory pathogens out of 200 Sample collected from sputum and swab throat. The isolation and identification of target pathogens were done using Cultural and biochemical Characterizations. 7(17.07%), 16(39.02%), 14(34.15%), 3(07.39%) and 1(02.44%) are positive of *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenza and Bordetella pertussis* respectively. For the synergistic antibacterial effect of both methanolic and ethanolic extracts of garlic and ginger tea, all respiratory pathogens that were isolated in the present study were showed the great antibacterial effect except *Bordetella pertussis. K. pneumoniae* has highest activity of 17±0.7mm at 150mg/ml in methanolic while *S. Aureus* in ethanolic with 15±0.2 at 100mg/ml. Lowest activity was found to be 07±0.2mm at 50mg/ml for both *H. influenza* and *Streptococcus pneumoniae*.

1. Introduction

Respiratory tract infection are the most common infectious diseases in human due to its common mode of transmission which is leading a heavy burden to public health.[1] It can be infected by variety of bacteria, both Gram negative and positive and the diseases that are caused may range from mild to severe that is Acute respiratory tract infection.[2] Respiratory infection may be associated with different types of virus and bacteria with different clinical manifestation from being asymptomatic to fatal.[3]

Nowadays the therapeutic mode of bacterial pathogens is more concerned in the use of medicinal plant particularly Synergistic way to overcome the antibacterial resistance strains. In previous findings both Garlic and Ginger had a scientific proof of their antibacterial effects from different researchers in the world. Therefore the present study has specified Target of Respiratory pathogens with evidence of their caused, *Streptococcus pneumoniae,*[4] *Haemophilus influenza,*[5] *Klebsiella pneumoniae,*[6,7] *Staphylococcus aureus,*[8] *Corynebacterium diptheriae,*[9] *Bordetella pertussis,*[10,11] *Chalamydophila pneumoniae,*[12] *and Burkholderia pseudomallei.*[13] The main aim of the research

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was to isolate bacteria associated with respiratory infection and using Synergistic effect to evaluate antibacterial activity of ginger and garlic tea extracts on isolates.

Scopes of the study

1. General bacterial culture, morphology observation, gram staining and microscopic examination.
2. Biochemical identification.
3. Collection and preparation of ginger and garlic tea extracts.
4. Antibacterial activity of synergistic ginger and garlic tea extracts on isolated pathogens.

2. Materials and Methods

2.1. Research area

Research area covered about three sites. Aminu Kano Teaching Hospital, Kano (AKTH), a place where bacterial samples are collected and transported to Bayero University Kano (BUK). Bacterial samples were processed at Microbiology laboratory, BUK. The last segment of antibacterial effects of plant was taken place at Assulfan Herbal research center, Herbal Company Nigeria limited.

2.2. Sample size

Sample size was calculated to be 200 from previous prevalence 18.91% of respiratory infection in Benin City, Nigeria.[14]

2.3. Study interval

January 2023 – August 2023.

2.4. Inclusion criteria

Patients (10 years to above) with serious coughing couple with chest burn and air blockage longer than 14 days before diagnosed and prescribed by physician (Patients are not on medication neither having a history of *Mycobacterium tuberculosis* infection).

2.5. Ethical approval

An ethical approval was obtained from Aminu Kano Teaching Hospital Kano (AKTH), Kano State based on the consent of the Hospital Ethical Committee (HEC), under the consideration of Ministry of Health Kano, State.

2.6. Sample collection and transportation

Two hundred sample of Clinical sputum and swab throat was collected using clean wide mouthed container and sterile swab Stick respectively, all collection and labelling samples was done by Hospital laboratory personnel. The samples were then carried out and transported to Microbiology laboratory, Bayero University Kano for identification of target pathogens.

2.7. Bacterial culture

Five different types of media were prepared which included; Nutrient Agar, Blood Agar, Mackonkey Agar, Mannitol salt Agar and Chocolate Agar. Small portion of sputum was inoculated onto each prepared media using sterile wire loop in a zig zag motion. Swab throat was also inoculated as the same procedure and incubated at 37^0C for 24 to 48 hours as described by.[15]

2.8. Gram staining

All suspected colonies were Gram stained and observed microscopically for further clarification using standard procedure.[16]

2.9. Biochemical screening

Expected colonies were identified by the following biochemical Tests; Catalase, Oxidase, Coagulase, Mp/Vp Indole, Hydrogen sulphide, Motility, Urease, Hemolysis and Some sugars (Inulin, Mannitol, lactose).

2.10. Collection and preparation of extract (ginger and garlic tea powdered)

One hundred gram of Garlic and Ginger Tea powder was collected from package Department to research center of Assulfan herbal company. The sample was divided in to two part, each containing 50g of sample powder in a separate bama bottles and labelled as A and B. methanolic and ethanolic extracts were prepared using Cold maceration technique respectively according to the method.[17,18]

2.11. Synergistic effect of ginger and garlic tea extract against isolated respiratory pathogens

Synergistic antibacterial activity was described using standard procedure by.[19] The activity was also determined using agar well diffusion method on a prepared Mueller-Hinton Agar after 24 hours of incubation at 37^0C.

3. Results and Discussions

The present study covered about two sections, the first one we screened bacterial pathogens that may be responsible in the respiratory infections obtained from 200 samples of sputum and swab throat, the other part of study, we used Synergistic garlic and ginger tea extract to evaluate the Antibacterial activity of potential pathogens. The Bacterial screening involved different ways of analysis which included Cultural behavioral characteristics of the Pathogens (Table 1) and numerous biochemical characteristics (Tables 2 and 3) From 200 samples collected,

Table 1: Cultural characteristics

Isolates	Colony Appearance Color. Size(mm)	Gram Stain	Microscopic Appearance
K. Pneumoniae	MW. 3-4 PR. 2-4	Negative	Rod
S. aureus	GY. 1-3 W. 1-3	Positive	Spherical
S. pneumoniae	GR. 0.5-2	Negative	Lanced
H. influenzae	GR. 0.5-1	Negative	Rod
B. pertussis	GW. 0.5-1	Negative	Ovoid

Legends: MW= Mucous white on Nutrient Agar, PR= Pink Red on Mackonkey Agar, GY= Golden yellow on Mannitol Salt Agar, W= White on Nutrient Agar, GR= Gray on Chocolate Agar and Blood Agar, GW= Gray White on Mackonkey and Blood Agar.

Table 2: Biochemical characteristics

Isolates	Ct	Ox	Vp	Ur	Hs	Hm	Mt	Mp	Cg	M	L	I
K. pneumoniae	+	-	+	+	+	-	-	-	-	+	+	+
S.aureus	+	-	+	+	-	+	-	+	+	+	+	-
S. pneumoniae	-	-	-	-	+	+	-	+	-	-	-	+
H. influenzae	+	+	-	-	-	-	-	-	-	-	-	-
B. pertussis	+	+	-	-	-	+	-	-	-	-	-	-

Legends: Ct= Catalase, Ox= Oxidase, Cg= Coagulase, Hs= Hydrogen sulphide, Hm= Hemolysis, Mp= Methyl red, Vp= Vogue's proskauer, Ur= Urease Mt= Motility, F= Fermenter, I= Inulin, M= Mannitol, L= Lactose.

Table 3: Percentage occurrence of respiratory pathogens

Isolates	No. of isolates	% of occurrence
Klebsiella pneumoniae	7	17.07
Staphylococcus aureus	16	39.02
Streptococcus pneumoniae	14	34.15
Haemophilus influenzae	3	07.32
Bordetella pertussis	1	02.44
Total	41	100

Table 4: Synergistic effect of ginger and garlic tea extract on isolated pathogens.

Extracts	Conc.(mg/ml)	KP	SA	SP	HI	BP
Methanolic	150	17±0.7	15±0.7	11±0.9	15±0.0	GNF
	100	10±1.8	12±0.5	10±1.2	13±0.0	GNF
	50	08±0.6	07±0.2	GNF	09±0.7	GNF
Ethanolic	150	14±1.1	15±0.9	13±0.4	12±0.4	GNF
	100	14±0.4	16±0.2	08±1.6	GNF	GNF
	50	10±1.3	12±0.8	07±0.2	07±0.2	GNF
Erythro.	200	14±1.4	18±0.7	17±0.2	12±1.8	10±0.0

Legends: KP= *Klebsiellae pneumoniae*, SA= *Staphylococcus aureus*, SP= *Streptococcus aureus*, HI= *Haemophilus influenza*, BP= *Bordetella pertussis*, Erythro= Erythromycin, GNF= Growth Not Found.

41 isolates were recovered and it was found to be highest from swab throat than sputum, this was an argument with finding of Shailaja et al, who reported Sputum has highest than swab throat.[20] Also study revealed the presence of both Gram negative and positive bacteria with highest appearance of Gram negative strains covered 3/4 of isolates, this finding was an agreement with the study of Maclntyre et al, who reported a high prevalence of Gram negative bacteria from respiratory infection.[21] Refer to theTable 4, the highest percentage Occurrence of bacteria to the least was shown in the following order*; Staphylococcus aureus 16(39.02%), Streptococcus pneumoniae 14(34.15%), Klebsiella pneumoniae 7(17.07%), Haemophilus influenza 3(7.32%) and Bordetella pertussis 1(2.44%). The reason for high* Occurrence of *S. aureus* in this study was proved by many researchers that the isolate were the most common pathogens predominant in a different bacterial habitat.[22] while study showed that the fewer number of *B. pertussis* may be due to control by extensive vaccination program.[23]

For synergistic antibacterial effect of both methanolic and ethanolic extracts of garlic and ginger tea, *all respiratory tract pathogens that wereisolated in the present study were showedthe great antibacterial effect except Bordetella pertussis. K. pneumoniae* has the highest activity of 17±0.7mm at 150mg/ml in methanolic while *S. aureus* in ethanolic with 15±0.2 at 100mg/ml. The Lowest activity

was found to be 07±0.2mm at 50mg/ml for both *H. influenza* and *Streptococcus pneumoniae* . *Statistically using Anova variables, there are no significant differences based on susceptibility pattern of the isolates*.

4. Conclusion

Study revealed that, there is a presence of respiratory pathogens with high bacterial occurrences of *Staphylococcus aureus* from sample analyzed that may likely be an agent for the infection, However all isolated bacteria in the present study have a scientific evidenced to cause respiratory infection. For the antibacterial activity of synergistic Garlic and Ginger tea, both methanolic and ethanolic extract showed significant activity on all tested isolates except *Bordetella pertussis*.

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6. Conflict of Interest

None.

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