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Comparative antimicrobial activities of ethanolic extracts of leaves, seed and stem bark of *Mangifera indica* (Mango)

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Abstract
In traditional medicinal systems, different parts of the same plant may show therapeutic activities against a specific condition, but with different potencies. It is against this background that the current study seeks to compare the antimicrobial activities of different morphological parts of mango plant. Using the agar-well diffusion, and the broth dilution methods the antimicrobial activities of ethanolic extracts of the stem bark, leaf and seed of mango were investigated against some microbes. From the study, the seed extract exhibited the greatest antimicrobial activity of average diameters of zones of inhibition in the range of 14–19 mm for a 10mg/ml concentration, and 12–16 mm for a 5mg/ml. The bark and leaf extracts gave a range of 8–12 mm at the stated concentrations. The best minimum inhibitory concentration was against *S. aureus* and it was of 125µg/ml of the seed extract. The study has shown that the bark, leaf and seed extracts each of mango has antimicrobial activity, and that the seed has the most potent activity among the three morphological parts of the plant.

Keywords: Antimicrobial, mango, bark, leaf, seed, MIC

Introduction
The use of medicinal plants to treat diseases has been in existence for many centuries and continues to play significant roles in the healthcare needs of many, especially among people dwelling in sub-urban and rural areas in developing countries. Many plants are reputed to be useful in the treatment of infections, including those (infections) that defy orthodox antimicrobials. Some antimicrobial medicinal plants have been able to circumvent the actions of multi-drug resistant (MDR) micro-organisms [1, 2]. This makes such plants ideal candidates in the search for newer antimicrobial drugs to combat the problem of antimicrobial resistance. Ideal plants worth investigating include mango.
Mango (*Mangifera indica* Linn.) belongs to the plant family Anacardiaceae. It is widely distributed throughout the world and is mostly cultivated in the tropical regions. The popularity of mango comes from its edible fruit, which also serves as flavouring agent and fragrance. Various parts of the plant have found use in traditional medicine as dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative, diuretic. Additionally, they are used in the treatment or management of diarrhoea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and piles [3, 4]. In Cuba, the aqueous extract of the stem bark has been extensively used in management of cancer, diabetes, asthma, infertility, lupus, prostatitis, prostatic hyperplasia, gastric disorders, arthralgia, mouth sores and tooth pain [5].
Different morphological parts of the plant have been reported to have antimicrobial activities against some micro-organisms. For example, various solvent extracts of seeds of the plant have shown antimicrobial activity against microorganisms such as *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* [6, 7]. The leaf extracts of the plant have also shown antibacterial activity against *Salmonella typhi* [8]; *Staphylococcus aureus, Esherichia coli* and *Pseudomonas aeruginosa* [9]; and *Proteus vulgaris, Pseudomonas fluorescens, Shigella flexneri, Klebsiella pneumoniae, Salmonella typhi* [10]. Similarly, the bark extract of the plant has exhibited antimicrobial activity against *Staphylococcus aureus* [11] and *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Streptococcus pneumonia* [12]. In the search for agents to combat the current challenges of dealing with antimicrobial resistance, medicinal plants that have shown potency in managing various infections must be considered as potential lead. Medicinal plants, through the multiplicity of compounds present in a single morphological

part, may not be easy for micro-organism to develop resistance against. It is against this background that the antimicrobial activities of extracts from different morphological parts of the mango plant are being investigated to bring to light the part of the plant that has the most potent antimicrobial activity.

2. Materials and Method

2.1 Plant Material

The bark of a mango plant, as well as the leaves and seeds used in the study were collected from the same plant at Asafo, a suburb of Kumasi in the Ashanti Region of Ghana in March 2017. The samples were identified by an expert Pharmacognocist. The samples were each washed, chopped into pieces, air-dried and ground into course powder. Each was kept in a brown envelop until it was required for used.

2.2 Extraction of Plant Materials

Each of the samples was extracted with 96% ethanol. Hundred grams (100g) each of the coarsely powdered plant material was soaked in 400ml of ethanol for three (3) days with occasional shaking to facilitate the extraction of the constituents. Each mixture was filtered through a plug of cotton wool (followed by filtration through filter paper) labelled appropriately, and the filtrate allowed to dry by exposing it to air in a fume chamber.

2.3 Test Microorganisms

The microorganisms used in the study were Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus faecalis*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*) and the yeast-like fungus *Candida albicans*. These organisms were obtained from the Microbiology Laboratory of the Department of Pharmaceutical science, Kumasi Technical University.

2.4 Antimicrobial Assay

The cup plate method was used; 0.1ml of overnight broth culture of each microorganism, containing approximately 10^6cfu/mL, was introduced unto the surface of Nutrient Agar plate and distributed uniformly using a sterile cotton swab. Five wells, each of about 6mm diameter and 5mm depth, were made on the agar using a cork borer number 3 and labelled appropriately as B, S, L, G and MW representing bark, seed, leaves, gentamicin and water-methanol respectively. The extracts were dissolved in a mixture of methanol and water (1:9) to prepare two different concentrations, a 10mg/ml and a 5mg/ml. Gentamicin at a concentration of 0.1mg/ml and methanol-water (1:9) were used as the controls.

A volume of 0.1ml of each of the extracts (leaf, seed and bark) at a concentration of 10mg/ml was dispensed into their corresponding well. The same volume of the controls (Gentamicin and methanol-water mixture) was also dispensed into the corresponding wells. The plates were left on the laboratory bench for thirty (30) minutes before incubating for 24 hrs at 37ºC. The procedure was repeated using the 5mg/ml concentration of the extracts. The procedure was carried out in triplicates for each of the concentrations used.

The sensitivity of the test organisms to the extracts were determined by measuring the diameters of the zones of inhibition surrounding the wells using a ruler and the average of each recorded.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

Cultures of the organisms were prepared in Nutrient broth (Sigma) and incubated at 37°C. The bark, leaf and stem-bark extracts of the plant (mango) were each reconstituted in 1:9 ratio of methanol-water. Each was diluted with Nutrient broth to make up the required concentration. The MICs of the extracts and the controls were determined using the microtitre well dilution method [13] with triplicate wells and two (2) independent experiments. Each extract was serially diluted two-fold with Nutrient broth to present a dilution range of 8mg/mL to 0.008mg/mL in sterile 96 well microtitre plates. One hundred microlitres (100µL) of overnight broth culture of the organisms (10^5cfu/mL) was added to each well, and incubated at 37°C for 18h. Control wells did not contain the extracts, and the vehicle control wells contained scalar dilutions of the 10% methanol. Serial dilution of Gentamicin (from 2mg/mL to 0.002mg/mL) was used as the positive control. The plates were examined for growth after 18h of incubation.

3.0 Results and Discussion

3.1 Antimicrobial activities of the mango extracts

The antimicrobial activity of each of the mango extracts was assessed against each test microorganism and the results obtained for the 10mg/ml and 5mg/ml concentrations used are as shown in Table 1and Table 2 respectively. The MIC results are also shown in Table 3.

Table 1: Average zones of inhibition of mango leaf, seed and bark extract against the test microorganisms using 10mg/ml concentration.

	Average zones of Inhibition of the Sample (mm) (n=3)			
Microorganism	**Leaf**	**Bark**	**Seed**	**Gentamicin**
Streptococcus faecalis	11±0.5	11±1.0	17±0.3	27±1.5
Staphylococcus aureus	12±1.0	12±0.5	19±10	26±1.0
Escherichia coli	9±0.0	11±0.5	17±0.0	24±1.0
Klebsiella pneumonia	12±1.0	10±0.3	18±1.0	26±0.7
Candida albicans	12±0.3	9±0.3	14±1.0	NT

NT – not tested

Table 2: Average zones of inhibition of mango leaf, seed and bark extract against the test microorganisms using 5mg/ml concentration.

	Average zones of Inhibition of the Sample (mm) (n=3)			
Microorganism	**Leaf**	**Bark**	**Seed**	**Gentamicin**
Streptococcus faecalis	9±0.0	8±0.0	15±0.7	27±1.5
Staphylococcus aureus	11±1.0	7±0.0	14±0.7	26±1.0
Escherichia coli	9±1.0	9±0.7	15±0.5	24±1.0
Klebsiella pneumonia	12±0.5	9±0.0	16±1.5	26±1.0
Candida albicans	10±0.0	8±0.3	12±1.0	NT

Table 3: Minimum Inhibitory Concentrations of the extracts against the test organisms.

	MIC (mg/ml) of the Extracts and Gentamycin			
Microorganism	**Leaf**	**Bark**	**Seed**	**Gentamicin**
Streptococcus faecalis	2.00	>2.00	0.50	0.016
Staphylococcus aureus	2.00	>2.00	0.125	0.016
Escherichia coli	>2.00	>2.00	0.50	0.032
Klebsiella pneumonia	1.00	>2.00	0.50	0.032
Candida albicans	>2.00	>2.00	2.00	NT

In folklore medicine various morphological parts of plants are used in the management or treatment of diseases. These parts contain various phytochemicals which are responsible for the therapeutic activities of the plant. In an era where most drugs/medicines, especially the anti-infective products, no longer seem to have the initial potent activities they used to have, there is the need to search for newer pharmaceuticals with perhaps different mechanisms of actions. Plants, for centuries, have served the human person with potent medicines, in addition to being used for food and other domestic purposes. One common plant that has served man very well is *Mangifera indica* (mango). The commonest part of the plant is its exotic and highly patronised fruits which is noted for its antioxidant compounds, vitamins A, C and E and other useful nutrients.

The stem bark, leaves and seed in the fruits are also known to have useful activities including antimicrobials. In the current study, the potential antimicrobial activities of the mentioned morphological parts were compared to find out the part that has the most potent antimicrobial activity. The activities were compared using ethanol extract of each part and tested at a concentration of 5mg/ml and 10mg/ml against *Staphylococcus aureus, Streptococcus faecalis, Escherichia coli* and *Klebsiella pneumonia*, and the fungus *Candida albicans*. It was observed that the seed gave a relatively higher antimicrobial activity against all the micro-organisms than the leaf and the bark extracts, as observed from the all the Tables. In the local settings of Ghana, the parts of mango tree most often used for medicinal purposes are the stem bark and the leaves; and these are the parts that showed less antimicrobial activities. At a concentration of 5mg/ml of the seed extract, the lowest diameter of zone of inhibition observed was 12mm against *Candida albicans*. This figure (12mm) is the highest observed for the leaf and bark extracts even at a concentration of 10mg/ml. El-Gied and colleagues [6] carried out a similar work using concentrations ranging from 1.5–5mg/ml of mango seed ethanol and methanol extracts obtained through the use of Soxhlet apparatus; the inhibition zones were up to 18mm. This confirms that the seeds have activity at a relatively lower concentration.

The leaf extract, on the other hand, gave diameters of zone of inhibition in the range of 9mm - 12mm. This morphological part of the plant has also been reported to exhibit antimicrobial activity against different strains of bacteria. According to a study by Hannan and others [8] the acetone extract of mango leaves showed antimicrobial activity against multi-drug resistance and antibiotic sensitive strains of *Salmonella typhi*, which is known to be responsible for typhoid fever. In their study, a relatively higher concentrations of 50-50mg/ml was used, and the diameters of zones of inhibition obtained ranged from 15-26mm. Similarly, the bark of the plant also showed some level of activity against the microbes used in the current study; giving diameters of zones of inhibition ranging from 7mm–12mm. In a previous study, the bark exhibited antimicrobial activity against clinical bacterial isolates of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Streptococcus pneumonia*; showing diameters of inhibition zones in the range of 7mm - 16mm at a concentration of 50mg/ml from different solvent extractions [12].

The pharmacological activities of plants are due to phytochemicals present in the morphological part of the plant [14]. And there are seasonal variations in the amounts of secondary metabolites (constituents) present in plants, as such the time of collection of a medicinal plant may have a great influence on its biological activity. The geographical location of the plant, and the variety (type) of a particular species of the plant may all have an effect on the biological activity. There are several varieties of mango in Africa, and the constituents of each type may vary significantly thereby giving physiological activities at variable doses. From the literature, the doses of extracts of mango giving an antimicrobial activity vary, and this can be attributed to some of the reasons stated, namely time of collection, location of the plant and variety or type of the plant.

The seed kernel of mango has been found to contain gallic acid, methyl gallate, ethyl gallate, methyl *m*-digallate and *p*-hydroxybenzoic acid, and these compounds have been shown to contribute to the antimicrobial activity of the seeds [15]. In investigation of potential bioactivity of medicinal plants, including antimicrobial activity, it is prudent to use lower concentrations so that if it is found to be active at that concentrations then it could be selected as a probable candidate for further bioactivity studies. If larger concentrations are required give biological activity it would mean that so much of the plant material may be required in the management of a disease or condition in one person. Moreover, in isolating the pure compounds responsible for the biological activity, large amounts of the plant material may be needed to subject it through the various processes of isolation and testing, before structural elucidation of the compound. In most of the previous antimicrobial studies on the mango plant, it was observed that higher concentrations, especially for the leaves and bark, were administered to obtain an activity.

The relatively weak activity of the extracts against *Candida albicans* (a fungi) may be as a result of the cell wall structure of the fungi which is poorly permeable thereby preventing activity of the extract to produce greater zones of inhibition. The cell wall of fungi is poorly permeable, and it possesses both cell membrane and cell wall which consist of chitinous structure which does not allow easy penetration of bioactive substance [16]. Additionally, some species of fungi can excrete tannase, that can break down tannins into small molecules such as gallic acid, which could present limited antifungal activity by inhibiting the growth of some species of fungi only at higher concentrations [17].

In the study a mixture of methanol-water (1:9) was used as negative control; one part of methanol was used to dissolve each extract followed by nine parts of sterile water. This mixture did not show any antimicrobial activity indicating that the activity exhibited by the extracts indeed came from the extracts and was not augmented by the solvent used to reconstitute the extracts. Gentamicin, an aminoglycoside, which was used as positive control to compare the activities of the extracts is used to treat several types of bacterial infection [18]. It showed higher zones of inhibition (24–27mm) even though a relatively smaller concentration of 0.1mg/ml was used. It also gave an MIC in the range of 0.016-0.032mg/ml). Gentamicin is a refined product containing only one compound (which binds to 30S subunit of bacterial ribosome) interrupting with protein synthesis to inhibit the growth of microorganism [19]. Each of the morphological parts of the mango plant used in the study contain several compounds, and the biological activity may be coming from only one or few of these constituents/compounds.

Conclusion

Different morphological parts of mango exhibit antimicrobial activity. In a comparative study of the antimicrobial activities of the leaf, seed and stem bark of the plant, it emerged that the seed extract, though its use is limited, has the highest antimicrobial activity against both Gram-positive and Gram-negative bacterial and the Candida employed in the study. Both the leaf extract and that of the stem bark also gave some antimicrobial activity, they were not as much as that of the seed. The study has shown that ethanolic seed extract of mango has a more potent antimicrobial activity than the extracts of the stem bark and the leaf at the same concentration.

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