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# Antimicrobial Effects of *Anchomanes difformis* Extract- Pure Honey Mixture on Microorganisms Isolated From Sputum

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**ABSTRACT:** *This study investigates the inhibitory effect of Anchomanes difformis extract-pure honey mixture on bacteria and fungi isolates from sputum of infected patients of a hospital. Inhibitory zones against the bacteria by the extract ranges between 6mm and 25mm. Highest zone of inhibition (25mm),was recorded at 0.3g/ml concentration against Mycobacterium tuberculosis, while, the least (6mm) was recorded against Staphylococcus epidermidis at 0.1g/ml. concentration. Fungi inhibition zones range between 5mm and 16mm. The highest zone of inhibition(16mm) at 0.3g/ml was against Dictyostelium discoidium and Schzosaccharomyces spp, while, the least(5mm) was recorded against Didymium irridis at 0.1g/ml concentration. In comparison the effectiveness of the commercial antibiotics was high but no single antibiotic inhibits all the isolates. Anchomanes difformis extract-pure honey mixture has been discovered to posses high antimicrobial potentials against bacteria and fungi infections of human sputum. This study suggests the potential usage of Anchomanes difformis extract-pure honey mixture as an alternative cure for tuberculosis and other infections that may be caused by the tested organisms after thorough phamacognosis*.

**KEY WORDS:** *Anchomanes difformis,* pure honey, antimicrobial, sputum.

## I. INTRODUCTION

A large proportion of the population of developing countries uses traditional medicines, either as a result of the high cost of Western pharmaceuticals and health care, or because the traditional medicines are more acceptable from a cultural and spiritual perspective [1]. *Anchomanes difformis* is an herbaceous plant with prickly stem having huge divided leaf and spathe that arise from a horizontal tuber occurring in the forest of West Africa. It is sometimes called Forest Anchomanes in English while in South West Nigeria it is known as *Ogirisako,* (*Igbo* language) [2] and Langbodo in Yoruba language.

The rhizomes is eaten but only after special preparation that entails prolong washing and cooking of early shooting stage, aqueous extract of the tubers has been used to cure dysentery by traditional healers. The root leaves and stems are purgatives. It has also been reported in the treatment of kidney-pains, Oedemas and as diuretic for treating urethral discharge, jaundice and as poison antidote [3].

Honey is defined as the nectar and saccharine exudation of plants, gathered, modified and stored as honey in the honey comb by honey bees [4]. *Apis melifera.* Honey has been used as a medicine since ancient times and is still used in folk medicine. Ayurveda, the traditional Indian system of medicine, describe a honey as the nectar life. Honey is known to improve food assimilation and to be useful for chronic and infective intestinal disorders such as constipation, duodenal ulcers and liver disturbances. Honey is well known as remedy for cold and mouth, throat, or bronchial was used in this preparation. 5ml of pure honey was dispensed into a beaker already containing 5ml of *Anchomanes difformis* extract; these were properly agitated and kept in a refrigerator until it is needed.

Sputum is a matter that is expectorated from the respiratory tract such as mucus mixed with saliva and can be spit out from the mouth. It can be found to contain blood if in chronic, possibly from severe case of tuberculosis [5]. A sputum culture detects the presence of pathogenic bacteria in those who have bacterial pneumonia and lungs bronchial tubes are caused by fungi, viruses and parasite which are responsible for a variety of disease including plemonary tuberculosis, bacterial pneumonia, viral and mycoplasmal (a typical) pneumonia chronic bronchitis and bronchiectasis [6]. Sputum culture is done to find and identify bacteria or fungi that are causing pneumonia or tuberculosis of the lungs or airways leading to the lungs and symptoms of lungs infection may include difficulty breathing pain when breathing or cough that produces bloody or greenish brown sputum. Tuberculosis is common and often deadly infectious disease caused by mycobacterium.

In human *Mycobacterium tuberculosis* is a primary causative bacteria but also affect the central nervous system, the lymphatic system, the circulatory system, the gastrointestinal system, bones and even the skin, *Mycobacterium tuberculosis* is a respiratory infection commonly transmitted via the air to the lungs when

it thrives causing fever, cough and blood spitting [7]. Anyone can get bacteria respiratory infection but the elderly ones with suppressed immune systems. Those with damaged lung tissue, those who are exposed to lung irritants such as through smoking and those with conditions and disease that affect lung function, such as cystic fibrosis is at increase risk. Identification is step-by-step process that may involve several biochemical test and to observe the organism growth characteristics. Antimicrobial susceptibility testing is frequently required to guide the treatment of indentified pathogens and to determine whether the bacteria present are likely to respond to specific antibiotics [8]. The World Health Organization (WHO) has declared tuberculosis as a global emergency. It is estimated that one-third of the world's population is affected with *Mycobacterium tuberculosis.* Estimated 8-9million new cases occur each year with 2-3million deaths [9], [10]. This study investigates the antimicrobial activity of *A. difformis* rhizomes or mixture of *A. difformis* and honey on bacteria and fungi of public health importance.

## II. MATERIALS AND METHODS

**Sources and Processes of Bacteria**

Three bacteria; *Mycobacterium tuberculosis, Escherichia coli, Staphylococcus epidermidis* and 3 fungi; *Dictyostelium discoidium, Didymium iridis, Schizosaccharomyces* spp. earlier isolated and identified from sputum samples were collected from the Baptist Hospital, Ejigbo, Osun State.

**Collection and Identification of the Plant Materials**

A Fresh leaves and rhizomes of the plant were collected from Masifa, a town near Ejigbo in Ejigbo Local Government of Osun State, Nigeria. The plant was identified as *A. difformis* (Blume) Engl. (family= *Araceae*) at the Department of Botany, Ekiti State University, Ado-Ekiti, Nigeria.

**Preparation of the cocktail**

The rhizomes was collected and dried to a constant weight at 18°C in an enclosed air conditioned research laboratory. The rhizomes were sliced to pieces to ensure proper drying. The dried rhizomes were grounded to powder forms to increase the surface area for extraction. Rhizomes were extracted exhaustively by cold method of extraction, i.e. by soaking in a solvent for four days. The solvent used was water to get aqueous extract. About 100g of the grounded rhizomes powder yielded 20g of the extract. The extract was dried and then reconstituted using dimethylsulfoxide (DMSO). Varying weight (0.3g, 0.2g and 0.1g) of the extract was dissolved individually in 1ml of DMSO to give concentration of 0.3g/ml, 0.2g/ml and 0.1g/ml respectively. *Anchomanes difformis* extract-honey mixture was prepared in the volume ratio 4:1.

**Antimicrobial Assay**

Pure isolates of test organisms were inoculated in a nutrient broth for bacteria and Saubouraud Dextrose Agar (Oxoid) for fungi and incubated for 6 hours to ensure that the organisms were at their exponential phase of growth. The organisms were serially diluted using double sterilized distilled water and the $10^{-7}$ dilution corresponding to 0.5 MacFarland standard were used as the inoculum. Mueller Hinton Agar (Oxoid) for bacteria and Saubouraud Dextrose Agar (Oxoid) for fungi were used. The media were measured and dissolved in appropriate volume of distilled water, following the manufacturer's guideline; and was sterilized by autoclaving. 1ml of the standardized inoculum was mixed with the media in a sterile container to ensure that the test organisms were evenly distributed and poured into sterile Petri dishes and allowed to set. Each plate contains equal volume of the media. The antimicrobial activity of the crude extracts was determined in accordance with the agar-well diffusion method described by [11]. The plates were incubated at 37°C for bacteria and 28°C for yeast. The plates were observed for zones of inhibition after 24 h for bacteria. It was observed that the yeast produced discrete colonies within 24 hours, thus all the plates were read after 24hours. Two controls were used in this research: Organism viability control to check for the viability of the organisms. This implies that any clear zone of inhibition observed is due to the activity of the extract. All the organisms showed viability with colonies covering 100% surface of the plate. The second control is to test the activity of the solvent (DMSO) used to dissolve the extract to ensure that the activity is not due to action of the solvent on the organisms. The solvent showed zero activity on all the organisms. Plates were read by measuring observed clear zones (area without growth) of inhibition around the wells containing the extract. Measuring ruler in millimeter was used to take the measurement from the edge of the well to the end of the clear zone of inhibition. No measurement was taken if no clear zone of inhibition was observed. The estimation of MIC of the crude extracts was carried out using the method of Kinpelu and Kolawole [12].

**Antibiotics susceptibility testing**

Susceptibility testing was carried out on Mueller-Hinton agar using the disc diffusion method as described by Clinical and Laboratory Standard Institute (CLSI) (2008). The following commercial antibiotic disks (Kirby Bauer's disc) with their concentrations (in µg) were used: Pefloxacin 10µg, Gentamycin 10µg, Ampiclox 30µg, Zinacef 20µg, Amoxacillin 30µg, Rocephin 25µg, Ciprofloxacin 10µg, Streptomycin 30µg, Septrin 30µg, Erythromycin 10µg,

## III. RESULTS AND DISCUSSION

This study investigates the inhibitory effect of *Anchomanes difformis* extract-pure honey mixture on Bacteria and Fungi isolates from sputum of infected patients of a hospital. Inhibitory zones against the bacteria by the extract ranges between 5mm and 25mm. Highest zone of inhibition at 0.3g/ml was recorded against *M. tuberculosis*. While, the least was recorded against *S. epidermidis* at 0.1g/ml. concentration. Fungi inhibition zones range between 10mm and 16mm the highest zone of inhibition at 0.3g/ml was against *D.discoidium*, and *Schizosaccharomyces* sp. while, the least was recorded against *D. irridis* at 0.1g/ml concentration. These results were in line with findings of [1].

In comparison the effectiveness of the commercial antibiotics was high but no single antibiotic inhibits all the isolates.

The inhibitory effects of this cocktail also suggest presence of antibacterial and antifungal ingredients in the cocktail. Highest zone of inhibition on *Mycobacterium tuberculosis* suggests that the cocktail will be a very good checking measure of this bacterium on its patients or victims. This statement is in line with the finding of [13].

**Table 1: Inhibitory effects of the cocktail on the sputum microbial isolates.**

| Isolates | Inhibitory Zones at different concentration (mm) | | |
|---|---|---|---|
| | 0.1g/ml | 0.2g/ml | 0.3g/ml |
| *M. tuberculosis* | 15 | 20 | 25 |
| *E. coli* | 10 | 15 | 20 |
| *S. epidermidis* | 6 | 10 | 12 |
| *Dictyostelium discoidium* | 10 | 14 | 16 |
| *Didymium irridis* | 5 | 10 | 13 |
| *Schizosaccharomyces spp.* | 6 | 12 | 16 |

**Table 2: Antibiotic Sensitivity Pattern of the isolates**

| Antibiotics | *M. tuberculosis* | *S. epidermidis* | *E. coli* | *Dictyostelium discoidium* | *Didymium irridis* | *Schizosaccharomyces spp.* |
|---|---|---|---|---|---|---|
| **Pefloxacin** | 17mm | 16mm | 20mm | R | R | 10mm |
| **Gentamycin** | 18mm | 15mm | 11mm | R | R | 14mm |
| **Ampiclox** | R | R | R | R | R | 15mm |
| **Zinacef** | R | R | R | 6mm | R | R |
| **Amoxacillin** | 10mm | R | R | 10mm | 10mm | R |
| **Rocephin** | R | 20mm | R | 18mm | R | R |
| **Ciprofloxacin** | R | 14mm | 14mm | R | R | R |
| **Streptomycin** | R | R | 14mm | 10mm | 11mm | 14mm |
| **Septrin** | R | 10mm | 15mm | R | 10mm | R |
| **Erythromycin** | R | 15mm | 20mm | 11mm | R | 10mm |

**KEY:**
R= Resistant

## IV. CONCLUSION

*Anchomanes difformis* extract-pure honey mixture has been revealed to posses high antimicrobial potentials against bacteria and fungi infections of human sputum. Findings of this study suggest the potential usage of *Anchomanes difformis* extract-pure honey mixture as an alternative cure for tuberculosis and other infections that may be caused by the tested organisms after thorough phamacognosis.

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