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(RESEARCH ARTICLE)

Phytochemical screening and investigation on the antibacterial activity of fermented noni (*Morinda citrifolia*) fruit juice extracts on selected microorganisms in Makurdi Metropolis

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Abstract

Plant products are generally useful for medicinal purposes, one of which is the noni plant. Noni or pace (*Morinda citrifolia* L.) fruit juice was extracted using aqueous and methanol solvents, then fermented and screened for the presence or absence of bioactive constituents (phytochemicals) using the modified standard procedures. The major phytochemical constituents revealed were *alkaloids, anthraquinones*, *cardiac glycosides*, and *flavonoids, phenolic* compounds, *saponins, tannins*, reducing sugar, *saponnins,* carbohydrate and *terpenoids*. Bacterial cell viability and MIC values were also determined by turbidity test. The lowest concentration of the extract with clear suspension was considered the MIC values. The lowest concentration of the extract in the post-incubation suspensions which did not harbour any bacterial growth upon spotting on MHA after overnight incubation at 37° C was considered the MBC values. The test was performed in triplicates alongside antibiotics ciprofloxacin (5μg) as a positive control at a concentration of 0.1mg/ml. Antibacterial activity was perfumed against three bacteria (*Salmonella typhi, Escherichia coli, and Staphylococcus aureus*) by agar-well diffusion method. Activity was observed for both methanolic and aqueous extracts of *Morinda citrifolia* L. fruit juice against the organisms tested. The extracts had both bacteriostatic and bactericidal activity against the test microorganisms. The Minimum Inhibitory Concentration (MIC) test revealed that the bacterial isolates were inhibited between concentrations of 50mg/ml and 25mg/ml for the methanolic extract, and between 100mg/ml and 50mg/ml for the aqueous extract of the fermented fruit juice. The extract was bactericidal from the range of 100mg/ml to 200mg/ml for both extracts, though with the exception of S. aureus which was bactericidal at 50mg/ml for methanolic extract.

Keywords: Phytochemicals; Activity; Bacteria; Inhibitory; Concentration; Aqueous; Extract

1. Introduction

A medicinal plant is any plant which has substances in its organs that can be used for therapeutic purposes or which are precursors for chemo-pharmaceutical synthesis [1]. Natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine. Over the past century, the phytochemicals in plants have been a pivotal pipeline for pharmaceutical discovery. The importance of the active ingredients of plants in agriculture and medicine has stimulated significant scientific interest in the biological activities of these substances [2]. One of such plant with abundance of phytochemicals is *Morinda citrifolia* (*Rubiaceae*).

In the past, a large number of antimicrobial compounds were discovered from synthetic and natural products for the treatment and control of infectious agents [3]. However, only a few of them were reachable to the needy world's market [4].

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The emergence of multidrug-resistant bacteria has further worsened the accessibility and affordability of many currently prescribed antibiotics worldwide [5]. As a result, it reduces the effectiveness of the treatment regimens and increases morbidity, mortality, and health care costs [6]. According to CDC report, each year in the United States, at least 2 million people acquire serious infections with bacteria that are resistant to one or more of the antibiotics used for the treatment of infections. The total economic cost of antibiotic resistance was estimated as high as $20 billion in direct healthcare and $35 billion dollars loss in productivity in a single year. The situation is further complicated in low-income countries by lack of effective surveillance systems, laboratory diagnostics, and access to appropriate antimicrobials in the face of financial limitations [7].

If there were no successful efforts to intervene in terms of looking for new drugs, the number of deaths will rise to ten million and costs the world up to $100 trillion by the year 2050 [8]. To this effect, the search for an innovative antibiotic from natural products is ultimately an important segment of modern medicine to overcome the socio-economic and health impact caused by multidrug-resistant microbes [9].

The therapeutic agents derived from plants are justified by the emergence of diseases and the growth of scientific knowledge about herbal medicines as important alternatives or complementary treatment for diseases [10]. Many studies have shown that medicinal plants contain coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectin, polypeptides, and polyacetylenes [11].

These bioactive compounds are used as a starting point for antibiotics synthesis in order to treat infectious diseases [12].

Plants are generally useful as medicine, one of which is the noni plant. Noni or pace (*Morinda citrifolia* L.) is a medicinal plant that has been in demand in recent years. Noni is a tropical and wild plant. Noni can grow on the beach to an altitude of 1500 m (above sea level), both infertile and marginal lands. Its distribution is broad, covering the entire South Pacific archipelago, Malaysia, Indonesia, Taiwan, the Philippines, Vietnam, India, Africa, and the West Indies. Noni (*Morinda citrifolia* L.) or pace, kudu (Java), kudu (Sundanese), koddhu (Madura), tibah (Bali) originated from Southeast Asia. Other names for this plant are noni (Betawi, Hawaii), nono (Tahiti), nonu (Tonga), ungcoikan (Myanmar), and ach (Hindi). Noni tree reaches 3–8 m high, has white hump flowers. The fruit is a compound fruit, which is still young, shiny green, and has spots, and when it is old, it is white with black spots [4].

Noni plants can begin to bear fruit about 9 months to 1 year after planting. Fruits can be harvested at this early stage, although they are generally small and few. Some farmers choose to forgo harvest during the first or second years in favour of pruning back the branches instead [4]

Literature reveals that Noni has traditionally been used for colds, flu, diabetes, anxiety, and high blood pressure, as well as for depression and anxiety. All plant parts are used for a variety of illnesses in Samoan culture, and noni is one of the most frequently used Hawaiian plant medicines. Plants are generally useful as medicine, one of which is the noni plant. Noni or pace (*Morinda citrifolia* L.) is a medicinal plant that has been in demand in recent years. Noni is a tropical and wild plant [13].

Noni has traditionally been used for colds, flu, diabetes, anxiety, and high blood pressure, as well as for depression and anxiety. Extracts of noni seed have been shown to possess bioactive compounds that exhibit antioxidant, anti-mutagenic, anti-tumor, anti-inflammatory, anti-allergic, anti-viral, anti-fungal, anti-microbial, and anti-carcinogenic properties. Bioactive compound-rich noni fruit seed could be a potential source of functional foods [14].

During the last couple of decades the concepts of food in the developed world have been changing from past emphasis on survival, hunger satisfaction, and malnutrition to promising use of foods to promote better health and well-being to emasculate the risk of chronic illness and several kind of diseases such as cardiovascular diseases, certain types of cancers and obesity. An important part of this new “Healthy Eating Concept” is functional foods. Functional foods can be defined as a food which beneficially affects one or more target functions in the body, besides adequate nutritional effects, in a way that is relevant to either improved stage of health and well-being or reduction of diseases. The first wave of these "functional food" products, which involved noni juice had been commercialized in the USA in 1990s and are increasingly distributed all over the world [15].

The products derived from *Morinda citrifolia* fruits and leaves are being sold as capsules, teas, and juice, although the fruit juice being the predominant form. The various products, especially noni juice is being promoted for its nutraceutical and therapeutical properties, thereby emerging as major product of health and wellness industry. It has recently been the object of many claims concerning its therapeutically properties [14].

One of the popular claims by [16] was that noni fruit has the presence of an active "alkaloid" named xeronine, and is said to be resulting from a precursor perxeronine for which no structure was given. The author described a wide range of potential indications for noni fruit juice, including high blood pressure, menstrual cramps, hypertension, gastric ulcers, sprains, injuries, mental depression, atherosclerosis, blood vessel problems, drug addiction, relief of pain and many others. Various publications have shown that it can be used to relieve different diseases. The observed beneficial effects may result from certain compounds such as alkaloids, scopoletin, damnacanthal and lots of other reported molecules [15].

Some companies producing flavoured noni juices by addition of other fruit juices to render the product more embellish and yummy for making it more acceptable in market. Flavour compounds were also extracted from noni fruit juice [17]. In response to this demand, some countries including India have increased the commercial cultivation of noni [15].

Chemical composition differs largely according to the part of the plant. To date, several classes of metabolites have been described in different parts, including acids, alcohols and phenols, anthraquinone, anthraquinone glycosides, carotenoids, esters, flavonoids, iridoids, ketones, lactones, lignans, nucleosides, triterpenoids, sterols and several minor compounds. Anthraquinones are the major phenolic compounds that have been identified and isolated from different parts of *M. citrifolia* [18].

Among the reported anthraquinones, damnacanthal appear quite unique with respect to their function for its anti-cancer and anti-HIV activity [19], while similar anthraquinone damnacanthal is a potent tyrosine kinase and topoisomerase II inhibitor [18]. [20] identified and isolated an extremely potent quinone reductase inducer, 2-methoxy1, 3, 6-trihydroxyanthraquinone, with a 40 fold higher potency than the well-known inducer, sulforaphane. The reported molecule provides quinone reductase induction mediated protective activity against chemical carcinogenesis. Although, the concentration is very low in the fruit, but its biological properties make it a promising biomolecule to prevent initiation phase of cancer. [21] Isolated an anthraquinone, 4-dihydroxy-2-methoxy-7- methylanthraquinone in fruits and established that this compound was a good candidate as an anti-wrinkle agent due to its strong induction of biosynthetic activity of extracellular matrix components [18].

Widespread claims of the therapeutic effect have encouraged researchers worldwide to further investigate some of these potentials. By virtue of this, one can easily find out several research articles on the Singh biological activities and pharmacological actions of *M. citrifolia* based on modern scientific investigation [18].

The [22] reported *M. citrifolia* antibacterial activity against certain infectious bacterial strains such as *Pseudomonas aeruginosa*, *Proteus morgaii*, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella* and *Shigella*. He accentuated that the antimicrobial effect observed may be due to the presence of phenolic compounds, such as acubin, L-asperuloside, and alizarin in the fruit, as well as some other anthraquinone compounds in roots [23]. Another study by [24] showed that an acetonitrile extract of the dried fruit inhibited the growth of *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli*, and *Streptococcus pyrogene* [18].

Similar findings were also reported by [25] in the methanol and aqueous crude extracts of the fruit against *E. coli, Streptococcus species, Vibrio alginolyticus*, and *Vibrio harveyi [23]*. It has also been found that ethanol and hexane extract of this fruit provide protection against Mycobacterium tuberculosis. The major components identified in the hexane extract were *E-phytol, cycloartenol* and *stigmasterol* [18].

Again [25] showed that methanol extract of this fruit demonstrated zones of inhibition in a range of 7.7to 26 mm against *Vibrio cholerae*, *Klebsiella*, *B. subtilis*, *Lactobacillus lactis*, P. *aeruginosa, Salmonella typhi, E. coli, S. aureus, Streptococcus thermophilus, Shigella flexneri, V. harveyi, Chromobacterium violaceum, Aeromonas hydrophila, Salmonella paratyphi A*, while ethyl acetate extract demonstrated zones of inhibition in range of 5.7 to 15.7 mm against *L. lactis, S. typhi, B. subtilis, E. coli, V. harveyi, S. aureus, S. flexneri, V. cholerae, A. hydrophila, C. violaceum, S. paratyphi A* [26]; [27]; [18]. In a related report, it has been found that methanol extract of fruit demonstrated zones of inhibition in the range of 7 to 15 mm against *E. coli*, Streptococcus spp., *V. alginolyticus* and V. harveyi [18].

Furthermore, they showed that the antibacterial effect is greater when the fruit is ripe. Lately, antibacterial activities of the fruit juice was compared with that of sodium hypochlorite (NaOCl) and chlorhexidine gluconate (CHX) to remove the smear layer of Enterococcus faecalis from the canal walls of endodontic ally instrumented teeth. The data from this experiment indicated that noni fruit juice and NaOCl treatment have similar effects [18]. Topical treatment of pain and bruising is one of the most common uses of the noni plant in tropical alternative medicine. Some animal studies suggest that noni possesses possible anti-inflammatory activity [28]. Among the natural intercanal medicaments, *M. citrifolia* gel consistently exhibited good inhibition followed by aloe vera gel and papain gel. Another study showed that iridoids from noni fruit appear to be active against yeasts, Gram negative, and Gram positive bacteria [18].

Recent research examined the mechanisms involved in immunological properties of Tahitian Noni Juice (TNJ) and noni fruit juice concentrates (NFJC) in mice. The result showed that both modulate the immune system via the activation of the CB2 receptors and suppression of the IL-4, but increasing the production of IFN-gamma cytokines [29]. In a related report, it has been found that ethanol precipitation of the fruit contains a polysaccharide-rich fraction which showed antitumour activity in the Lewis lung carcinoma model in mice. It also stimulate the release of several potential mediators, including TNF-α, IL-1β, IL-10, IL-12 p70, IFN-γ and nitric oxide, but had no effect on IL-2 and suppressed IL-4 [30].

Phytochemicals are bioactive compounds found in vegetables, fruits, cereal grains and plant based beverages such as tea and wine. Phytochemical consumption is associated with a decrease in risk of several types of chronic diseases due to impact of their antioxidant and free radical scavenging effects [31]. They are non-nutritive plant chemicals that have protective or disease preventive properties [32].

Plants such as vegetables, fruits, spices, medicinal herbs, etc. have been used to cure many diseases since ancient times. Synthetic drugs which are easily readily available and effective to cure diseases have disadvantages and are harmful compared to traditional folk medicines. Diversity of secondary metabolites isolated from different plants have proved these compounds anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and other activities[33].

Plants produce many chemical compounds which include alkaloids, glycosides, flavonoids, tannins, triterpenoids, steroids, saponins, diterpenes, resins, volatile oils, etc. [34]. These are secondary metabolites called "phytochemical compounds". Plants containing phytochemical compounds supplement the needs of human body by acting as natural antioxidants. The consumption of fruits and vegetables has many health benefits which includes medicinal properties and high nutritional value [35].

These phytochemical compounds have immense industrial, commercial, and medical applications and therefore have gained special importance in all countries. Phytochemicals already naturally occurring in leaves, root and stem bark of Medicinal plants and they have defence mechanisms and can protect from various diseases [36]. The main aim of phytochemical screening is to identify the nature of the compounds present in a given plant extract, which may be responsible for the observed biological effect. Medicinal actions of some species of plants are as a result of the effect of the plant constituents on some of the organs of the human body. They increase the body's resistance to disease, retard or ease the process of Natural ageing. These compounds are responsible for a green therapeutic effect and they frequently serve as model for the synthesis of new medicine [37].

2. Material and methods

2.1. Sample collection / Preparation of noni fruit extracts

Fresh Noni fruits was collected and washed thoroughly under running tap water and rinsed in distilled water, air-dried at room temperature under shade (23–27° C), and reduced to appropriate size. The powdered plant materials was packed in a plastic bag and kept until extraction. The powdered plant materials was weighed by sensitive digital weighing balance and a total of 1.2 g of powdered aerial was macerated with 80% methanol (250 g in 1500 mL) in an Erlenmeyer flask and separately soaked in 500ml of distilled water for three days at room temperature (23–27° C). After three days of frequent agitation, the extract was separated from the marc using gauze and the resulting liquid was filtered using Whatman filter paper No. 3 (Whatman Ltd., England). The residue was re-macerated and repeated three times to exhaustively extract the fruit material.

The filtrates obtained from the successive maceration was concentrated under reduced pressure using a rotary evaporator (Hamato, Japan) followed by a hot air oven (Medit-Medizin Technik, Germany) set at 40°C. Extraction was repeated seven times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using a Rotary evaporator at 40° C. After the removal of the organic solvent, the aqueous residue was placed in a lyophilizer until non-polar solvents were removed and the extracts became dried. The extract was further dried by freeze-drying (in order to make it more concentrated) using a lyophilizer (Labfreez, China). After drying, the amount of dried extract obtained was harvested and transferred into airtight bottles, where the resulting dried mass was packed into a glass vial and stored in a desiccator over silica gel and stored in a refrigerator at −4° C until use [38].

2.2. Sterility test of the plant extracts

Each fruit extract of methanol and aqueous was tested for the growth of microbes. This was carried out by inoculating 0.5ml of each of them on sterile Mueller Hinton Agar and incubated at 37° C for 18–24 hrs. The plates were observed for growth. The absence of growth in the extracts after incubation indicates sterility and evaluated for antimicrobial activity as indicated in CLSI guidelines [39].

2.3. Culture media

Nutrient Agar, TSY Broth, MacConkey, Muller Hinton Agar (MHA), Muller Hinton Broth (MHB), Blood Agar (BA), Mannitol Salt Agar (MSA), Chocolate Agar, and biochemical reagents for bacteria was obtained from the Department of Microbiology, Laboratory, Federal University of Agriculture Makurdi (FUAM), Nigeria.

2.4. Test microorganisms

The reference bacterial species; American type cell culture (ATCC) of *Escherichia coli* (ATCC25922), *Salmonella typhi* (ATCC 19615), *Staphylococcus aureus* (ATCC 25923) was collected from the Microbiology Laboratory, FUAM. All laboratory works were performed in accordance with CLSI guidelines.

2.5. Modern antibiotics

The antimicrobial discs used for this research was the products of Liofilchem® Inc. (Liofilchem, Clinical and Industrial Microbiology Company) produced in 2017. The modern antibiotic disc employed for this work was Ciprofloxacin (5µg/disc), as a control. This was done in accordance with the CLSI guideline protocols [39].

2.6. Determination of MIC and MBC values

After preliminary screening of plants for their antimicrobial activity, those which revealed potent antibacterial effects were further tested to determine MIC and MBC against multidrug-resistant gram-negative and gram-positive bacteria. This was determined by the MHB broth micro-dilution method in accordance with the protocol and procedure given by CLSI guidelines and the modified protocol of Wiegand.

Bacterial cell viability and MIC values was determined by turbidity test. The lowest concentration of the extract with clear suspension was considered as the MIC values. The lowest concentration of the extract in the post-incubation suspensions which did not harbor any bacterial growth upon spotting on MHA after overnight incubation at 37° C was considered as the MBC values. The test was performed in triplicates alongside antibiotics ciprofloxacin (5µg) as a positive control at a concentration of 0.1mg/ml for bacteria.

2.7. Phytochemical screening

Standard preliminary phytochemical qualitative analysis of the extract was carried out for the various plant constituents and screened for the presence or absence of biologically active compounds or secondary metabolites using standard procedures. As a result, the major phytochemical constituents considered were alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenolic compounds, saponins, tannins, and terpenoids in 80% methanol extracts using modified standard procedures [38].

Exactly 200mg plant material was boiled in 10mL methanol and filtered. Then, 1% HCl was added followed by 6 drops of Dragendorff reagent. The brownish-red precipitate was taken as a piece of evidence for the presence of alkaloids. Towards the end, an alkaloids test was applied on few extracts by diluting 2.5mg of the extract with 2.5ml of 1% HCl in a tube and boiled. Then, 1ml of the filtrate was added to 1ml of dilute ammonia. Finally, 1ml of chloroform ($CHCl_3$) was added and shaken gently to reveal the alkaloid base. 1mg of each extract was reacted with 2ml benzene, shaken properly, and filtered through Whatman's no. 1 filter paper. Then, the filtrates was allowed to react with 2.5ml of 10% ammonia solution and shaken properly. The presence of pink, red, or violet color in ammonia solution in the lower phase indicated the presence of anthraquinones. 1.25mg of each extract was allowed to react with 0.5ml chloroform and mixed carefully. 0.5ml of concentrated sulfuric acid was then carefully added to form a lower layer. The reddish-brown color at the interface indicated the presence of a steroidal ring, the glycone portion of cardiac glycosides. 7.5mg of each dry extract was dissolved in 0.5ml of ethanol, concentrated HCl, and magnesium turnings. A yellowish coloration indicates the presence of flavonoids.The crude fruit extract was treated with 3 to 4 drops of ferric chloride solution, or dissolving 5mg of dry extract in 0.5ml of 1% ferric chloride solution. The formation of bluish-black color indicates the presence of phenolic compounds. 2.5mg of the fruit extract was allowed to react with 5ml water and shaken properly in a test tube. Samples showing froth were warmed. Persistent foam formation indicates the presence of saponin. 2.5mg fruit extract was boiled in 5ml of water in a test tube and then filtered through Whatman's no. 1 filter paper. Two to three drops of 0.1% ferric chloride added and read for brownish green or a blue-black precipitate indicating a positive result. About 0.5ml of the chloroform extract of the dried extracts was evaporated to dryness on a water bath and heated with 3ml of concentrated sulfuric acid for 10 minutes on a water bath. The gray color indicates the presence of terpenoids.

2.8. Statistical Analysis

Data obtained from phytochemical analysis were analyzed using the SPSS software. The experimental results (replicates) were expressed in mean standard deviation. The data was subjected to one-way analysis of variance (ANOVA) and difference between the samples was determined.

3. Results

The Results of the Phytochemical Screening of the methanol and aqueous extract of the fermented noni juice is shown in **table 1**. The phytochemicals present were *alkaloids, cardiac glycosides, and flavonoids, phenolic* compounds, *saponins, tannins,* reducing sugar*, saponnins, anthraquinones,* carbohydrate and *terpenoids*.

Table 1 Results of the Phytochemical Screening of the methanolic and aqueous extract of Noni fruit juice

Phytochemicals	Aqueous Methanol
Alkaloids	+ +
Cardiac glycosides	+ +
Carbohydrate	+ +
Reducing sugar	+ +
Tannins	+ +
Saponins	+ +
Anthraquinone	+ +
Flavonoid	+ +
Steroids	+ +
Terpenoids	+ +
	+ +

Key; **+ =** present in trace amount; - = not detected

Table 2 Zones of Inhibition of the Noni fruit juice Methanol Extracts on test organisms at 200mg/ml - 25mg/ml concentration

Test organisms	200	Mean ± SD / 100	50	25
Escherichia coli	18.00±2.00	12.33±1.53	8.33±1.53	4.33±0.58
Salmonella spp	24.67±2.52	20.00±2.00	15.00±1.00	11.00±1.00
Staphylococcus spp	19.67±1.53	15.00±1.00	10.67±1.15	4.33±1.53
Ciprofloxacin	30.00±1.00	30.00±1.00	30.00±1.00	30.00±1.00

KEY: Ciprofloxacin Positive control

Diameter of zones of inhibitions of the fermented fruits juice extracts of *Morinda citrifolia* against the sampled bacterial isolates is shown in **table 3**. The control (ciprofloxacin) had the widest zones of inhibition (30.0±1.00) compared to the fermented aqueous fruit juice extracts, though the fermented aqueous fruit juice extracts of noni fruit also had appreciable zones of inhibitions of varying degrees depending on the bacterial isolate. At highest concentration of

200mg/ml, *Escherichia coli* had (16.00±1.73), *Salmonella typhi* (18.67±1.15) and *Staphylococcus aureus* (15.67±0.58). At the least conc. 25mg/ml *Eschericia coli* had (5.33±1.15), *Salmonella typhi* (9.33±1.15) and *Staphylococcus aureus* (6.33±1.53).

Result of the MIC and MBC of Antibacterial Activity of Aqueous and Methanol fermented Noni fruit juice extracts at Various Concentrations on the Bacterial Strains is presented in **table 4.** The extracts had both bacteriostatic and bactericidal activity against the test microorganisms. The Minimum Inhibitory Concentration (MIC) test revealed that the bacterial isolates were inhibited between concentrations of about 50mg/ml and 25mg/ml for the methanolic extract, and between 100mg/ml and 50mg/ml for the aqueous extract of the fermented fruit juice. The extract was bactericidal from the range of 100mg/ml to 200mg/ml for both extracts, though with the exception of *S. aureus* which was bactericidal at 50mg/ml for methanolic extract.

Table 3 Zones of Inhibition of the Noni fruit juice Aqueous Extracts on test organisms at 200mg/mL - 25mg/mL concentration

Test 0rganisms	**200**	**Mean ± SD** **100**	**50**	**25**
Escherichia coli	16.00±1.73	12.00±2.00	9.00±1.00	5.33±1.15
Salmonella spp	18.67±1.15	16.33±0.58	12.67±1.15	9.33±1.15
Staphylococcus spp	15.67±0.58	13.33±1.15	9.67±1.15	6.33±1.53
Ciprofloxacin	30.00±1.00	30.00±1.00	30.00±1.00	30.00±1.00

Key: Ciprofloxacin=control

Table 4 Result of the MIC and MBC of Antibacterial Activity of Aqueous and Methanol Noni fruit juice extracts at Various Concentrations on the Bacterial Strains

Test organisms	**MIC**		**MBC**	
	Aqueous	Methanol	Aqueous	Methanol
Escherichia coli	50	25	200	100
Salmonella typhi	100	50	200	200
Staphylococcus aureus	50	25	100	50

Key: MIC= Minimum Inhibitory Concentration; MBC= Minimum Bactericidal Concentration

4. Discussion

The result obtained from this study revealed that *Morinda citrifolia* fruit juice extracts contain various amounts of phytochemicals, *Tannins, saponins, flavonoids, alkaloids*, *cardiac glycosides*, carbohydrates, reducing sugar, *steroids*, *terpenoids* and *Anthraquinone.* The result agree with [3] and [18] who accentuated that, considerable number of medicinal plants contain some active phytochemical components such as phenolics, flavonoids, terpenes, coumarins, etc, which induce different biological activities in animals including antioxidant, anti-inflammatory and anti-cholinesterase effects. It is also known that the curative effect of any medicinal plant is correlated to its active phytochemical constituents. Phytochemicals are constituents of plant that may have different important roles in science of medicines. These constituents (phytochemicals) of the plant are found in different concentrations depending on the process of the synthesis and accumulation in the cells of the plants.

In respect to having screened the plant for phytochemicals it also agree with the opinion of [23] that, phytochemical analysis is important in ensuring that the crude use of plant products with consequent effects are reduced or moderated while the positive effects of such plant products are maximized. [35], reported that active molecules present in the noni fruit have been emphasized by certain authors: xeronine by Heinicke, terpenes such as asperuloside, aucubine, essential oils, β-Dglycopyranose pentacetate, asperulocide tetra acetate and ascorbic acid, alizarin carbonate, rubichloric acid and chrysophanol; resins of sterols are also quoted. Others also report the presence of anthraquinones, glycosides, phenolic compounds, resins of β-sitosterols and ursolic acid. Salicin, having anti-inflammatory and pain-relieving properties, was originally extracted from the bark of the white willow tree and later synthetically produced to become the staple, over-the-counter drug aspirin. Toxicity tests are very important to science. Some tests are designed specifically to detect a particular effect or changes in physiology (such as skin and eye irritancy, skin sensitization and mutagenicity studies).

Information from toxicity tests can be used to provide classification for a chemical, for example to assign **a**ppropriate warning labels for containers, and, where necessary, instructions for use of such substances. The measurement of the activities of enzymes in tissues and body fluids plays a significant and well known role in investigation and diagnosis of diseases. The result from this work suggested that fruits of *Morinda citrifolia* are not toxic after an acute exposure in laboratory mice. From the result obtained, extracts of fruits of *Morinda citrifolia* screened showed antibacterial activity. Though the antimicrobial activity varied according to extraction solvents used and the test organisms. This result agree with the report of [18] who opined that fruits of *Morinda citrifolia* has been extensively evaluated for medical and therapeutic potentials and it proved encouraging.

The result is also in consonance with that of [14] who reported that similarly, the aqueous extract of the stem bark of fruits of *Morinda citrifolia* were used for the treatment of diarrhea in poultry and humans and it also produced some anti-ulcer activity. The effect was almost the same with that of the antibacterial drug (Ciprofloxacin) used as a control. Fruits of *Morinda citrifolia* could be effective and suitable for general usage against some pathogenic bacteria of humans. The inhibitory effect of fruits of *Morinda citrifolia* extracts on test organisms demonstrated an encouraging result since both MIC and the MBC at low concentration were effective.

The results of the MIC and MBC agree with the report and definition of [10] as follows, these terms (MIC and MBC), in respective order explain the lowest concentration of antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation. The minimum bactericidal concentrations (MBCs), the lowest concentration of antimicrobial that will prevent the growth of an organism after sub-culture on to antibiotic free media. It is also in line with [23] by the expression that, the MBC on the other hand is adjudged to be complementary to the MIC; whereas the MIC test demonstrates the lowest level of antimicrobial agent that inhibits growth, the MBC demonstrates the lowest level of antimicrobial agent that results in microbial death. Other studies on the extract of fruits of *Morinda citrifolia* confirm the antibacterial activity or effect of the plants extract, for instance [23] reported that, the extract of fruits of *Morinda citrifolia* has antibacterial effect on both gram positive and gram negative bacteria like *Staphylococcus aureus, Escherichia coli and Salmonella typhy.*

Result from this study strongly agree with that of [18] who also reported that, there has been an earlier report on the antibacterial activity of fruits of *Morinda citrifolia*, stem bark and oil from its leaves. [22] also reported antibacterial activity of *M. citrifolia* against certain infectious bacterial strains such as *Pseudomonas aeruginosa*, *Proteus morgaii*, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella* and *Shigella*. [18] also accentuated that another study by [24] showed that an acetonitrile extract of the dried fruits inhibited the growth of *P. aeruginosa, B. subtilis, E. coli*, and *Streptococcus pyrogene.*

5. Conclusion

The fermented extracts of fruits juice of *Morinda citrifolia* had antibacterial activities against the sampled bacteria such as *Salmonella typhi, Escherichia coli*, and *Staphylococcus aureus*. The antibacterial activity was dose reliant. The optimum activity was achieved at higher dosage. Conclusively, the extract of fermented fruits juice of *Morinda citrifolia* had antibacterial effect on both gram positive and gram negative bacteria like *Staphylococcus aureus, Escherichia coli, and Salmonella typhy.* However over dilution of the extract may reduce its potency.

Recommendations

- There is need for expansion of studies on fruits of *Morinda citrifolia* to include more enteric and other bacteria in order to ascertain the broadness of the plant's activity
- It is also important to study the anti-parasitic and antifungal effects of fruits of *Morinda citrifolia*
- Advanced studies on the medicinal use of fruits of *Morinda citrifolia* is encouraged. This is with the view to fine-tuning the possibility of commencing clinical trials by local pharmaceutical companies
- Inter-departmental collaborative studies on fruits of *Morinda citrifolia* can assist in bringing out a better breed of the plant (Botany) as well as its application in veterinary and human medicine

Adjuvant studies of fruits of *Morinda citrifolia* with other plants or products are encouraged as these can assist in obtaining the best combined action of the medicinal effect of the plant

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical Approval

Ethical approval was duly obtained from the Benue State Health Management Board with an Issuance of an ethical clearance certificate from the ethics committee. All participants were informed of the details of the study before samples were collected.

Statement of Informed Consent

Informed consent was obtained from all individuals who participated in this study.

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