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ASSESSMENT OF THE ANTIBACTERIAL PROPERTIES OF COMBINED AQUEOUS EXTRACTS OF FRESH GARLIC, GINGER, BITTER COLA AND HONEY

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ABSTRACT

Ginger, garlic, bitter cola and honey have been used for a long time in the treatment of diseases due to their potential antimicrobial activities. This study was carried out to investigate the antibacterial effect of a combined extract from fresh samples of garlic, ginger and bitter cola and honey using the Agar well diffusion method. The organisms are Escherichia coli, Streptococcus pneumonia and Klebsiella pneumonia. A commercial drug, Gentamicin was used as control. The results shows that all the bacterial species were sensitive to the mixture within the concentration range of 12.5 – 100mg/ml. The maximum antibacterial activities were 22 mm, 23 mm and 26 mm for Streptococcus pneumonia, Klebsiella pneumonia and Escherichia coli respectively. Escherichia coli exhibited the highest sensitivity (26 mm). It was also observed that the higher the concentration, the higher the level of sensitivity. The minimum inhibitory analysis indicates that the mixture inhibited the growth of all the pathogens within the concentration range of 3.125 - 50mg/ml. This research has justified the use of bitter cola, ginger, garlic and honey in traditional medicine in the treatment of infectious diseases caused by micro-organisms.

Key words: Antibacterial, ginger, garlic, bitter cola, honey.

INTRODUCTION

The use of plant extracts in the treatment of diseases has attracted a lot of interest over the years. This is as a result of the fact that micro-organism are developing resistance to many drugs and as such, created a situation where some of the common and less expensive antimicrobial agents are losing effectiveness (Beutler, 2019; Derouiche, 2020). In view of this, there is an urgent need to find the alternatives to chemotherapeutic drugs in disease treatment particularly those of plants origin which are easily available and have considerably less side effects (Chibuzo, 2019). In the past, man has used plants totreat common infectious diseases and even long before mankind discovered the existence of microbes; the idea that certain plant had healing potentials was well accepted (Chibuzo, 2019; Ozioma,&Chinwe, 2019). A medicinal plant is one in which one or more of its parts contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Jain,Khatana&Vijayvergia, 2019). A number of plants have been used in traditional medicine for many years due to their antimicrobial properties (Manandhar, Luitel&Dahal, 2019). Specifically, the medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human or animal body (Najmi, Javed, Al Bratty, &Alhazmi, 2022). The most important of these bioactive constituents which are mainly secondary metabolites are alkaloids, flavonoids, tannins and phenolic compounds (Kaur, & Ahmed, 2021; Agidew, 2022). These phytochemicals are toxic to microbial cells. Medicinal plants generally contain a number of compounds which may be potential natural antibacterials for the treatment of common infections (TatliCankaya, &Somuncuoglu, 2021). Plant derived medicines are relatively safer than synthetic alternative, offering profound therapeutic benefits and more affordable treatment (Berdigaliyev&Aljofan, 2020).

Bitter cola (Garcinia kola) is a flowering plant found mostly in the tropical rain forest region of Central and West Africa. In folkloric medicine, every part such as the seed, stem, and leaves has medicinal value. The seeds are edible and are consumed as an adjuvant to the true kola (cola nitida) and for medicinal purposes (Omodamiro, Ajah&Ewa-Ibe, 2020). In ethnomedicine, Garcinia kola has been used as a purgative, antiparasitic and antimicrobial agent for throat infections, diarrhea, bronchitis and as an aphrodisiac (Kumari, Kumar, & Kumar, 2019)). The plant contains the following active ingredients flavonoids, saponins, apigenin, kolaviron, bifiavonoid-ametoflavone, tannins and resins (Ugwu,Ezeonu,Mbah-Omeje, Agu&Onuorah, 2017). The stem bark and seeds are used for acute fever, cough, and liver disorders and as an anti-vomiting agent (Ugwu et. al, 2017). It is also used as a remedy for inflammation of respiratory tract bronchitis, throat, stomach ache and gastritis (Buba, Okhale, &Muazzam, 2016; Kumari et al, 2019). The seed extract is very efficacious for hepatitis antiseptic and is active against gram positive and negative bacteria (Kigigha,Selekere&Izah, 2018). The decoction of the root is used as aphrodisiac, evacuant and as an anti-cancer agent.

Garlic (Allium Sativum) belongs to a family of Allianceae. Garlic is widely use in culinary, as well as in medicine (Oosthuizen, Reid &Lall, 2018; Nasir,Fatma, Neshat, & Ahmad, 2020). It has a pungent hot flavor but mellows and improves with cooking. It has been utilized to fight infections such as cold, cough, asthma, diarrhea, flu, headache, sore throat, abdominal discomfort and respiratory tract infections (Poddar, Sarkar, Choudhury, Chatterjee & Ghosh, 2020; Aware &Rohane, 2021).

Ginger (Zingiberofficinale) is a flowering plant whose rhizome, ginger root or ginger is a herbaceous perennial which grows annual pseudostems, about one meter tall bearing narrow leaf blades. Ginger extracts is one of those medicinal plants that played a huge role in the treatment of different diseases. Ginger has strong properties that kill or inhibit the growth of pathogenic

bacteria. Investigations have also revealed that ginger has active components that inhibit the multiplication of digestive bacteria (Yadufashije, Niyonkuru, Munyeshyaka, Madjidi, &Mucumbitsi, 2020).

Honey is a sweet liquid made by bees using the nectar from flowers. Honey has high levels of monosaccharides, fructose, and glucose. It contains about 70 to 80 percent sugar, which provides its sweetness (Mora, &Dando, 2021; Sharma, Thakur,Rana, Devi, &Bajiya, 2023). Honey also has antiseptic as well as antibacterial properties and used in managing chronic wound and combating infections.

The antimicrobial activities of ginger, garlic, bitter cola and honey against various pathogens have been studied (Amalu, Chukwueze,&Ugwu, 2014; Deepa,&Vrinda, 2015;Kigigha,Selekere&Izah, 2018; Hbibi, Sikkou, Khedid, El Hamzaoui, Bouziane&Benazza, 2020). The use of these agents in the treatment of disease has become an important area of interest over the years. This is as a result of the fact that microorganism are developing resistance to many drugs. This study therefore intends to test the efficacy of a combination of ginger, garlic, bitter cola and honey against some selected bacteria.

EXPERIMENTAL

Materials

The following materials were used for the research;

(i) Weighing balance

(ii) Oven

(iii) Mortar and pestle

(iv) Distilled water

(v) Incubator

(vi) Nutrient agar

(vii) Gentamycin

Sample collection and preparation

Fresh samples of garlic, bitter cola, ginger and honey were purchased from the market. The garlic, bitter cola and ginger were thoroughly washed separately and sliced after which they were dried in an oven at 70^0C. The samples were then ground separately, after which 2.5g of each of the samples were weighed into a 200ml beaker containing 100cm^3 of distilled water and stirred thoroughly. The sample was then filtered to obtain the crude extract which was then mixed with 50 cm^3 of honey and stirred. This mixture was then packaged in a sample bottle prior to analysis.

Analysis of Samples

- **Invitro-antibacterial test using the agar diffusion method**

(a) Standardization of Inoculums

Three (3) microorganisms namely: Escherichia coli, Streptococcus pneumonia and klebsiella pneumonia were used for testing the antibacterial activity. The organisms were collected from a clinical laboratory. The pathogens were sub-cultured to nutrient agar (NA) slants using a wire wop (done aseptically) and incubated by turbidity. The turbidity produced was adjusted to match 0.5 Mc Farmland (100^8Cfu/ml) which was further adjusted to 10^5 (Cfu/ml).

(b) Inoculation of the Plates and Application of the Extract

To inoculate the plate, one drop of the adopted sub-cultured broth was applied to the surface of the nutrient agar with microbes. One microbe was incubated to one plate making a total of three (3) microorganisms. After 30 minutes four wells were punched on the plate using a

sterile cork borer of 5 mm diameter, two for the water extract, one for negative and one for positive control. A 0.1 ml of the solvent (equivalent to 20mg of the extract) was dropped into each of appropriate labeled wells into the remaining two wells, distilled water and the tricycle of the same concentration as the extract was to serve as negative or positive controls for the bacteria.

The inoculation was left on the table for 1 hour to allow for proper diffusion. Agar plate were incubated aerobically at 37^0C while the sabouraud dextrose agar was incubated for 48 hours at 25^0C. Zone of inhibition produced after incubation was measured by linear measurement of diameter.

(c) Minimum Inhibitory Concentration

The minimum, inhibitory concentration was determined using the tube dilution method by preparing different concentrations of the mixture (100mg/ml). Cleaned test tubes where taken and the volume of medium made up to 20ml with nutrient broth. The control was prepared with 2ml of nutrient broth without the extract. Both were then sterilized at a temperature of 121^0C for 15 minutes in an autoclave.

After sterilization the medium was allowed to cool and 0.2ml of overnight culture of each organism was dispensed into sterile medium and incubated for 48 hours. The activity was measured by turbidity for inhibition and growth of the bactericidal.

(d) Antimicrobial Assay

20ml of media was poured in petriplates and allowed for solidification. The microbial culture was made using sterile cotton swab and labeled. The wells were made in the media with the help of a cork borer with centers of at least 24mm. The recommended concentration of 50ml of the test sample and 100mg/ml of water was introduced in the respective wells. Other wells

were supplemented with reference antibacterial drug (Gentamicin).The agar plates were incubated aerobically at 37^0C for 24 hours. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with that of the drug, Gentamicin.

RESULTS

The results of the minimum inhibitory concentration and antibacterial activity are presented in tables 1-2 below.

TABLE 1: RESULTS OF THE MINIMUM INHIBITORY CONCENTRATION (MIC)

ORGANISM	CONCENTRATION OF EXTRACT (%)						EXTRACT	MIC (%)
	100	**50**	**25**	**12.5**	**6.25**	**3.125**		
EC	-	µ	+	+	+	+	FRESH	50
SP	-	µ	+	+	+	+	FRESH	50
KP	-	µ	+	+	+	+	FRESH	50

Key: **EC-***Escherichia coli*, **SP-***Streptococcus pneumoniae*, **KP-***Klebsiellapneumoniae*,

Key

(+)= Visible turbidity

(-)= No visible turbidity

(µ) = min inhibitory concentration

Control: Gentamicin 20mg/ml

TABLE 2: RESULTS OF THE ANTIBACTERIAL ACTIVITY

ORGANISM	CONCENTRATION OF EXTRACT (%)/DIAMETER OF ZONE OF INHIBITION (MM)				EXTRACT	CONTROL
	100	**50**	**25**	**12.5**		**Gentamycin (20 mg/ml)**
EC	26	24	15	12	FRESH	33

SP	22	18	14	8	FRESH	24
KP	23	20	18	10	FRESH	32

EC-*Escherichia coli*, **SP**-*Streptococcus pneumoniae*, **KP**-*Klebsiellapneumoniae*

DISCUSSION

Minimum Inhibitory Concentration

The minimum inhibitory analysis of thefresh crude extracts from ginger, garlic, bitter cola and honey mixture was tested using three (3) bacterial pathogens namely;Escherichia coli, Streptococcus pneumonia andKlebsiellapneumonia. The results (Table 1) indicates that the mixtureinhibited the growth of all the pathogens only within the concentration range of3.125 - 50mg/ml. These results are in line with similar analysis carried by Ogodo and Ekeleme, (2013). They investigated the antibacterial activity of ginger and garlic extracts using cold water and ethanol extracts on Escherichia coli, Staphylococcus aureus, Salmonella species and Bacillus cereus.

A related study was also carried out by Deepa and Vrinda (2015). They investigated the antibacterial effect of ginger and garlic extracts on Escherichia coli and Listeria moonocytogenes by spectrophotometric method. They observed that both spices exhibited bacteriostatic effect against both test organisms. The inhibitory effect increased with increase in the concentration of the extracts. The garlic extract was more effective against E.coli thanL.moonocytogenes. The minimum inhibitory effect of the ginger extract was found to be 8.7mm and 8.5mm for E. coli and L.moonocytogenes respectively whereas in the case of garlic extract, it was 9.1mm and 8.0mmrespectively. They concluded that both spices possess a good potential to act as natural preservatives.

Antibacterial Activity

The results of the antibacterial analysis (Table 2) indicates that all the bacterial species were sensitive to the mixture within the concentration range used(12.5 – 100mg/ml). The maximum antibacterial activities were 22mm, 23mm and 26mmforstreptococcus pneumonia, klebsiellapneumonia and escherichia coli respectively. E. coli exhibited the highest sensitivity (26mm). It was also observed that the higher the concentration, the higher the level of sensitivity. Gentamicin was used as a control.These findings are also in line with similar studies carried out by Ogodo and Ekeleme (2013) who investigated the antibacterial activity of garlic, cloves and ginger rhizomes on selected food-borne pathogens using the agar well diffusion method. The organisms were Escherichia coli, Staphylococcus aureus, Salmonella species and Bacillus cereus. Their results also demonstrated different levels of sensitivity to the extracts.

CONCLUSION

The antibacterial activity of crude extracts from a mixture of fresh ginger, garlic and bitter cola in honey was evaluated using three (3) bacterial pathogens namely;Escherichia coliStreptococcus pneumonia and Klebsiella pneumonia. A commercial drug, Gentamicin was used as control. The data obtained from this findings revealed that the synergy of bitter cola, garlic, ginger and honey has medicinal potentials as antibacterial agents. It was also discovered that the inhibition of the bacterial growth was dose dependent. Finally, the research has justified the use of bitter cola, ginger, garlic and honey in traditional medicine in the treatment of infectious diseases caused by micro-organisms.

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