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# Evaluation of the Phytochemical and Antimicrobial potential of the Leaf Extracts of *Bryophyllum pinnatum* L.and *Citrus aurantifolia* Sw. and their Synergy.

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**ABSTRACT:** The aim of this research is to comparatively study the phytochemical and antimicrobial properties of *Bryophyllum pinnatum* and *Citrus aurantifolia* leaf extracts and their synergy. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa, Aspergillus niger, Mucor mucedo, Penicillium notatum and Candida albicans,* were used as test organisms and the antimicrobial activity of the extracts was determined by the agar-well diffusion method. Synergistic antibacterial activity ranged from 0.0 ± 0.0 to 2.4 ± 0.6, 11.3 ± 0.9 to 23.5 ± 1.1, 16.7 ± 0.3 to 27.3 ± 0.6 and 8.7 ± 0.9 to 22.7 ± 0.9, for aqueous, ethanol, methanol and acetone extracts respectively. Extracts of *C. aurantifolia* were more effective against the test organisms than *B. pinnatum* extracts, except the aqueous extract. Synergistic antifungal activity of the aqueous extract was 0.0 ± 0.0 mm for all the test fungi, the synergistic antifungal activity ranged from 8.7 ± 0.6 mm to 14.0 ± 0.9 mm, 10.0 ± 0.9 mm to 21.7 ± 0.6 mm and 0.0 ± 0.0 mm to 20.0 ± 0.6 mm for the ethanol, methanol and acetone extract respectively. Larger zones of inhibition were observed in the methanol extract of the synergy than the other extracting solvents. The synergy gave higher zones of inhibition neither *B. pinnatum* extract nor *C. aurantifolia* extract could give. It was also observed that the extracts compared well with the standard antimicrobial agents used as positive control. The phytochemical analysis of the extract revealed the presence of phytochemical constituents which conferred antimicrobial property on the plants. From the foregoing, the methanol extract of the synergy is considered the most effective in the treatment of infections caused by the test organisms. 

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***KEY WORDS*:** *Bryophyllum pinnatum*, *Citrus aurantifolia*, Synergy, Antimicrobial, Phytochemical constituents.

## Introduction

According to the World Health Organization, a medicinal plant is any plant in which one or more of its organs contain substances that can be used for the synthesis of useful drugs (WHO, 1977). Plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al.*, 2000). Medicinal plants contains biologically active chemical substances such as saponins, tannins, essential oil flavonoids, alkaloids and other chemical (Sofowora, 1996) which have curative properties. These complex chemical substances of different composition are found as secondary plant metabolite in one or more of these plants (Kayode and Kayode, 2011). There are several published reports describing the antimicrobial activity of various crude plant extracts either in single or in combinations (Igoli *et al.*, 2005). It is estimated that there are about 2.5 million species of higher plants and the majority of these have not yet been examined for their pharmacological activities. Herbal extracts are fast becoming popular as natural antimicrobial preservatives or additives (Cox *et al.*, 2010).

In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Piddock and Wise, 1989; Singh *et al*., 1992; Mulligen *et al*., 1993; Davis, 1994; Robin *et al*., 1998). However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antimicrobials. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants (Mandal *et al*., 2009; Basualdo *et al*., 2007). Being new, such compounds may not have the problem of microbial resistance.

Plant-based antimicrobials represent a vast untapped source. The use of plant extract for medicinal treatment has become popular especially now when people are beginning to realize that the effective life span of antimicrobials is limited and ove-prescription

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and misuse cause microbial resistance (Alam *et al.*, 2009). Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harborne and Baxter, 1995). In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Grosvenor *et al.*, 1995; Ratnakar and Murthy, 1995; David, 1997; Saxena, 1997; Nimri *et al.*, 1999; Saxena and Sharma, 1999). Presently, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or ayurvedic medicines (Murugesan *et al.*, 2011; Jabeen *et al*., 2007; Banso, 2009; Ahameethunisa and Hopper, 2010).

*Citrus aurantifolia* (Lime) is a small fruit from the Citrus family; it comes either sour or sweet naturally. Sour limes possess a greater sugar and citric acid content than lemons and feature an acidic and tart taste (Bina *et al*., 2010). The nutritional profile includes information on a full array of nutrients including carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids, amino acids and more. Limes contain unique flavonoid compounds that have antioxidant and anti-cancer properties. These flavonoids have been shown to stop cell division in many cancer cell lines and are perhaps most interesting for their antibiotic effects (Tomotake, 2006). *C. aurantifolia* exhibits bioactive activities for colds, fevers, sore throats, sinusitis, bronchitis and asthma (Khan *et al.*, 2012).

*Bryophyllum pinnatum* (*Kalanchoe pinnatum* or *Bryophyllum calycinum*), belongs to the family crassulaceae, and it is commonly known as sprouting leaf. It is found in the tropical Africa, India, China, America and Australia (Devbhuti *et al.*, 2012; Gill, 1992). The leaves and leaf juice have been used traditionally as anti-inflammatory, antipyretic, antimicrobial, anti-oxidant, antitumour, antidiabetic, anti-ulcer, antiseptic, hypocholosterolemic, and cough suppressant (Ali *et al.*, 2013). The leaves and bark are not sweet, astringent to the bowels, analgesic, and useful in diarrhea and vomiting (Quazi *et al.*, 2011). The plant is good source of ascorbic acids, riboflavin, thiamine and niacin.

Traditional preparation of medicinal plants with antimicrobial activities have been extensively used in the West African regions (Adesuyi *et al*., 2012; Dunford *et al*., 2000; Mboto *et al*., 2009; Mythilypriya *et al*., 2007). Combined therapy is traditionally used to increase antimicrobial activity and reduce toxic effects of agents (Tallarida, 2001). This study was carried out to evaluate the phytochemical properties and biotherapeutic potential of *Bryophyllum pinnatum*, *Citrus aurantifolia* and their synergy, using different extracting solvents.

## MATERIALS AND METHODS

*Collection of plant materials*: Fresh *B. pinnatum* and *C. aurantifolia* leaves were obtained from home gardens in Benin City, Edo State, Nigeria and identified in Department of Plant Biology and Biotechnology of the University of Benin, Benin City, identification was confirmed with appropriate literature (Akobundu and Agyakwa, 1998; Keay, 1989). The leaves were air-dried, grinded and made into a fine powder using laboratory mortar and pestle and kept in a sterile air-tight container to avoid contamination.

*Preparation of extract*: Fifty grammes each of dried pulverized leaf powder was dissolved in 500 ml each of distilled water (to make aqueous extract) for 24 hrs and centrifuged at 3000 rpm to enable paper diffusion of the active ingredients into the extraction medium. Filtration was later carried out using Whatman's (No. II) filter paper and the filtrate was evaporated to dryness using steam water-bath at 100 $^{o}$C. This procedure was further carried out with ethanol, methanol and acetone to obtain ethanol extract, methanol extract and acetone extract respectively. The extracts were now stored at 4 $^{o}$C in a refrigerator. Combination of both plants was used in the synergistic assessment.

*Test Organisms:* Bacterial cultures of the test organisms, *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the Department of Medical Microbiology, University of Benin teaching hospital, Benin City, Nigeria. Their identity was confirmed using cultural, morphological and biochemical test as described by Cheesebrough *et al*. (2002). They were maintained on nutrient agar slants at 4 $^{0}$C. These test bacteria have been previously described (Prescott *et al*., 2008 Akinnibosun *et al*., 2008a, b). Fungal species from laboratory stock of the Department of Microbiology, University of Benin, Benin City, identified and characterized based on their morphological characteristics and microscopic analysis by using taxonomic guides and standard procedures (Gilman, 1944; Barnett and Hunter, 1972; Ellis, 1976; Domsch *et al*., 1980), were also used as test organisms (*Aspergillus niger, Mucor mucedo, Penicillium notatum and Candida albicans*).

*Phytochemical screening of the extracts:*
Phytochemical screening of the extracts was carried out according to methods described by Odebiyi and

Sofowora, (1978) and Trease and Evans, (1989) as follows:

*Test for Alkaloids*: Five grams of evaporated extract was boiled with 5 ml of 2 % HCL on a steam bath for 5 mins, the mixture was filtered after cooling, and the filtrate was shared into 3 test tubes A B and C. 1 ml portion of filtrate was treated with 2 drops of Mayer's reagent, a creamy white precipitate was observed. To confirm this result, 1 ml portion of the filtrate was treated with Dragendoff's reagent which gave a red precipitate to indicate the presence of alkaloids.

*Test for Flavonoids:* Five grams of extracts was introduced into a test-tube containing 10 ml ethyl acetate solution and heated in boiling water for 1 min, the mixture was filtered and 4 ml of filtrate was shaken with 1ml of 1 % aluminum chloride solution and left to stand for 10 mins. The formation of a yellow colouration in the presence of 1 ml of dilute ammonia solution, indicated the presence of flavonoids.

*Test for Saponins:* One gram of extract was boiled with 5ml of distilled water for 5 mins and the mixture was filtered while hot. To 1 ml of filtrate, two (2) drops of olive oil was added, the mixture was shaken and observed for the formation of emulsion, then 1 ml of the filtrate was diluted with 4ml of distilled water. The mixture was shaken and observed for the formation of stable frothing on standing, which indicated positive for saponins.

*Test for Tannins***:** To 2 g of the sample, 5 ml of 45 % ethanol was added and boiled for 5 mins. The mixture was cooled and filtered. To 1 ml of the filtrate, three (3) drops of lead acetate solution was added. The formation of gelatinous precipitate indicates the presence of tannins. Also as a confirmation test, 1 ml of filtrate was treated with 0.5 ml of bromine water and the formation of a pale brown precipitate indicates the presence of tannins.

*Test for glycosides:* Two grams of samples were mixed with 30 ml of distilled water and boiled for 5 mins in a water bath. The mixture was cooled and filtered. To 5 ml of the filtrate, 0.2 ml of Fehling's solution A and B were added and boiled further in a water bath for 2 mins. A brick red colouration which indicates the presence of glycosides was noticed.

*Test for Reducing Sugar:* About 5 g each of the dried samples was introduced into a test tube and equal amount of Fehling's solution A and B were added. The mixture was boiled over a burner and observed for colour change. The colour changed from deep blue to brick red, indicating the presence of reducing sugar.

*Test for Steroids***:** About 2 ml each of concentrated sulphuric acid ($H_2SO_4$) and acetic anhydride were poured into 5ml each of the aqueous extract samples. The colour changed from violet indicates the presence of steroids.

*Determination of Antimicrobial Activity***:** The crude extracts were screened for antimicrobial activity by determining the zone of inhibition against the test organisms using agar-well diffusion method. Sterile Mueller-Hinton agar plates were inoculated with prepared inoculum with sterile cotton swab. Then with the help of sterile cork borer, wells were made in the inoculated media plate. 50 µl of the working solution/ suspension of different concentration were transferred into the well with the help of micropipette. The control was also placed in the separate well at the same time. After proper incubation, the plates were viewed for the zone of inhibition, which is suggested by clear areas without growth around the well.

## RESULTS AND DISCUSSION

Microbial resistance to several antibiotics is becoming a source of challenge and concern to public health. In view of the increasing rate of antimicrobial drug resistance ravaging not only the African continent but the world at large, alternative, effective and affordable substitutes are essential if bacterial infections are to be properly controlled. Phytochemicals are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms. Many plants containing alkaloids and flavonoids have diuretic, anti-inflammatory and analgesic effects. Alkaloids are capable of reducing headache associated with hypertension. It has been reported that alkaloids can be used in the management of cold, fever and chronic Catarrh. Flavonoids are known for their antioxidant activity and hence they help to protect the body against cancer and other degenerative diseases (Jindal *et al.* 2012). Tannins are known to exhibit antiviral, antibacterial and antitumor activities. Saponin is used as hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory and weight loss. The presence of these phytochemicals (steroids, tannins, reducing sugars, flavonoids, alkaloids, saponins and cardiac glycosides) in *B. pinnatum* and *C. aurantifolia* used in this study (Tables 1 – 3) supports their use as medicinal plants. These chemical constituents could be responsible for their antibacterial activity (Gill, 1992). Different plant parts contain a complex of chemicals with unique

biological activity (Farnsworth and Bingel, 1977), which is thought to be due to toxins and secondary metabolites, which act as attractants or deterrents (Fisher, 1991). Over the years, these bioactive principles have been exploited in tradomedical practice for the treatment of various ailments (Adebanjo *et al.,* 1983).

Antimicrobial resistance of pathogenic bacteria to current synthetic drugs has necessitated the investigation into new, safe, efficient and cost-effective antimicrobial agents as alternative agents for controlling the infectious diseases (Khan *et al.,* 2012). The extent of sensitivity of the test organisms to the plant fractions was assessed by measuring the zone of inhibition after 24 hrs incubation. Table 4 shows the antimicrobial activity of *B. pinnatum* leaf extract using different extracting solvents. The results revealed that the ethanol extract of *B. pinnatum* was most effective against the test organisms than the other extracting solvents. *S. aureus* showed the highest susceptibility (17.3 ± 1.2 mm) to *B. pinnatum* ethanol extract, while *P. aeruginosa* showed the least susceptibility (8.3 ± 0.9 mm). The results also revealed that all the test fungi were resistant to the different extracts except *C. albicans.* The ethanol extract of *B. pinnatum* was the most effective against the *C. albicans,* while acetone extract was the least effective compared to the other extracting solvents. This is in agreement with the observations of Ammara *et al*., 2009, who concluded that the stronger extraction capacity of ethanol could have been responsible for the higher antimicrobial activity. Table 5 shows antimicrobial activity of *C. aurantifolia* leaf extract using different extracting solvents. The results revealed that the methanol extract of *C. aurantifolia* was most effective against the test organisms than the other extracting solvents. This explains the reason for the highest antimicrobial activity of *C. aurantifolia* using methanol as the extracting medium. The stronger extraction capacity of methanol for *C. aurantifolia* could have been responsible for the higher antifungal activity. The biologically active components in the plant could have been enhanced in the presence of methanol (Tshesche, 1970). *C. albicans* showed the highest susceptibility (18.7 ± 0.9 mm) to *C. aurantifolia* methanol extract, while *P. notatum* showed the least susceptibility to methanol extract (8.0 ± 0.9 mm). The aqueous extract had the least effect on the test organisms, followed by the acetone extract. Acetone extract of *C. aurantifolia* was only effective against *C. albicans* (11.7± 0.9 mm). *S. aureus* showed the highest susceptibility (25.3 ± 0.9 mm) to *C. aurantifolia* ethanol extract, while *P. aeruginosa* showed the least susceptibility (12.7 ± 0.9 mm).

Table 6 shows antimicrobial activity of *Bryophyllum pinnatum* and *Citrus aurantifolia* synergy using different extracting solvents. The results revealed that the methanol extract of *Bryophyllum pinnatum* and *C. aurantifolia* synergy was most effective against the test organisms than the other extracting solvents. This explains the reason for the highest antimicrobial activity of the synergy using methanol as the extracting medium. The stronger extraction capacity of methanol could have produced greater active constituents responsible for the higher antifungal activity of the synergy (Bankole, 1992). *C. albicans* showed the highest susceptibility (21.7 ± 0.6 mm) to methanol synergy extract, while *P. notatum* showed the least susceptibility (10.0 ± 0.9 mm). *S. aureus* showed the highest susceptibility (27.3 ± 0.6 mm) to methanol synergy extract, while *P. aeruginosa* showed the least susceptibility (16.7 ± 0.3 mm). *K. pneumoniae* and *P. aeruginosa* were particularly resistant to the aqueous extract. The aqueous extract had the least effect on the test organisms, compared to the other extracting solvents. This could be due to the inability of the aqueous extract to fully extract all the bioactive ingredients as shown in table 1. Acetone extract of the synergy was only effective against *C. albicans* (20.0 ± 0.6 mm). The synergy of *B. pinnatum* and *C. aurantifolia* leaf extracts gave higher zones of inhibition neither *B. pinnatum* extract nor *C. aurantifolia* extract could give. This showed that both leaf extracts acted synergistically against the test isolates (Ates and Erdogrul, 2003; Adwan *et al.*, 2010). The results of this synergy is supported by Prekesh *et al.*, 2006a and Dawoud *et al.*, 2013. The additive and synergistic effects of phytochemicals enhanced the antibacterial effect of the synergy extract (combined) extract (Matchimuthu *et al.*, 2008) According to Cain *et al*. (2003), synergistic activity suggest different mode of action of the combining components. The extract synergy compared well with the standard antimicrobial agents which also acted as positive control (Table 7). The synergy, therefore has shown potential antimicrobial effect against the test organisms and can therefore be used in the treatment of infections caused by the test organisms.

**Table 1:** Phytochemical constituents of *Bryophyllum pinnatum* leaf extract using different extracting solvents.

| Constituents | Aqueous extract | Ethanol extract | Methanol extract | Acetone extract |
|---|---|---|---|---|
| Saponins | + | + | + | + |
| Flavonoids | + | + | + | + |
| Steroids | _ | + | + | + |
| Alkaloids | + | + | - | + |
| Tannins | _ | + | + | _ |
| Cardiac glycosides | _ | + | + | + |
| Reducing sugars | + | + | + | + |

Key:
+ = Present
- = Absent

**Table 2:** Phytochemical constituents of *Citrus aurantifolia* leaf extract using different e xtracting solvents**.**

| Constituents | Aqueous extract | Ethanol extract | Methanol extract | Acetone extract |
|---|---|---|---|---|
| Saponins | + | + | _ | _ |
| Flavonoids | + | + | + | + |
| Steroids | + | + | + | _ |
| Alkaloids | + | + | + | _ |
| Tannins | + | + | + | + |
| Cardiac glycosides | + | + | + | _ |
| Reducing sugars | + | + | + | + |

Key:
+ = Present
- = Absent

**Table 3:** Phytochemical constituents of *Bryophyllum pinnatum* and *Citrus aurantifolia* synergy using different extracting solvents

| Constituents | Aqueous extract | Ethanol extract | Methanol extract | Acetone extract |
|---|---|---|---|---|
| Saponins | + | + | + | + |
| Flavonoids | + | + | + | + |
| Steroids | + | + | + | + |
| Alkaloids | + | + | + | + |
| Tannins | + | + | + | + |
| Cardiac glycosides | + | + | + | + |
| Reducing sugars | + | + | + | + |

Key:
+ = Present

**Table 4:** Antimicrobial activity of *Bryophyllum pinnatum* leaf extract using different extracting solvents (Zone of inhibition in mm)

| Test organisms | Aqueous extract | Ethanol extract | Methanol extract | Acetone extract |
|---|---|---|---|---|
| ***S. aureus*** | 11.5 ± 0.9 | 17.3 ± 1.2 | 12.7 ± 0.6 | 10.7 ± 0.9 |
| ***E. coli*** | 9.0 ± 0.6 | 12.7 ± 0.9 | 9.3 ± 0.9 | 7.0 ± 0.6 |
| ***K. pneumoniae*** | 0.0 ± 0.0 | 10.0 ± 1.2 | 6.7 ± 0.9 | 4.7 ± 0.9 |
| ***P. aeruginosa*** | 0.0 ± 0.0 | 8.3 ± 0.9 | 5.0 ± 1.2 | 5.0 ± 1.2 |
| ***A. niger*** | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| ***M. mucedo*** | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| ***P. notatum*** | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| ***C. albicans*** | 0.0 ± 0.0 | 12.7 ± 0.9 | 8.7 ± 0.9 | 7.3 ± 0.9 |

**Table 5:** Antimicrobial activity of *Citrus aurantifolia* leaf extract using different extracting solvents (Zone of inhibition in mm)

| Test organisms | Aqueous extract | Ethanol extract | Methanol extract | Acetone extract |
|---|---|---|---|---|
| ***S. aureus*** | 11.5 ± 0.9 | 22.7 ± 0.9 | 25.3 ± 0.9 | 15.3 ± 0.9 |
| ***E.coli*** | 9.0 ± 0.6 | 16.7 ± 0.9 | 22.0 ± 0.6 | 12.0 ± 0.6 |
| ***K. pneumoniae*** | 0.0 ± 0.0 | 12.7 ± 0.9 | 17.0 ± 1.2 | 8.0 ± 0.6 |
| ***P. aeruginosa*** | 0.0 ± 0.0 | 11.0 ± 1.2 | 12.7 ± 0.9 | 5.3 ± 0.9 |
| ***A. niger*** | 0.0 ± 0.0 | 10.7 ± 0.9 | 13.0 ± 1.2 | 0.0 ± 0.0 |
| ***M. mucedo*** | 0.0 ± 0.0 | 14.0 ± 0.6 | 15.0 ± 0.6 | 0.0 ± 0.0 |
| ***P. notatum*** | 0.0 ± 0.0 | 8.7 ± 0.9 | 8.0 ± 0.9 | 0.0 ± 0.0 |
| ***C. albicans*** | 0.0 ± 0.0 | 16.3 ± 0.9 | 18.7 ± 0.9 | 11.7 ± 0.9 |

**Table 6:** Antimicrobial activity of *Bryophyllum pinnatum* and *Citrus aurantifolia* synergy using different extracting solvents (Zone of inhibition in mm**)**

| Test organisms | Aqueous extract | Ethanol extract | Methanol extract | Acetone extract |
|---|---|---|---|---|
| ***S. aureus*** | 1.7 ± 0.9 | 23.5 ± 1.1 | 27.3 ± 0.6 | 22.7 ± 0.9 |
| ***E.coli*** | 2.4 ± 0.6 | 18.3 ± 0.7 | 26.7 ± 0.9 | 17.3 ± 0.6 |
| ***K. pneumoniae*** | 0.0 ± 0.0 | 15.6 ± 0.8 | 20.3 ± 0.9 | 11.6 ± 0.8 |
| ***P. aeruginosa*** | 0.0 ± 0.0 | 11.3 ± 0.9 | 16.7 ± 0.3 | 8.7 ± 0.9 |
| ***A. niger*** | 0.0 ± 0.0 | 10.6 ± 0.4 | 14.0 ± 0.9 | 0.0 ± 0.0 |
| ***M. mucedo*** | 0.0 ± 0.0 | 12.0 ± 0.7 | 17.3 ± 0.5 | 0.0 ± 0.0 |
| ***P. notatum*** | 0.0 ± 0.0 | 8.7 ± 0.6 | 10.0 ± 0.9 | 0.0 ± 0.0 |
| ***C. albicans*** | 0.0 ± 0.0 | 14.0 ± 0.9 | 21.7 ± 0.6 | 20.0 ± 0.6 |

**Table 7:** Zone of Inhibition (mm) of Standard antimicrobial agent against the test organisms (Positive control).

| Test organisms | Ciprofloxacin | Nystatin |
|---|---|---|
| ***S. aureus*** | 26.0 | ND |
| ***E. coli*** | 33.0 | ND |
| ***K. pneumoniae*** | 24.0 | ND |
| ***P. aeruginosa*** | 29.0 | ND |
| ***A. niger*** | ND | 10.0 |
| ***M. mucedo*** | ND | 18.0 |
| ***P. notatum*** | ND | 10.0 |
| ***C. albicans*** | ND | 21.0 |

**Key: ND = Not determined**

*Conclusion***:** This study has shown that combinations of extracts demonstrated synergistic and additive effects on microorganisms. The synergy is better, as microbial tolerance is less likely to develop against substances having more than one type of mode of action. Differential antimicrobial activity of the extracts against different bacteria and fungi was due to the presence of different active phyto-compounds which made the test organisms to be susceptible. It is therefore recommended that the synergistic use of medicinal plant extracts be encouraged to prevent drug resistance and treat the emerging and re-emerging diseases caused by the bacterial and fungal species.

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