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ANTIMICROBIAL POTENCY OF THE AQUEOUS EXTRACT OF LEAVES OF TERMINALIA *CATAPPA*

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ABSTRACT

Antimicrobial efficacy of the aqueous extract of leaves of Terminalia cattappa was investigated against Staphylococcus. aureus (gram positive), E.coli , (gram negative), Klebsiella pneumonia (gram negative) and Candida albicans using the Disc diffusion assay. Antimicrobial potency of the plant aqueous extract against the pathogenic microorganism followed the sequence: Klebsiella pneumonia > Staphylococcus aureus > Escheria. coli > Candida. albicans. Antimicrobial potency was also found to be less than standard antibiotics, Ampicillin, Nystatin, Penicillin under standard conditions.

Keywords: Antimicrobial efficacy, Terminalia catappa, Disc diffusion, Staphylococcus aureus , E.coli , Klebsiella pneumonia, Candida albicans

INTRODUCTION

This paper focuses on the antimicrobial (antibacterial and antifungal) properties of leaf extract of leaves of *Terminalia catappa*, from the coastal plain of the Guyana flora and its possible use as an/herbal medicine. Antimicrobial properties were investigated against *S. aureus* (gram positive), *E.coli* (gram negative), *Klebsiella pneumonia (gram negative* and *C.albicans* strains using the Disc diffusion assay.

There is an urgent need to intensify research in herbal medicine and drug discovery, considering the presence of incurable diseases such as HIV AIDS and the threat of new emerging disease such as SARS, bird flu etc. Over the years, plants extracts and fractionated plant extracts have been a good source of herbal medicines and natural products/ phytochemicals [1-31]. Guyana has a rich biodiversified flora whose crude extracts, both organic and aqueous are currently been screened for their antimicrobial activity[20-30]. Also, the specified plants parts fractionated or screened for natural products whose antimicrobial activity can also be investigated and compared with the crude extracts. Following this, clinical trials of crude extracts or fractionated natural products can lead to the formulation of an herbal plant cream or herbal medicine. A few herbal medicine shops are established in Guyana and the "bush" medicine man is still an important figure in Guyana's culture. Plants are known to synthesize antimicrobial natural products whose structure usually correlates with biological activity [20-34]. However, there are a large number of plants in Guyana whose antimicrobial activity need urgent investigations. Besides used as an herbal cream, following clinical trials, crude plant extracts can be chromatographed, leading to the isolation and purification of new and known bioactive natural products/phytochemicals, whose medicinal activity can also be investigated [1,2,8,13,32,34]. As an example, novel diterpenes (1) – (6), Fig. 1.0 were isolated from *Calceolaria pinfolia*. These compounds were found to be active against *S.aureus*, methicillin resistant MRSA, *Bacillus subtilis* (BS) and *Eschericia coli* (EC) [34].

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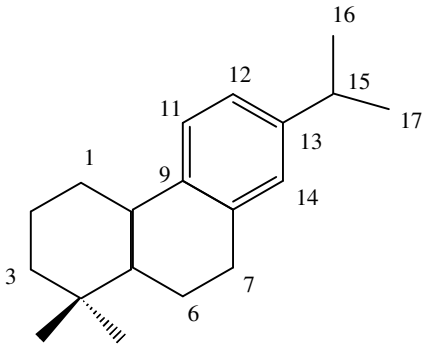

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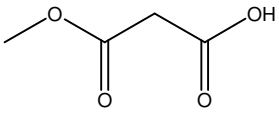
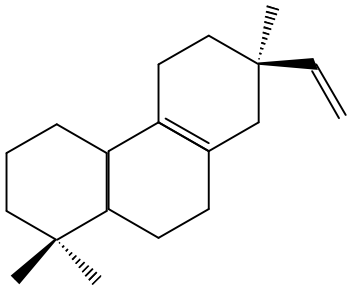
3. R = 



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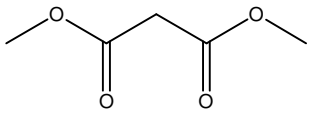
5. R = 

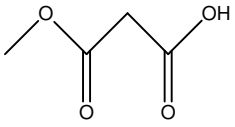
6. R = 

Fig. 1.0

Pathogenic microorganism investigated were *Escherica coli (EC), Staphylococcus aureus* (*SA), Klebsiella pneumoniae and C. albicans (CA). Escherica. coli* can cause several intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and gram-negative pneumonia[35]. *Staphylococcus aureus,* the yellow type can cause furuncles (boils), carbuncles (a collection of furuncles)[36]. In infants, *Staphylococcus aureus* can cause a severe disease Staphylococcal scalded skin syndrome (SSSS). Staphylococcal *endocarditis* (infection of the heart valves) and pneumonia may be fatal. *Staphylococcus aureus* can cause food poisoning. *Candida albicans* is a diploid fungus (a form of yeast) and is a casual agent of opportunistic oral and genital infections in humans[37-38]. It is responsible for the infectious disease, candidiasis, thrush etc.

Klebsiella pneumoniae is a gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines[39]*. K.*

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pneumoniae can cause the disease *Klebsiella pneumonia*. They are responsible for destructive changes to human lungs inflammation and hemorrhage with cell death, necrosis that sometimes produces a thick, bloody, mucoid sputum . Klebsiella infections are mostly seen in people with a weakened immune system. The most common infection caused by Klebsiella bacteria outside the hospital is pneumonia in the form of bronchopneumonia and bronchitis. These patients have an increased tendency to develop lung abscess, cavitation, empyema, and pleural adhesions. It has a high death rate of about 50% even with antimicrobial therapy. The mortality rate can be 100% for persons with alcoholism and bacteremia. Klebsiella also cause infections in the urinary tract, lower biliary tract, and surgical wound sites. The range of clinical diseases includes pneumonia, thrombophlebitis, urinary tract infection (UTI), cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, bacteremia and septicemia. Other interesting infections from *Klebsiella* are rhinoscleroma and ozena.

Morphological Description and Scientific Classification of the Plant:

Terminalia catappa is a large tropical tree in the Leadwood tree family, *Combretaceae*. It grows to 35 metres (115 ft) tall, with an upright, symmetrical crown and horizontal branches. It has corky, light fruit that is dispersed by water. The nut within the fruit is edible when fully ripe,tasting almost like almond. The leaves are large, 15–25 cm long and 10–14 cm broad, ovoid, glossy dark green and leathery. They are dry-season deciduous; before falling, they turn pinkish-reddish or yellow-brown, due to pigments such as violaxanthin, lutein, and zeaxanthin. The flowers are monoecious, with distinct male and female flowers on the same tree. Both are 1 cm in diameter, white to greenish, inconspicuous with no petals. They are produced on axillary or terminal spikes [40].

Natural Products and folkloric data:

The leaves contain several flavonoids such as kaempferol or quercetin), Fig. 2.0, several tannins(punicalin, punicalagin or tercatin), saponines and phytosterols. The leaves and also the bark are used in different traditional medicines for various purposes. In Taiwan, fallen leaves are used as a herb to treat liver diseases. In Suriname, a tea made from the leaves is prescribed against dysentery and diarrhea. The leaves are thought to contain agents for prevention of cancers, although they have not demonstrated anticarcinogenic properties and antioxidant as well as anticlastogenic characteristics. The leaves kept in an aquarium is said to lower the pH and heavy metal content of the water. It's also believed that it helps prevent fungus forming on the eggs of the fish[40].

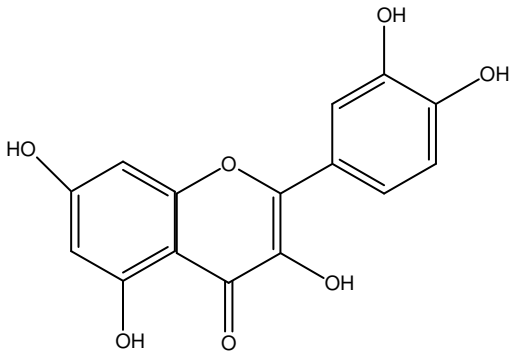

Fig.2.0, Quercetin

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Scientific classification:

Table 1.0. The scientific classification of the plant is given below:

Binomial name	*Terminalia catappa*
Kingdom	Plantae
Division	Magnoliphyta
Class	Magnoliopsida
Order	Myrtales
Family	Combretaceae
Genus	*Terminalia*
Species:	*T. catappa*

Source of microorganisms:

For the bacterial organisms, gram negative bacteria used was *Staphylococcus aureus* (ATCC 25923). For the fungi, yeast of the *Candida albicans* (ATCC 1023) species was investigated. These microorganisms were stored in a refrigerator at the microbiology laboratory at John's Science Campus, Berbice.

Reference and Control:

The references were antibiotic in nature. *Ampicillin* , Penicillin, *Nystatin*. *Ampicillin* was choosen as the reference for all bacterial species used: *E.coli* and *S. aureus*. *Nystatin* was used as the reference for the fungus, *Candida.albicans.* The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion [40-41].

Antimicrobial tests:

The aqueous extract of the plant was investigated for its antimicrobial activity using the Disc diffusion method [40-41] techniques under aseptic conditions.

Aseptic conditions:

The aseptic chamber consists of a wooden box (1m x 1m x 0.5m) with a door which was cleaned with 70% ethanol and irradiated with short wave UV light for 1 hour.

Disc Diffusion Assay.

The Disc diffusion assay was done using the Kirby Bauer's method [40-41]. Molten agar was poured into a 90 mm diameter sterile petri dish to a depth of 4 mm (about 25 ml per plate) on a level surface so that the depth of the medium is uniform. An innoculum containing bacterial or yeast cells were applied onto nutrient agar plates. On each plate, four discs were also applied. One of these disc is a reference one on which the positive control was applied. The positive control is Ampicillin/Pennicillin and Nystatin for the bacterial and fungal strains respectively. The positive control was inoculated on a separate plate. The reference antibiotic disc contained about 200mg antibiotic/ml. The discs (5-6mm) were made from a filter paper using a perforator. Each disc was impregnated with the anticipated antimicrobial plant extract at appropriate concentration of 200 mg/ml using a microlitre syringe. The antimicrobial compound is expected to diffuse from the disc into the medium. Each disc was then incubated at 37°C in an inverted position. Incubation was 24 and 48hrs for bacterial and Fungal species respectively. Following overnight incubation, the culture was examined for areas of no growth around the disc (zone of inhibition). The diameter of the zone of inhibition was measured. It is anticipated through the antimicrobial activity of plant extract, no area of

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growth will be induced around the disc. Interpretation of susceptibility are made by comparing the sizes of zones of inhibition to a standard reference table [40-41].

RESULTS

Table 1.0: Diameter of zone of Inhibition induced by the Aqueous extract of *Terminalia catappa*

Diameter of Zone of inhibition (mm)	Area of zone of Inhibition (mm^2)	Type of Extract	Microbial Strains	Volume of Extract applied to Disc
18	254.71	Aqueous	*Klebsiella pneumoniae*	34 uL
14.5	165.13	Aqueous	*Eshceria coli*	34uL
16.5	213.82	Aqueous	*Staphylococcus aureus*	34uL
10.1	80.08	Aqueous	*Candida albicans*	34uL

Table 3.0:

Diameter of Zone of inhibition (mm)	Area of zone of Inhibition (mm^2)	Type of Antibiotics	Microbial Strains	Volume of Extract applied to Disc
9.0	62.62	Ampicllin	*Staphylococcus aureus*	34 uL
10.5	86.54	Penicillin	*Staphylococcus aureus*	34 uL
8.0	50.27	Penicillin	*Eshceria coli*	34 uL
7.5	44.18	Ampicillin	*Klebsiella pneumonia*	34 uL
7.0	38.48	Penicillin	*Klebsiella pneumonia*	34 uL
6.5	33.18	Ampicllin	*Eshceria coli*	34 uL
6.0	28.26	Nystatin	*Candida albicans*	34uL

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Fig. 3.0a Antimicrobial activity of Aqueous Extract of Terminalia catappa

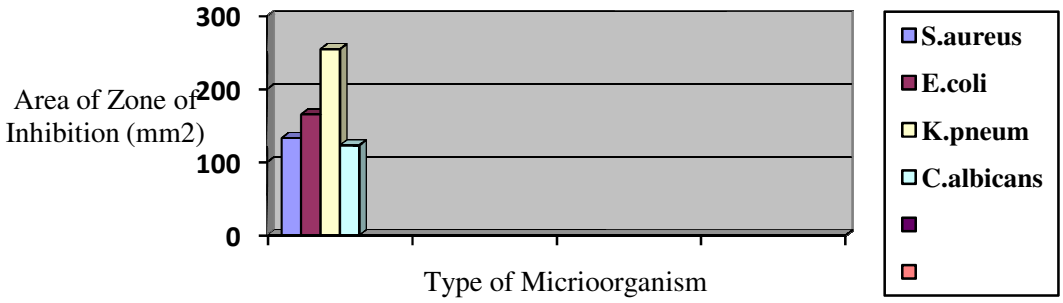
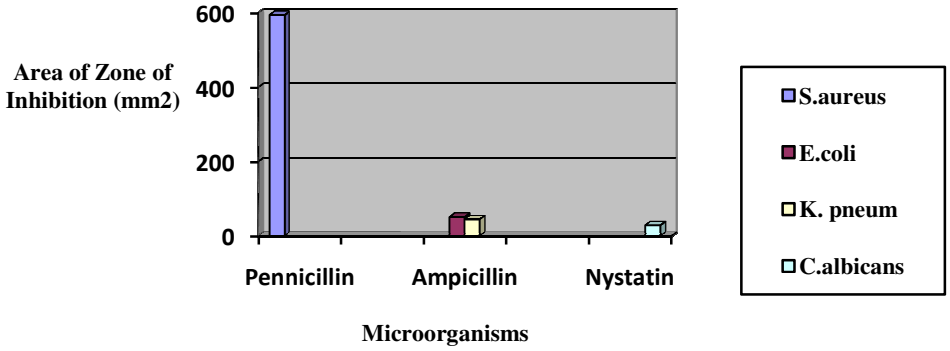


Fig.3.0b. Antimicrobial study of Standard Antibiotics against Pathogens



DISCUSSION

Antimicrobial activity of the aqueous extract of *Terminalia catappa* was investigated using the Kirby Bauer Disc diffusion method [40-41]. The diameter of the zone of inhibition was measured and the area of zone of inhibition was calculated. Larger the diameter of zone of inhibition, greater is the antimicrobial activities. The inhibition zones produced by different antimicrobials against the same organism vary in size due to differences in antimicrobial molecular structures. A larger zone of inhibition is produced by an antimicrobial that diffuses rapidly and a smaller zone by one that diffuses more slowly. Bacteria or fungal strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains grow up to the edge of the disc Microbial organisms strains investigated were *Staphylococcus. aureus*, *Klebsella pneumonia*, *Escheria.coli* and *Candida albicans.* The plant extract was administered at 34uL per disc at a concentration of 0.1g per 10ml. The results, Table 2.0, indicates that the largest zone of inhibition of 254.71mm^2 was induced against *Klebsiella pneumonia* and the least of 80.08 mm^2 against *Candida albicans*. The antimicrobial potency of the aqueous extract of the plant against the four microbial strains followed the sequence: *Klebsiella*

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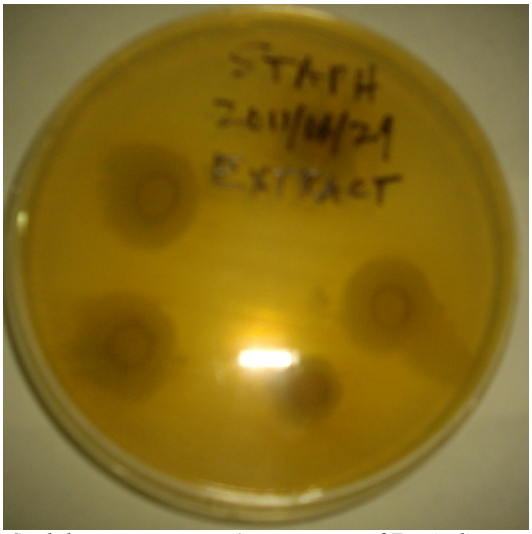
pneumonia > *Staphylococcus aureus* > *Escheria. coli* > *Candida. albicans* i.e the extract was found to be more antifbacterial than antifungal.

The control experiment with solvents indicates that solvents induced negligible zone of inhibition (< 5 mm^2). This indicates that the area of zone of inhibition shown in Table 3.0 is due to the plant's antimicrobial natural products/phytochemicals rather than to a solvent effect.

Interestingly, the aqueous extract induced zones of inhibition that are greater than that of the standard antibiotics: Ampicillin, Penicillin and Nystatin. Fig. 3.0 (a) shows a plot of the zone of inhibition versus the type of microorganism for the aqueous extract whereas Fig. 3.0 (b) shows a plot of the zone of inhibition versus standard antibiotics, Penicillin, Ampicillin and Nystatin. Fig. 4.0. is a pictorial representation of the effect of the administration of aqueous extract of *Terminalia catappa* against *Staphylococcus aureus*.



Staphylococcus aureus vs. Aqueous extract of *Terminalia catappa*)

Fig. 4.0. The effect of the administration of aqueous extract of *Terminalia catappa* against *Staphylococcus aureus*.

CONCLUSION

Antimicrobial activity of the aqueous plant extract was investigated and its effect of the aqueous extract against microbes followed the sequence: *Klebsiella pneumonia* > *Staphylococcus aureus* > *Escheria. coli* > *Candida. albicans.* Also, the microbial potency of the aqueous extract seems to be greater than that of standard antibiotics, Penicillin, Ampicillin and Nystatin. Clinical trials of the aqueous extract should be undertaken to establish this plant as a potential antibacterial herbs worldwide and should be commercialised

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