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ANTIBACTERIAL ACTIVITIES AND BI-HERBAL ETHANOLIC LEAF EXTRACT OF MISTLETOE (*PHORADEMDRON SEROTINUM*) AND MANGO (*MANGIFERA INDICA*) AGAINST SELECTED GASTROINTESTINAL PATHOGENS

Ojeleye F.S., * Arogundade E.O., Umar M., Jakheng S.P.E., Oko J.O., Albert K.A., Ojo, S.A., Nathaniel J., and Amuta I.C.
Nigerian Institute of Leather and Science Technology (NILEST), Zaria, Nigeria
***Correspondence: sbonire@gmail.com, +2348069554680**

ABSTRACT

The antibacterial activities of bi-herbal ethanolic leaf extract of Mistletoe (Phorademdron serotinum) and mango (Mangifera indica) against selected gastrointestinal pathogens were carried out. Selected phytochemical compounds of each plant extract were analysed using standard methods. The percentage yield of mango ethanolic leaf extract was 31.0%, with mistletoe ethanolic leaf extract recording a high yield of 41.1%. Each plant's ethanolic leaf extract was tested against the bacterial isolates singly and combined. The antibacterial activity of mango ethanolic leaf extract ranges from 11.00mm to 24.00mm, while that of Mistletoe ethanolic leaf extract ranges from 9.00mm to 24.00mm. All the phytochemicals screened were present in both plants' leaf extracts, except glycosides and oxalate, which were absent in mango ethanolic leaf extract. The plant extracts showed greater potency strength with each bacterial isolate when tested. The minimum inhibitory concentration of mango ethanol leaf extract against all the test organisms was 50mg/ml, except Bacillus cereus, which recorded a MIC of 12.5mg/ml. The MIC of Mistletoe ethanolic leaf extract against the test organisms was 25mg/ml, except for Streptococcus pneumoniae and Vibrio cholerae, which recorded MICs of 12.5mg/ml and 6.25mg/ml, respectively. Conversely, the MIC of bi-herbal extracts against the test organisms showed promising inhibitory potential of 6.25mg/ml, except in Staphylococcus aureus and Klebsiella pneumoniae, where each recorded MIC of 12.5mg/ml. The organisms were sensitive to each extract tested separately but were more sensitive to the extract when tested combined than when tested separately.

KEYWORDS
Antibacterial, phytochemistry, extraction, mango, mistletoe

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INTRODUCTION

Plant-derived products like gums, oils and extracts were used for therapeutic purposes before the introduction of modern drugs and continue to provide health coverage for over eighty per cent of the world's population (Oladunmoye *et al*., 2006). Serious attention is being given to medicinal plants, as evidenced by the recommendation by the World Health Organization (WHO) in 2015. WHO emphasised the need to include traditional remedies within national drug policies, as plants serve as the best sources of various drugs; it is essential to study plants to explore their properties, safety and efficacy. The first plant compound with antimicrobial activity was reported in the 1930s, and now, many plant compounds are readily available from herbal suppliers and natural food stores (Bally *et al.*, 2017). In Africa, self-medication with these substances is common and growing in popularity. Reasons can be attributed to the easy accessibility and affordability of plants compared to commercial drugs.

Contrary to the belief that natural medicine has no ill effects, several people have been hospitalised by consuming plants with unknown properties. It has been known that plants are the first and only genuine medicines ever used by man (Marchese & Shito, 2017). However, until recently, the practices of herbs in Nigeria have been kept in secrecy and shrouded in dreaded magical incantations, rituals, and sacrifices. It is now apparent that the potency of the plants and their parts does not depend on such an exhibition. Using plants for medical purposes is an integral part of the culture and tradition in Africa. Thus, about 80% of the population depends directly on traditional medicine for primary health care (Ekhaise & Okoruwa, 2017). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, especially among gastrointestinal pathogens, mainly due to the indiscriminate use of commercial antimicrobial drugs commonly employed in treating infectious diseases (Eloff, 2018). The number of resistant strains of microbial pathogens has been growing since penicillin resistance and multi-resistance pneumococci caused a significant problem in South Africa in 1977 (Eloff, 2018; Marchese & Shito, 2017).

Phoradendron is a genus of Mistletoe (Plate 1) native to the Americas' warm temperate and tropical regions. The centre of diversity is the Amazon rainforest (Coder, 2008). Phoradendron is the largest genus of Mistletoe in the Americas and possibly the largest of mistletoes in the world.

Traditionally, the genus has been placed in the family Viscaceae. However, genetic research acknowledged by the Angiosperm Phylogeny Group shows this family to be correctly placed within a larger circumscription of the sandalwood family, Santalaceae (Kujit, 2003). They are woody hemi-parasitic shrubs with branches 10–80 cm (3.9–31.5 in) long, which grow on other trees. The foliage is dichotomously branching, with opposite pairs of leaves; these are relatively large, 2–5 cm (0.79–1.97 in) long, green and photosynthetic in some species (e.g., *P. leucarpum*), but minimal in some others (e.g., *P. californicum*). Although they can photosynthesise, the plant relies on its host for some nutrients (Hiller, 2010).

Plate 1: Fresh leaf of Mistletoe in the present study

Mangifera indica, commonly known as mango (Plate 2), is a species of flowering plant in the sumac and poison ivy family Anacardiaceae. Mangoes are believed to have originated from the region between northwestern Myanmar, Bangladesh, and northeastern India. It is a large fruit tree, capable of growing to a height and crown width of about 30 metres (100 ft) and trunk circumference of more than 3.7 metres (12 ft). *M. indica* were domesticated separately in South Asia and Southeast Asia since ancient times, resulting in two distinct genetic populations in modern mangoes - the "Indian type" and the "Southeast Asian type". Mangoes have since been introduced to other warm regions (Bally *et al*., 2017). Over 500 varieties of mangoes are known, many of which ripen in summer, while some give a double crop. The fruit takes four to five months from flowering to ripening (Morton, 2007).

Plate 2: Mango tree displaying fresh leaves used in the present study

The development of antimicrobial agent resistance has forced scientists to search for antibacterial substances from alternative sources such as medicinal plants. This has also made medicinal plants receive much attention as an alternative therapy against synthetic drugs. Traditionally, mistletoe extracts have been used against various diseases, such as disorders in the female reproductive system, cancer, arthritis, rheumatism, epithelial tumours, hypertension, asthma, nervousness and epilepsy (Evans, 2015). Synthetic drugs are often the option for chemotherapy. However, most synthetic drugs kill not only targeted cells but also normal cells, and most have severe side effects. There is a need to search for more efficacious antibacterial agents with fewer side effects; therefore, there is an urgent need for novel treatment options with improved features.

It has been estimated that about 70% of the third-world population is almost entirely dependent on traditional medicines for maintaining general health and combating many diseases (Kuhn, 2015). The present study can increase public awareness of natural remedies for treating bacterial infections. This study aimed to assess the antibacterial activities of bi-herbal ethanolic extract of *Phorademdron serotinum* (Mistletoe) leaves and *Mangifera indica* (Mango) against gastrointestinal pathogens to extract and ascertain the phytochemicals present in the ethanolic extracts of *Phorademdron serotinum* (Mistletoe) leaves and *Mangifera indica* (Mango) leaves; to assess the antibacterial activities of *Phorademdron serotinum* (Mistletoe) leaves and *Mangifera indica* (Mango) leaves; and to evaluate the synergistic effect of *Phorademdron serotinum* (Mistletoe) leaves and *Mangifera indica* (Mango) leaves.

MATERIALS AND METHODS

The plant samples were collected from the Division of Agriculture College (DAC) and Ahmadu Bello University (ABU), Samaru-Zaria. They were transferred to the Herbarium section of the Biological Science Department, Ahmadu Bello University, Samaru-Zaria, for authentication and identification. The collected plant samples were washed, dried under shade and pounded using mortar and pestle into powdered form ready for analysis.

Extraction of Plant Materials

The plant extract to study the antimicrobial potential of *Phorademdron serotinum* (Mistletoe leaves) and *Mangifera indica* (Mango leaves) using ethanol was prepared as adopted by Umar *et al*. (2016) with a minor modification. Each plant's dried and powdered leaves were sieved using a sterile sieve. Exactly 100g of each powdered plant material was transferred into a beaker containing 150 of ethanol, allowed to soak for 48 hours, and sieved using a paper sieve. The extraction solvent was removed by evaporation. The extract was then stored in an airtight container until required for use. Each extract's percentage yield was measured using a weighing balance (Mettle 166®, USA) and recorded as a percentage of the total weight of each plant's material used for the extraction.

Qualitative Phytochemical Analysis

Phytochemical screening was carried out using established protocols as described by (Harbone, 1998). A stock solution of the extracts with a 1mg/mL concentration was prepared and used.

Test for tannins

Exactly 2mL of the ethanolic extract solution was stirred with 2mL of distilled water, and a few drops of $FeCl_3$ solution were added. The formation of a green precipitate indicated the presence of tannins.

Test for saponins

Exactly 5mL of ethanolic extract solution was mixed vigorously with 5mL of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for flavonoids

Exactly 1 ml of ethanolic extract solution was added to 1 ml of 10% lead acetate solution. The formation of a yellow precipitation was taken as a positive test for flavonoids.

Test for terpenoids

Exactly 2mL of the ethanolic extract solution was dissolved in 2mL chloroform and evaporated to dryness. A volume of 2ml of concentrated sulphuric acid was then added and heated for two (2) minutes; the formation of a greyish colour indicated the presence of terpenoids.

Test for glycosides

Exactly 2mL of each extract solution was dissolved in 2mL of chloroform. A volume of 2mL of sulphuric acid (Sigma-Aldrich®, 7664-93-9, USA) was carefully mixed gently. Reddish brown colours indicate the presence of a steroidal ring (that is, a glycone portion of glucoside).

Test for alkaloids

Exactly 3mL of ethanolic extract solution was stirred with 3mL of 1% HCl in a steam bath. One drop each of Mayer's and Wagner's reagents was then added to the mixture. The turbidity of the resulting precipitate was taken as evidence of the alkaloids' presence.

Test for steroids

Exactly 2mL of acetic anhydride was added to 2mL of H_2SO_4 (Sigma-Aldrich®, 7664-93-9, USA). A colour change from violet to blue indicates the presence of steroids.

Test for Anthraquinones

A mass of 0.5 g of the extracts was boiled with 10% HCl for a few minutes in a water bath. It was filtered and allowed to cool. An equal volume of chloroform was added to the filtrate. A 10 per cent ammonia drop was added to the mixture and heated. The formation of a rose-pink colour indicates the presence of anthraquinones.

Test for Resin

Exactly 0.2g of extract was treated with 1.5 mL of 96% ethanol. The alcoholic extract was poured into 2 ml of distilled water in a beaker. A precipitate indicated the presence of resins.

Test for Phenol

Exactly 1mL of the extract was placed in a clear dried test tube, and 1mL of 5% ferric chloride was added to the test tube. Blue colourations indicated the presence of phenol.

Preparation of Test Organisms

The test organisms for this study are clinical gastrointestinal pathogens that include *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus cereus, Vibrio cholerae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, which were obtained from the Microbiology Laboratory of the Department of Microbiology, Ahmadu Bello University (ABU), Zaria. All the growth media used were prepared aseptically using the manufacturer's specifications. Each test organism was inoculated on nutrient agar and incubated at 37°C for 24 hours. Each of the cultures was sub-cultured to obtain pure isolates. The purified colonies of each test organism were standardised by using regular saline suspension of the organisms to a turbidity matching 0.5% McFarland standard (10^8cfu/ml).

Preparation of Plant Extract Concentrations.

Each extract was prepared in a diffuse concentration of 100mg by dissolving 1g of the extract in 10 mL of EDTA to obtain a 100mg/mL concentration. A ten-fold serial dilution was used to obtain

half the strength of each dilution. A successive dilution was used to obtain concentrations of 50mg/mL, 25mg/mL, 12.5mg/mL, and 6.25mg/mL respectively (Umar *et al*., 2016).

Antimicrobial Assay

Mueller-Hinton agar and broth used in this analysis were prepared according to the manufacturer's standard, sterilised at 121℃ for 15 minutes, and cooled. Here, the agar plate surface was inoculated by spreading a volume of 0.1mL of the microbial inoculum over the entire agar surface, and it was allowed to absorb. Wells 5mm in diameter and 2cm apart were bored in the culture media with a sterile cork-borer. The various concentrations were dispensed into each well to fullness (Shaidi, 2017). The already prepared extract was incorporated into the well and diffused. Inhibition zones around the wells were measured in millimetres after 24 hours of incubation. The control drugs (ciprofloxacin 30µg and septin 30µg) were also used to serve as a positive control, and blank EDTA served as a negative control (Umar *et al*., 2016).

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the broth dilution method. Exactly 2mL of the sterilised broth was dispensed into 16 test tubes (4 for each organism at different concentrations). A volume of 0.2 mL of the extract was added to the various tubes, and 0.1mL of the test organisms was added and then incubated at 37℃ for 24 hours. The lowest concentration in the tube showing the absence of turbidity is recorded as the extract's minimum inhibitory concentration (MIC).

Minimum Bacteriocidal Concentration (MBC)

A 20 mL of molten Mueller Hinton agar was dispensed into four Petri dishes and allowed to solidify. The petri dishes were each labelled with the name of the organisms and based on the concentrations in the test tubes from the MIC tests that showed no turbidity. A sterilised wire loop was used to obtain inoculum from the respective tubes and inoculated by streaking method on the surface of the solidified agar. This was repeated for each concentration and was incubated at 37℃ for 24 hours. Following incubation, the plates inoculated from the lowest concentration showing no colony growth were recorded as the minimum Bactericidal Concentration (MBC).

RESULTS

Table 1 shows a quantitative analysis of the phytochemical analysis of the ethanolic extract of *Phorademdron serotinum* (Mistletoe) and *Mangifera indica* (Mango) leaves. The result reveals that all the phytochemicals investigated on the plant were present except for glycosides and oxalate in the ethanolic extract of *Mangifera indica*.

Table 1: Phytochemical analysis of *Phorademdron serotinum* and *Mangifera indica* leaves extracts

S/N	Parameters	Mango	Mistletoe
1	Alkaloids	+	+
2	Steroid	+	+
3	Tannins	+	+
4	Phenol	+	+
5	Terpenoid	+	+
6	Anthraquinone	+	+
7	Glycoside	−	+
8	Saponins	+	+
9	Flavonoids	+	+
10	Resin	+	+
11	Oxalate	−	+

Key: += Present; −= Absent

The results showed that *Phorademdron serotinum* extract contained alkaloids, Steroids, tannins, phenols, terpenoids, anthraquinones, saponins and flavonoids. The phytochemical screening of the *Mangifera indica* leaf extract showed alkaloids, Steroids, tannins, phenols, terpenoids, anthraquinones glycosides, saponin and flavonoids. This result agrees with the findings of Dushimemaria *et al*. (2017), who reported the presence of alkaloids, phenols, flavonoids, saponins, and tannins in *Mangifera indica* leaf extract. Coder (2008) reported a wide range of plant secondary metabolites in the mistletoe leaf extract.

Table 2 shows the percentage yield of the bi-herbal extract of mango leaves

Table 2: Percentage yield of the bi-herbal extract of mango and mistletoe leaves

Samples	Percentage Yield (%)
Mango leaves	31.0
Mistletoe leaves	40.4
Bi-herbal (mango leaves and mistletoe leaves)	53.0

The results showed that *Phorademdron serotinum* and *Mangifera indica* yielded 49.4% and 41.0%, respectively, while the bi-herbal/combined extracts (1:1) yield of *Mangifera indica* was 53%. This agreed with the findings of Stanley (2015), who reported a yield of more than 20% by extraction using ethanol as solvent. This is probably due to the polarity of ethanol, giving it more extraction ability than other non-polar and less polar solvents.

Table 3 shows the antimicrobial screening of the ethanolic extract of *Phorademdron serotinum* (Mistletoe) and *Mangifera indica* (Mango) leaves at different concentrations. The result revealed that the extract exhibited some degree of inhibition of the test organisms.

Table 3: Antibacterial activity of *Phorademdron serotinum* (Mistletoe) and *Mangifera indica* (Mango) leaf extracts.

Test Organism	**Zone of Inhibition (mm) at Various Concentrations of the Extract 100 (mg/ml)**			**Control (mm)**	
	Mangifera indica	***Phorademdron serotinum***	**Bi-herbal**	**Sxt**	**Cpx**
Staphylococcus aureus	24.00	22.00	27.00	28.00	43.00
Streptococcus pneumoniae	14.00	17.00	22.00	16.00	37.00
Bacillus cereus	11.00	9.00	23.00	10.00	31.00
Vibrio cholerae	12.00	14.00	23.00	25.00	29.00
Klebsiella pneumoniae	16.00	12.00	21.00	30.00	34.00
Pseudomonas aerugenosa	22.00	24.00	30.00	23.00	34.00

Key: Sxt= Septrin; Cpx= Ciprofloxacin

Antibacterial screening of combined plant extracts, *Phorademdron serotinum* (Mistletoe leaves) and *Mangifera indica* (Mango leaves), show that the zone of inhibition of antibacterial screening of the extract has various inhibitory effects against the test organisms, which recorded activities against *Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus, Vibrio cholera, Klebsiella pneumoniae and Pseudomonas aeruginosa* at a range between 9.00mm to 24.00mm. The bi-herbal extract has the highest zone of inhibition of 27mm at a concentration of 100mg/mL against *Staphylococcus aureus,* followed by *Mangifera indica* extract, which recorded a zone of inhibition of 24mm at a similar concentration against *Staphylococcus aureus*. The zone of inhibition of the standard drug, i.e., septrin, recorded 10mm against *Bacillus cereus*, which was less potent than the activity of the bi-herbal (23mm) extract.

The bi-herbal extract has the highest zone of inhibition against *Streptococcus pneumoniae*, which is 22mm at 100mg/mL. In contrast, *Phorademdron serotinum* (Mistletoe leave) extract recorded a zone of inhibition of 17mm at 100mg/ml concentration against *Streptococcus pneumoniae,* followed by *Mangifera indica* (Mango leave) extract, which has a zone of inhibition of 14mm at similar concentration against *Streptococcus pneumoniae*. The zone of inhibition of the standard drug, i.e. Ciprofloxacin, is 30mm against *Streptococcus pneumoniae,* showing the broad-spectrum nature of Ciprofloxacin.

The bi-herbal extract has the highest zone of inhibition against *Bacillus cereus* at 23mm at 100mg/ml. In contrast, *Mangifera indica* leaf extract has a zone of inhibition of 11mm at 100mg/ml concentration against *Bacillus cereus*, followed by *Phoradendron serotinum* leaf extract has a zone of inhibition of 9mm at a similar concentration against *Streptococcus pneumoniae*. The zone of inhibition of the standard drug, i.e.., Ciprofloxacin, is 30mm against *Bacillus cereus*. The bi-herbal extract has the highest zone of inhibition against Vibro cholerae, which is 23mm at 100mg/ml. In contrast, *Phorademdron serotinum* leaf extract has a zone of inhibition of 14mm at 100mg/ml concentration against *Vibro cholerae,* followed by *Mangifera indica* (Mango leave) extract, which has a zone of inhibition of 12mm at a similar concentration against *Vibrio cholerae*. Hence, the standard drug's inhibition zone, i.e. Ciprofloxacin, is 30mm against *Vibrio cholerae*.

The bi-herbal extract has the highest zone of inhibition against *Klebsiella pneumoniae* at 21mm at 100mg/ml. In contrast, *Mangifera indica* leaf extract has a zone of inhibition of 14mm at

100mg/ml concentration against *Klebsiella pneumoniae*, followed by *Phorademdron serotinum* leaf extract, which has a zone of inhibition of 12mm at a similar concentration against *Klebsiella pneumoniae*. Hence, the standard drug's inhibition zone, i.e. Ciprofloxacin, is 30mm against *Klebsiella pneumoniae*. The bi-herbal extract has the highest zone of inhibition of 30mm at 100mg/ml against *Pseudomonas aeruginosa*.

In contrast, *Phorademdron serotinum* leaf extract has a zone of inhibition of 24mm at 100mg/ml concentration against *Pseudomonas aeruginosa*, followed by Mango leaf extract, which has a zone of inhibition of 22mm at a similar concentration against *Pseudomonas aeruginosa*. The standard drug's inhibition zone, i.e. Ciprofloxacin, is 30mm against *Pseudomonas aeruginosa*. This agrees with the findings of Martinez and Watson (2006) and Wettberg *et al*. (2019), who reported the activity of mango and mistletoe leaf extracts against Gram-positive and Gram-negative bacterial species.

Other studies reported that *Mangifera indica* leaf extract has been recognised as a rich source of bioactive compounds with potential pharmaceutical and nutraceutical applications and has attracted increasing interest from the research (Oak & Deshpande, 2019). Furthermore, it was reported that complete *Phorademdron serotinum* leaf extract is more potent at inhibiting tumour cells, and there is synergistic action between different groups of mistletoe compounds. The bi-herbal plant extracts show bi-directional activity in treating cancer and gastrointestinal diseases.

Table 4 depicts the result of the Minimum Inhibitory Concentration of *Phorademdron serotinum*, and *Mangifera indica* extract at different concentrations.

Minimum inhibitory concentration (MIC) is the minimum concentration in (mg/L) of extract that inhibits the growth of the bacteria or exhibits a bacteriostatic effect. The MIC provides information concerning the susceptibility or resistance of a test organism to the antibacterial agent or extract and can help make correct treatment decisions (Adhikary, 2011). This result shows that the extract has various inhibitory effects against the test organisms*: Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus, Vibro cholerae, Klebsiella pneumoniae and Pseudomonas aeruginosa*. The *Magnifera indica* leaves extract recorded a minimum inhibitory concentration of 12.5mg/ml against *Staphylococcus aureus*, and the *Phoradendron serotinum* has a similar

inhibition concentration against *Staphylococcus aureus*. However, the bi-herbal plant extracts have a minimum inhibitory concentration of 6.25mg/ml against *Staphylococcus aureus*. *Staphylococcus aureus* is the most pathogenic; it typically causes skin infections and sometimes pneumonia, endocarditis, and osteomyelitis. It commonly leads to abscess formation. Some strains elaborate toxins that cause gastroenteritis, scalded skin syndrome, and toxic shock syndrome, but they can efficiently be inhibited by various ethnomedicinal herbs (Kubacher *et al.*, 2017).

Table 4: Minimum inhibitory concentration of *Phorademdron serotinum* and *Mangifera indica* extracts

Test microbes	**Concentration of Bi-herbal Extract of Mango Root and Mistletoe Leave in mg/ml**														
	Mango leaves					**Mistletoe leaves**					**Bi-herbal**				
	100	50	25	12.5	6.25	100	50	25	12.5	6.25	100	50	25	12.5	6.25
S. aureus	–	–	–	+	++	–	–	–	+	++	–	–	–	–	+
S. pneumoniae	–	–	–	+	++	–	–	–	–	–	–	–	–	–	–
B. cereus	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–
V. cholerae	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–
K. pneumoniae	–	–	–	+	+	–	–	–	–	+	–	–	–	–	–
P. aeruginosa	–	–	–	+	++	–	–	++	+	++	–	–	–	–	+

Key: -= no turbidity; += mild turbidity; +++= high turbidity; ++= moderate turbidity: ++++= very high turbidity

The ethanolic leaf extract of *Mangifera indica* recorded an inhibitory concentration of 12.5mg/ml against *Streptococcus pneumoniae*, followed by the Bi-herbal plant extract with an inhibitory concentration of 6.25mg/ml against *Streptococcus pneumoniae*. *Phoradendron serotinum* has a clear inhibitory concentration against *Streptococcus pneumoniae*. *Streptococcus pneumoniae* has emerged as one of the most essential *Streptococcus* species. In addition, several recent investigations have reported the recovery of *Streptococcus* resistant to the antibacterial drugs currently available, such as penicillin G, Ciprofloxacin and ofloxacin (Choi *et al.*, 2016).

However, Martinez and Watson (2006) reported the sensitivity of Streptococcus species to mistletoe leaf extract.

The ethanolic leaves extract of *Phoradendron serotinum* has an inhibitory concentration of 25mg/ml against *Bacillus cereus*, followed by the *Mangifera indica* with an inhibitory concentration of 6.25mg/ml against *Bacillus cereus*. Hence, Bi-herbal plant extract has an apparent inhibitory concentration against *Bacillus cereus*. Developing bi-herbal plant extract against antibacterial resistance in *Bacillus* species is a significant problem and reason for treatment failure in clinical settings. Hence, there is a need to develop newer antibacterial molecules and modify existing antibacterials to make them more effective and less toxic.

Mangifera indica has an inhibitory concentration of 12.5mg/ml against *Vibrio cholerae*, followed by the *Phoradendron serotinum* with an inhibitory concentration of 6.25mg/ml against *Vibrio cholerae*. Bi-herbal plant extract has an apparent inhibitory activity against *Vibrio cholerae*. The ethanolic leaves extract of *Phoradendron serotinum* has an inhibitory concentration of 25mg/ml against *Klebsiella pneumoniae*, followed by the *Mangifera indica* with an inhibitory concentration of 12.5mg/ml against *Klebsiella pneumoniae*. Thus, Bi-herbal plant extract has a minimum inhibitory concentration of 6.25mg/ml against *Klebsiella pneumoniae*. Also, the ethanolic extract of *Mangifera indica* has an inhibitory concentration of 12.5mg/ml against *Vibrio cholerae*, followed by the *Phoradendron serotinum* at the same concentration against *Vibrio cholerae*. Hence, Bi-herbal plant extract has a clear inhibitory ac against *Vibrio c*ooperativity. This agrees with the findings of Wettberg *et al*. (2019), who reported the application of mango leaf extract in treating diseases caused by Gram-negative and Gram-positive bacteria.

Table 5 shows the Minimum Bacteriocidal Concentration of the bi-herbal extract of *Phorademdron serotinum* and *Mangifera indica* extract at different concentrations.

Table 5: Minimum Bacteriocidal Concentration of Bi-herbal Extract of *Phorademdron serotinum* and *Mangifera indica*

Test microbes	Concentration of Bi-herbal Extract of Mango Root and Mistletoe Leave in mg/ml													
	Mango leaves					Mistletoe leave					Bi-herbal			
	100	50	25	12.5	6.25	100	50	25	12.5	6.25	100	50	25	12.5	6.25
S. aureus	–	–		++	+++	–	–	–	++	+++	–	–	–	++	
S. pneumoniae	–	–		++	+++	–	–	–	–	+++	–	–	–	++	
B. cereus	–	–		–	++	–	–	++	+++	+	–	–	–	–	
V. cholerae	–	–		++	++	–	–	++	+++	++	–	–	–	+++	
K. pneumoniae	–	–		++	++	–	–	–	++	+++	–	–	–	+	
P. aeruginosa	–	–		++	+++	–	–	–		+++	–	–	–	–	

Key: - no turbidity; += mild turbidity; ++= moderate turbidity; +++= high turbidity; ++++= very high turbidity.

The *Phoradendron serotinum* (Mistletoe leaves) has the minimum bacteriocidal concentration of 50mg/ml against *Bacillus cereus*, followed by *Magnifera indica* (Mango leaves), which has the minimum bacteriocidal concentration of 12.5mg/ml against *Bacillus cereus*. Thus, the bi-herbal plant extracts have the minimum bacteriocidal concentration of 6.25mg/ml against *Bacillus cereus*. This coincides with the findings of Wettberg *et al*. (2019), who reported both bacteriostatic and bactericidal effects of mango leaf extracts against various bacterial species.

Thus, *Mangifera indica* (Mango leaves) recorded the minimum bacteriocidal concentration of 25mg/ml against *Vibro cholerae*, followed by *Phoradendron serotinum* (Mistletoe leaves), which has the minimum bacteriocidal concentration of 12.5mg/ml against *Vibro cholerae*. The bi-herbal plant extracts have the minimum bactericidal concentration of 6.25mg/ml against *Vibro cholerae*.

The *Phoradendron serotinum* (Mistletoe leaves) has the minimum bacteriocidal concentration of 50mg/ml against *Klebsiella pneumoniae*, followed by *Mangifera indica* (Mango

leaves), which has the minimum bacteriocidal concentration of 25mg/ml against *Klebsiella pneumoniae*. The bi-herbal plant extracts have the minimum bacteriocidal concentration of 12.5mg/ml against *Klebsiella pneumoniae*.

The *Mangifera indica* (Mango leaves) and *Phoradendron serotinum* (Mistletoe leaves) have the minimum bacteriocidal concentration of 25mg/ml against *Pseudomonas aeruginosa*. Hence, the bi-herbal plant extracts have the minimum bacteriocidal concentration of 6.25mg/ml against *Pseudomonas aeruginosa*. This concurs with the study of Martinez and Watson (2006), who confirmed the bactericidal effect of mistletoe leaf extracts.

CONCLUSION

The study to assess the antibacterial activities of Bi-herbal ethanolic extract of *Phorademdron serotinum* (Mistletoe) leaves, and *Mangifera indica* (Mango) leaves validates the saying "Derivatives from plants have been said to have therapeutic properties" as stated by the WHO (2015). The extract of *Phorademdron serotinum* leaves, and *Mangifera indica* leaves were tested separately on *Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus, Vibrio cholerae, Klebsiella pneumoniae and Pseudomonas aeruginosa* and both were also tested synergistically to examine their effect on the test organisms.

The test organisms were sensitive to each of the plant's ethanolic extracts with a clear zone of inhibition around the agar well. When tested synergistically on each bacteria isolate, the plant extract showed greater potency strength. In addition, the study revealed that the *Phoradendron serotinum* and *Mangifera indica* extracts could be used together as they demonstrated a synergistic effect. Both plant extracts had a minimum inhibitory concentration of 25mg/ml when tested individually. However, when used together, their minimum inhibitory concentration was significantly reduced to 2.25mg/ml. This suggests combining these plant extracts may be a more effective treatment against bacterial infections. When tested separately, the minimum bactericidal concentration for both plants was 12.5mg/ml; for both plants used synergistically, the minimum inhibitory concentration was 6.25mg/ml.

It can then be concluded that *Phorademdron serotinum* and *Mangifera indica* extract can be used for the treatment of bacterial infection caused by gastrointestinal pathogens such as

Staphylococcus aureus, Streptococcus pneumoniae, Bacillus cereus, Vibrio cholerae, Klebsiella pneumoniae or *Pseudomonas aeruginosa*. However, the plant extract can be used synergistically to treat and eradicate pathogens effectively.

Recommendations

In subsequent studies that will be carried out in the future on similar study, it is recommended that the researchers should consider the following:

1. The use of various solvents to enhance the effect of extraction.
2. The combination of different parts of plant extract should be tested on different organisms to test the effects because organisms have begun to develop resistant genes to some plant extract.
3. The testing of Bi-herbal extract on fungi isolates should be considered.
4. The inhibitory and antagonistic relationships between the plants used in synergy should be studied.

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