Potential inhibitory effects of some African tuberous plant extracts on Escherichia coli, Staphylococcus aureus and Candida albicans

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Abstract
Potential inhibitory effects of three African tuberous plant extracts of Zingiber officinale Rosc., Curcuma longa L. and Dioscorea bulbifera L. was investigated. Ethanol and cold water were used as solvents for extraction. Three human pathogens, Escherichia coli, Staphylococcus aureus and Candida albicans were employed in this study. The potential inhibitory effects of the ethanol and aqueous extracts of Zingiber officinale, Curcuma longa and Dioscorea bulbifera on the test organisms were conducted using the disc-diffusion method of antimicrobial assay. Phytochemical screening of the plants was conducted using different standard methods. Standard antibiotics disc were used as positive control while discs impregnated in sterile distilled water were used as negative control. The ethanol extracts proved to be more potent than the aqueous extract. The potency of the extracts varied with solvent of extraction and concentration of the plant extracts. The minimum inhibitory concentration (MIC) of the ethanol extract ranged between 0.5-1.0 mg/ml on E. coli, Staphylococcus aureus and Candida albicans. For the aqueous extract, MIC was 1.0 mg/ml on E. coli and ranged between 0.5-1.0 mg/ml on S. aureus and C. albicans. The solvent used for extraction varied significantly (P<0.05) to the three test organisms. Staphylococcus aureus was more susceptible to the extract while Candida albicans was least inhibited. In all, Concentration of 1.5 mg/ml gave the maximum inhibition on the three test organisms. Phytochemical (qualitative) screening of the plants revealed the presence of biologically active chemical compounds such as tannins, Phenols, Saponins, alkaloids, flavonoids and Steroids/ triterpenes. The quantitative determination of the phytochemicals present revealed different levels of concentrations of the phytochemicals present. The significance of these findings are discussed in relation to phytochemicals as a means of disease control and the substitution of plant extracts as potential antimicrobial drug to the resistance of the human pathogens.

Keywords: Inhibition, Curcuma, Dioscorea, Zingiber, Pathogen.

INTRODUCTION

Medicinal plant is a therapeutic resource used by the traditional population of the African continent specifically for healthcare and which may also serve as precursors for the synthesis of useful drugs (Sofowora, 1993). Also, the World Health Organization (WHO, 2001), defined a medicinal plant as any herbal preparation produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological process which may be produced for immediate consumption or as a basis for herbal products. The term “Herbal drug” determines the part/parts of a plant used for preparing medicines (for example: leaves, flowers, seeds, roots, barks, stem, etc).

Studies on the use of plant extracts for control of diseases have shown the importance of natural chemicals (phytochemicals) as possible sources of non-phytotoxic and easily biodegradable alternative fungicides and antibiotics (Akueshi et al., 2002; Okigbo and Mmeka, 2008). Against this background, three African tuberous plants were selected for this research work based on ethnobotanical evidence of the plants to evaluate their potential inhibitory effects against Staphylococcus aureus, Escherichia coli and Candida albicans.

Zingiber officinale Rosc. (Ginger) of the family Zingiberaceae, is a herbaceous perennial plant which consists of an underground stem or rhizome, bearing...
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erect leafy shoots (Chandarana et al., 2005). *Curcuma longa* L. (Turmeric) of the family Zingiberaceae, is a perennial herb consisting of short and thick underground rhizomes and reduced stems, bearing tufts of large, broad, bright green leaves (Chandarana et al., 2005).

*Dioscorea bulbifera* L. belonging to the family Dioscoreaceae is a herbaceous annual climbing plant with aerial tubers or bulbils (Burkill, 1960). It is a monocotyledonous plant and the vine which is circular in cross section, twines anticlockwise. The leaf is simple and large. The leaves are arranged alternately and the base of the petiole is enlarged at the base encircling the node as auricles.

Many human pathogens require serious attention in developing countries like Nigeria (Okigbo and Omodamiro, 2006; Okigbo and Igwe, 2007). These include, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. There are various measures of control employed against these pathogens which include; sterilization, use of chemicals, use of antibiotics (Singleton 1997; Okigbo et al., 2005) and biological measures. However, the prevalence of microbial resistance to existing antimicrobial drugs underscores the need for the continuous search for new antimicrobials (Olorundare et al., 1998). One of the avenues for such search is to screen medicinal plants for microbical activities (Okigbo and Omodamiro, 2006).

This research work studied the potential inhibitory effects of three African tuberous plant extracts of *Zingiber officinale* Rosc., *Curcuma longa* L. and *Dioscorea bulbifera* L. on selected human pathogens. The effectiveness of aqueous and ethanol extracts of the tuberous plants were also studied against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, to access the possibility of inhibition of the diseases using plant extracts.

**MATERIALS AND METHODS**

**Sources of plant materials**

The test plants; *Zingiber officinale* Rosc. (UGl variety) and *Curcuma longa* L. (Vom variety) rhizomes were collected in September, 2007 from Ginger and other Root Crops program of the National Root Crops Research Institute (NRCRI), Umudike, Abia State, Nigeria. The bulbils of wild *Dioscorea bulbifera* L. was collected in September, 2007 from the bush adjacent, 2 km from the fence of the Eastern farm of NRCRI, Umudike, Abia State. The plants were identified and authenticated by Prof. C.U. Okeke of the Botany Department, Nnamdi Azikiwe University (N.A.U), Awk, Nigeria. The plants were assigned with voucher numbers as NAU/BOT/0118 for *Z. officinale*; NAU/BOT/0119 for *C. longa* and NAU/BOT/0120 for *D. bulbifera*.

**Test organisms**

The test bacterial pathogens, *Escherichia coli* and *Staphylococcus aureus* and a fungus, *Candida albicans*, used for the *in vitro* antimicrobial activity were obtained from the Diagnostic Laboratory of the National Veterinary Research Institute (NVRI) Umudike sub-station, Abia State, Nigeria.

**Sample preparations**

The ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) rhizomes and *Dioscorea bulbifera* samples were first examined visually for any sign of disease. The healthy samples were washed thoroughly under running tap water to remove residual soil debris. They were cut, spread on a laboratory tray and dried in the oven (Carbolite, England) at 65°C. The dried samples were ground into powdered form using a laboratory mill (Arthur Thomas, USA). The ground samples were sieved through 1 mm test sieve. The samples obtained were stored in air-tight bottles until required for analysis.

**Quantitative yield of extract from test plants**

To determine the quantitative yield of the extracts, the dish was weighed while empty and its weight recorded. The weight of the dish and its content after evaporation in the water bath was recorded. The yields of the extract were calculated using this formula:

\[
\% \text{ yield } = \frac{W_1 - W_3}{W_2} \times \frac{100}{20 \text{ g}}
\]

Where:
- \(W_1\) = weight of sample used
- \(W_2\) = weight of dish + pasty extract
- \(W_3\) = weight of empty dish.

**Extraction of plant material**

20 g of each sample of *Z. officinale*, *Curcuma longa* and wild *D. bulbifera* was weighed from the prepared samples stored in air-tight bottle. This was used for the extraction technique.

**Ethanol extraction and aqueous extraction (cold water)**

20 g of the samples were weighed into conical flasks and soaked in 150 ml of respective solvent. The top of the flasks were stopped with rubber corks and was agitated before it was left to stand for 24 h undisturbed. They were filtered with Whatman Filter Paper (No 42) into a beaker and the filtrate was evaporated over a
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water bath. The standard extract obtained was sealed with aluminum foils and stored in the refrigerator at 4°C until required for antimicrobial activity.

**Test for purity of extracts**

Each of the extracts obtained was tested to ensure its purity by streaking it separately onto sterile plates containing either Potato dextrose agar or nutrient agar. The plates were incubated at 37°C for 24 h (Cheesbrough, 2000) and was examined for possible growth of contaminants, the absence of which confirms the purity of the test extracts.

**Culture media preparation**

Culture media used were nutrient agar (NA) for bacteria and Potato dextrose agar (PDA) for fungi. These were prepared according to the specification by the manufacturers. The prepared medium were sterilized in the autoclave at 121°C and 1.0 kg/cm² for 15 min (Cheesbrough 2000; Jawetz et al., 2004).

**Preparation of antimicrobial discs**

The porous paper discs used in the various sensitivity tests were prepared using the method described by Cheesbrough (2000) and Jawetz et al. (2004). With the aid of an office paper perforator, circular discs (5mm) were cut from Whatman filter paper (No 1). The discs were boiled in distilled water for an hour, to remove any residual preservatives contained in the disc. The boiled discs were drained dry. They were put in McCartney bottle and sterilized in the autoclave (Cheesbrough, 2000) at 121°C, 1.0 kg/cm² for 15 min.

**Antimicrobial sensitivity testing**

The antimicrobial activity of the extract was determined by the agar disc diffusion technique delineated by Cheesbrough (2000) and Jawetz et al. (2004). The tests were conducted with the authenticated pure cultures of the test pathogens (E. coli, S. aureus and C. albicans) to determine their respective tolerance of the extract. Sterile agar plates were aseptically inoculated with a loopful of the test pathogens. Each inoculum was streaked evenly over the surface of the agar plate using a flame glass Jockey (Pelczar and Chan, 1977), by the spread Plate technique described by Singleton (1997).

Using a flamed pair of forceps, the antimicrobial sensitivity discs prepared were embedded in the respective reconstituted extracts. Reconstitution of the dry extracts was achieved by mixing it with drops of sterile distilled water to form a viscous paste. The mixture was left to stand for 3 h to allow the paper discs absorb the extract and was allowed to dry in the oven. The discs were carefully placed on the surface of the inoculated plates at a distance away from each disc to prevent over lapping, and allowed to stand for 5 min (to enable the extract permeate into the medium) before being incubated at 37°C for 24 h.

The plates were observed for the presence of inhibition zones around the extract-impregnated discs. The extent of inhibition was determined by measuring the diameter of the inhibition zone using a transparent ½ meter rule. Measurements were made across the paper discs thus including its diameter. The mean zone of inhibition of the three replicated tests (triplicate analysis) of the plant extracts on the test organism is expressed in millimeters. The discs were soaked /impregnated with an equivalent volume of sterile distilled water. This was used as a negative control.

**Antibiotic disc used**

An already made gram-positive and gram negative (Optun laboratories, Nig. Ltd, Aba, Nigeria) antibiotics sensitivity disc was bought from the pharmaceutical shopping store. Antibiotic disc used and their concentrations were as follows: Gram positive discs contained; Ciprofloxacin (10 µg), Norfloxacin (10 µg), Gentamycin (10 µg), Lincomycin (20 µg), Streptomycin (30 µg), Rifampicin(20 µg), Erythromycin (30 µg), chloramphenicol (30 µg), Ampiclox (20 µg) and Floxapen(20 µg). The gram negative discs contain, Tarivid (10 µg), Peflacine (10 µg), ciproflox (10 µg), Augumentin (30 µg), Gentamycin (10 µg), Stretomycin(30 µg), ceporex (10 µg), Nalidixic acid (30 µg), Seprin(30µg) and Ampicilin (30 µg). The gram positive disc was used on the culture of Staphyllococcus aureus while gram negative disc was used on Escherichia coli. The Antibiotic discs served as positive control.

**Minimum inhibitory concentration (MIC) determination**

The experiment was done with each extract to determine the least concentration which can inhibit the growth of each test pathogens in vitro. The MIC was determined by the disc diffusion method. To achieve this, each extract (from each test sample and test solvent) was reconstituted to contain specific amount of the extracts forming a series of concentrations (0.5 mg/ml, 1.0 mg/ml and 1.5 mg/ml) per discs. The disc bearing different concentrations of the different extract were used for the sensitivity tests. This was incubated for 24 h at 37°C. The least concentration which produced clear inhibition zone, was recorded as the minimum inhibitory concentration (MIC). Experiments were replicated three times and the mean value of each extract was observed and recorded.
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Phytochemical screening of the test plants

Both qualitative tests and quantitative tests were conducted using different standard methods as described by (Harborne, 1973; Pearson, 1976) to establish the qualitative presence of phytochemicals of interest.

Qualitative tests
1. **Test for Tannins**: The ferric chloride test described by Harborne (1973) was employed.
2. **Test For Flavonoids**: The presence of flavonoids in the test samples was determined by the acid alkaline test described by Harborne (1973).
3. **Test for Saponins**: The presence of saponins in the test samples was determined by the froth test as well as the emulsion test described by Harborne (1973).
4. **Test for Alkaloids**: The presence of alkaloids in the test samples was determined using the tests described by Harborne (1973).
5. **Test for Sterols/Triterpenes**: 2 mls of ethanol extract was dispensed into a test tube and 1 ml of acetic anhydride was added to the mixture. 1 ml of concentrated hydrogen tetraoxosulphate (VI) acid (H$_2$SO$_4$) was added carefully by the side of the test tube. A brown coloration at the interface of the two layers was indicative of the presence of sterols /triterpenes. The test was repeated two more times and was conducted against a blank that did not contain the sample.
6. **Direct test for Hydrogen Cyanide (HCN)**: 1g of the sample was weighed into a conical flask and 100ml of water was added. Picrate paper was suspended over the mixture by hanging. This was allowed to incubate overnight. Change in color was observed from yellow towards orange. The picrate paper was eluted in 60 ml of water. The absorbance was read at 540 nm in a spectrophotometer (Jenway 6061, England).

Quantitative tests
Quantitative analysis of the test samples was carried out using the methods described by Harborne (1973), Pearson (1976).

1. **Determination of Flavonoids**: This was determined by gravimetry using the method of Harborne (1973).
2. **Determination of Tannins**: The Follins-Dennis spectrophotometric method (Pearson 1976) was used.
3. **Determination of Alkaloid**: The quantitative determination of alkaloid was carried out by the alkaline precipitation through gravimetric method described by Harborne (1973).

4. **Determination of Saponins**: Saponin Content of the sample was determined by double solvent extraction gravimetric method (Harborne 1973).
5. **Determination of Phenols**: The Follins Dennis method described by Pearson (1976) was used to determine the phenol content of the samples.

Statistical analysis
The statistical analysis used in this research work are based on methods in standard textbook (Wahua, 1999) and a computer package (SAS, 1999). These include standard deviation of means, analysis of variance (ANOVA) and correlation analysis with appropriate test of significance. Least significant difference (LSD) at 0.05 probability level was used to separate the means. The design and analysis of each replicated experiment is shown in the appendix.

RESULTS

Yield of extracts
The yield of the extracts (ethanol and aqueous) was calculated and recorded as percentage of each plant material (Table 1 [Supplementary data]). The ethanol extract gave the highest yield in Zingiber officinale (5.25%) and Dioscorea bulbifera (4.06%) while the least yield (3.46%) was recorded by Curcuma longa. The aqueous (cold water) extract of Z. officinale gave (4.06%) yield followed by D. bulbifera and C. longa with (3.88%) and (2.54%) respectively (Table 1). The extraction medium affected the percentage yield of the extracts significantly (P<0.05). Z. officinale and D. bulbifera had higher yield than C. longa and the differences between them were significant (P < 0.05).

Antimicrobial activity of the plant extracts on the test organisms
The results of the sensitivity tests on Staphylococcus aureus, Escherichia coli, and Candida albicans using extracts of Zingiber officinale, Curcuma longa and Dioscorea bulbifera showed that the organisms were sensitive to all the plant extracts. The antimicrobial activities of the ethanol and cold water extracts of the plant materials showed that the plant extracts inhibited all three test organisms in vitro.

The ethanol extract of Zingiber officinale produced the highest zone of inhibition on Staphylococcus aureus, Candida albicans and Escherichia coli (Table 2 [Supplementary data]). The aqueous extracts of Zingiber officinale and Dioscorea bulbifera inhibited the growth of Escherichia coli much more intensely than the ethanol extract. On the other hand, the ethanol extract of Z. officinale, C. longa and D. bulbifera were
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more potent than the aqueous extracts on *Staphylococcus aureus* and *Candida albicans* (Table 2).

There was significant difference (P<0.05) in the mean zone of inhibition produced by the ethanol extracts of *Z. officinale*, *C. longa* and *D. bulbifera* on the inhibition of *S. aureus* and *C. albicans* in vitro (Table 2). Also the effect of all three plant extracts on the inhibition of *S. aureus* and *C. albicans* was not significant (P>0.05), implying that the three extract, *Z. officinale*, *C. longa* and *D. bulbifera* equally inhibited the growth of the test organisms effectively (Table 2).

In the negative control where sterile distilled water was used, there was no inhibition. The antibiotics discs used as positive control showed activity against the test bacterial microorganisms. The mean zone of inhibition of each drug is recorded in (Table 3 [Supplementary data]).

**Minimum inhibitory concentration (MIC) test**

The MIC was determined with the ethanol and aqueous extracts of the plant samples using the agar disc diffusion method. The ethanol extracts of *Z. officinale*, *C. longa* and *D. bulbifera* showed different zones of inhibition when different concentrations of the extract was used against *E. coli*, *S. aureus* and *C. albicans* (Table 4 [Supplementary data]). Also the aqueous extracts of the plant material gave varying zones of inhibition in different concentrations on the test organisms (Table 5 [Supplementary data]).

**Effect of extraction medium and concentration rates on the test organisms**

The effect of concentration of extracts on the test organisms was significant (P<0.05). The diameter of the zone inhibition increased as the concentration of the extracts increased (i.e. 50mg/ml > 100mg/ml > 150mg/ml).The interaction of extraction medium and concentration of extracts was significant (P<0.05) on the inhibition of the test organisms, *S. aureus*, *E. coli* and *C. albicans*.

Aqueous extracts of *Z. officinale* gave the highest inhibition of *E. coli* followed by *D. bulbifera*, *C. longa* had the least inhibition on *E. coli*. The inhibitory effect of *Z. officinale* was significantly (P<0.05) higher than that of *D. bulbifera* and *C. longa* extracts (Table 6 [Supplementary data]). The ethanol extract of *D. bulbifera* gave the highest zone of inhibition on *E. coli* followed by *Z. officinale* while *C. longa* had least inhibition on *E. coli*. There was no significant difference (P>0.05) between the inhibitory effect of *D. bulbifera* and *Z officinale*.

The aqueous extracts of *Zingiber officinale* had the highest inhibitory effect on *Staphylococcus aureus* followed by *C. longa* and *D. bulbifera* (Table 7 [Supplementary data]). The inhibitory effect of *Z. officinale* was significantly (P<0.05) higher than that of *C. longa* and *D. bulbifera* (Table 7).

Aqueous extracts of *C. longa* had the highest effect on *C. albicans*, followed by *Z. officinale* and *D. bulbifera* (Table 8). The inhibitory effect of *C. longa* was significantly (P<0.05) higher than that of *D. bulbifera* and *Z. officinale* (Table 8). The ethanol extract of *Z. officinale* gave the highest inhibition zone on *Candida albicans*. This was significantly (P<0.05) different from the inhibitory effect of *C. longa* extract and no inhibition by *D. bulbifera*. Extract concentration of 150mg/ml gave the highest inhibitory effect. This was significantly different (P<0.05) from the inhibitory effect of concentration of 100 mg/ml and 50 mg/ml (Table 8 [Supplementary data]).

**Phytochemical screening of test plants**

**Qualitative test**

Phytochemical analysis of *Zingiber officinale*, *Curcuma longa* and *Dioscorea bulbifera*, tested positive for the presence of saponins, flavonoids, steroids/triterpenes, alkaloids, tannins and phenols.

**Quantitative test**

The phytochemical constituents (Table 9 [Supplementary data]) showed that the quantity of alkaloids in *C. longa* was significantly (P<0.05) higher than that in *Z. officinale* and *D. bulbifera*. *Z. officinale* contained more flavonoid than *C. longa* and *D. bulbifera* and the difference between the flavonoid content of *Z. officinale* and *C. longa* is significant (P<0.05). *C. longa* contained saponin which is significantly (P<0.05) higher than that of *Z. officinale*. There was no significant difference (P>0.05) between the saponin contents of *Z. officinale* and *D. bulbifera*. The hydrogen cyanide (HCN) content of *D. bulbifera* was significantly (P<0.05) different from *C. longa* and *Z. officinale*. The tannin content of *C. longa* was significantly (P<0.05) higher than that of *D. bulbifera* (0.53%) and *Z. officinale* (0.67%). More phenol was detected in *D. bulbifera* (0.17%) than *C. Longa* (0.11%) and *Z. officinale* (0.08%) and the differences between them were significant (P<0.05) (Table 9).

**Correlation coefficient (r) of phytochemical properties of the test plants**

Correlation analysis (Table 10 [Supplementary data]) showed that the correlation between the following pairs of phytochemicals are significant at 0.05 level of probability. Flavonoid and tannin (r=-0.7809). At (P<0.01) level of probability, flavonoid and saponin (r=-0.8645), flavonoid and alkaloid (r=-0.8012), phenol
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and HCN (r=0.8735), Saponin and HCN (r=0.8113), alkaloid and HCN (r=0.9153) and HCN and tannin (r=0.9229) are significant (P<0.01). Also saponin and alkaloid (r= 0.9646), saponin and tannin ( r=0.9656) and alkaloid and tannin (r=0.9970) are significant at (P<0.10) level of probability.

A positive and significant correlation coefficient indicates that any two phytochemicals are likely to be present in the test plant, while a negative and significant correlation coefficient indicates that as one phytochemical increases, the other decreases. While phenol/HCN, saponin/alkaloid, saponin/ tannin and alkaloid/tannin are likely to occur in the same plant samples; flavonoid/saponin, flavonoid/alkaloid, flavonoid/tannin, saponin/ HCN, alkaloid/HCN and HCN/tannins are not likely to occur together.

DISCUSSION

The potential inhibitory effects of three African tuberous plant extracts of Zingiber officinale Rosc; Curcuma longa L. and Dioscorea bulbifera L. was evaluated on the selected human pathogens – Escherichia coli, Staphylococcus aureus and Candida albicans. The results of this study show that the ethanol and aqueous extracts of the plants were inhibitory on the test organisms in vitro. This is in line with observations made by Chandarana et al. (2005); Okigbo and Igwe (2007).

The yield of the extracts differed with the plant material, solvent of extraction and extraction technique. The ethanol extracts gave higher yield in Zingiber officinale than Dioscorea bulbifera and Curcuma longa. This may be because ethanol is an organic solvent and will dissolve organic compounds better, hence liberate the active compounds required for antimicrobial activity (Okigbo and Emoghene, 2004; Okigbo et al., 2005; Ekwenye and Elegalam, 2005). Also aqueous extracts of Zingiber officinale and Dioscorea bulbifera gave higher yields than Curcuma longa.

The potential inhibitory effects of the extracts on Staphylococcus aureus, Escherichia coli and Candida albicans varied with solvent of extraction. It was observed that the ethanol extracts of Dioscorea bulbifera gave the widest zone of inhibition on Staphylococcus aureus followed by Zingiber officinale with and least in Curcuma longa extracts. The aqueous extracts of Z. officinale showed more potency against E. coli than the ethanol extracts. Comparatively, the control drug (i.e. commercial standard antibiotics) used as positive control, gave wider zones of inhibition than the plant extracts. This may be that the active compound of the crude extracts diffuses slowly through the paper disc. Likewise, the sterile paper discs used as negative control did not show any inhibition on the test organisms. This is in line with observations made by Chandarana et al. (2005); Okigbo and Igwe (2007).

The plant extracts were more inhibitory to Staphylococcus aureus followed by Escherichia coli and Candida albicans. This is in accordance with reports of Desta (1993); Okigbo and Mmeka (2008). They reported that plant extracts show stronger retardation effects on the gram positive test strain than on the gram negative ones. It can also be observed in the standard antibiotics used in which Gentamycin and Streptomycin had more effect on Staphylococcus aureus than on Escherichia coli.

The antimicrobial property of these plants justifies their use by traditional medical practitioners for the treatment of various ailments (Gill, 1992; Okigbo and Mmeka, 2006). In humans, Zingiber officinale is thought to directly act on the gastrointestinal system to reduce nausea (Yamahara, 1990). It has anti-inflammatory (Verma et al., 1994), antioxidant (Shobana and Naidu, 2000) and antimicrobial (Foster 2000; Wood et al., 2001) properties. According to Gill (1992), the juice of the rhizome with honey is a very efficacious remedy for cough and asthma. Curcuma longa has antioxidant (Remirez-Tortosa et al., 1999); anti-inflammatory (Wood et al., 2001), anti tumor, anti spasmodiac, anti-HIV and anticancer (Manikandan et al., 2006) properties.

The inhibitory effects of the plant extracts were highly dependent on concentration as reported by Ekwenye and Elegalam (2005), Okigbo and Igwe (2007). At higher concentration wider zone of inhibition was produced which reduced as the concentration reduces. This coincides with observation made by Okigbo and Omodamiro (2006) in the effects of aqueous, petroleum-ether, ethanol and chloroform/methanol extracts on the diameter zone of inhibition (mm) at varying concentrations (mg/ml) of both dry and fresh leaf extracts of pigeon pea (Cajanus cajan (L.) Millsp) on some human pathogens.

The Minimum Inhibitory Concentration (MIC) was done using the disc diffusion method. The aqueous extracts of Zingiber officinale, Curcuma longa and Dioscorea bulbifera had 1.0 mg/ml as the minimum concentration to inhibit E.coli. Staphylococcus aureus was inhibited by all the test concentrations of Z. officinale, and Curcuma longa. With the ethanol extracts; Z. officinale inhibited all the test organisms at all concentrations. Curcuma longa inhibited E.coli and C. albicans at a minimum concentration of 1.0 mg/ml. There was no inhibition on Candida albicans by the three test concentrations of the ethanol extracts of Dioscorea bulbifera. The effect of extraction media and concentration of the extracts of Z.officinale, C. longa and D. bulbifera on the inhibition of Staphylococcus aureus, E. coli, and Candida albicans was pooled...
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together. The interaction of extraction medium and concentration of extracts was significant (P<0.05) on the inhibition of the test organisms. In all, the aqueous and ethanol extracts at 150 mg/ml concentration gave the highest inhibitory effect on the test organisms.

The inhibitory effect of plant extracts of Zingiber officinale, Curcuma longa and Dioscorea bulbifera is probably due to the presence of phytochemicals (ie bioactive ingredients) which are antimicrobial agents that are inhibitory to the growth of these pathogens (Etani 1998; Okigbo and Ajalie, 2005). Phytochemical (qualitative) screening of the plants revealed the presence of alkaloids, flavonoids, tannin, saponin, phenols and steroids/triterpenes. Equally, phytochemical (quantitative) determination showed enormous quantity of alkaloids in Curcuma longa, Zingiber officinale and Dioscorea bulbifera. Alkaloids are reported to be toxic and often elucidate strong physiological changes in the body; hence they are widely used in medicine (Harborne, 1973; Sofowora, 1993) for their analgesic, antispasmodics and antibacterial effects (Stary, 1998; Okwu and Okwu, 2004). Flavonoids are antioxidants and also provide anti-inflammatory activity (Okwu, 2005). Tannins possess astringent properties while saponins are used as expectorant and emulsifying agents in medicine (Okwu and Okwu, 2004; Okwu, 2005). Phenols are antimicrobial agents which are inhibitory to the growth of pathogens (Okwu and Okwu, 2004).

The phenolic compounds, Curcuminoids present in Curcuma longa (Chandarana et al., 2005) is the major yellow pigment that has been isolated from the ground rhizome of Curcuma species (Manikandan et al., 2006). Curcumin exhibit a wide array of pharmacological and biological activities which include antioxidant (Kunchandy and Rao, 1990) and anti-inflammatory activity (Shih and Lin, 1993). Curcuma longa (turmeric) oil isolated from CRTO (i.e. Curcumin removed turmeric oleoresin) was found to be both antibacterial (Negi et al., 1999) and antifungal (Jayaparakasha et al., 2001).

A correlation of the different phytochemical constituents of Zingiber officinale, Curcuma longa and Dioscorea bulbifera against one another indicates a positive and significant relationship between various pairs of phytochemicals. This shows that they are likely to be present in the test plant. These phytochemicals confer antimicrobial activity on the plant extracts. The difference in antimicrobial properties of the plant extracts is attributable to the age of the plant used, freshness of the plant materials, physical factors (temperature, light, water) and contamination by field microbes (Calixo, 2000; Okigbo and Omodamo, 2006; Okigbo and Igwe, 2007).

CONCLUSION

From the results obtained in this research work, it is evident that Zingiber officinale, Curcuma longa and Dioscorea bulbifera possess potential inhibitory activity against human pathogens in vitro to varying degrees. The activities of these extracts were below those of standard antibiotics. This relatively poor performance was attributed to the fact that it was the crude extract that was used. Hence, the need to isolate possibly by purification the various phytochemical groups in the extracts.

Therefore, it is recommended that further investigations should be done on the chemical nature of the active principles of the test plants. This knowledge will help pharmaceutical chemists to alter the structures of these principles in order to enhance their lethality on the test organisms. Also further investigations can combine the plant extracts for possible synergistic effects.

References


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