

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY



Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index

Phytochemical Screening, Proximate Composition and Mineral Element Analysis of *Neocarya macrophylla* (Gingerbread) Plum and its Effects on Microorganisms

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HISTORY

Received: 24th June 2022 Received in revised form: 24th July 2022 Accepted: 28th July 2022

KEYWORDS

Antimicrobial *N. macrophylla* Minerals Antibiotics Antifungal

ABSTRACT

The need to increase the production and utilization of locally available food and antimicrobial resources has been discussed at different national and international forum. Fresh Neocarva macrophylla fruits were obtained from Birnin Kebbi central market in Kebbi state and it was transported to Biochemistry Department at Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria. Where the fruit of N. macrophylla was transformed or crushed through stigmasterol extraction with 70% of methanol. The compound, stigmasterol, indicated varying actions of antimicrobial activity against the microbes tested. Susceptibility test result showed inhibition ranging from 23 mm to 30 mm against all the organisms, which are S. aureus (24 mm), Salmonella Typhimurium (23 mm), P. aeruginosa (26 mm), E. coli (28 mm), Streptococcus pyogenes (25 mm), B. subitilis (23 mm), A. niger (29 mm), C. albicans (24 mm), and C. kruseii (23 mm). Creating zone of inhibition. The ash content was 6.70±0.05., moisture 14.23%±0.10., lipids 6.70% ±0.05., fibre 10.15%± 0.57., crude protein 1.015%±0.127., carbohydrate 51.33%±1.025. The pulp also contains low concentrations of magnesium (2.843±0.025) and very low concentrations of iron (0.0856 ± 0.002) , manganese (0.0122 ± 0.048) , copper (0.0087 ± 0.002) , and zinc (0.0024 ± 0.001) , which are important micro elements required by body for proper functioning. The result obtained indicate that Neocarya macrophylla fruit pulp of pharmaceutical and medical significances that are useful in Combating antibiotics resistance infections, nutritional rich in terms of minerals and carbohydrate composition.

INTRODUCTION

Improving prognosis and monitoring response to therapy is gradually becoming more difficult in combating certain infections ranging from asymptomatic, pre-clinical to clinical stage infection. Antimicrobial-resistant microbes are becoming a threat in the health care sector [1]. Recent literature had shown that the fruit of *Neocarya macrophylla* can be used alternatively in place of conventional drugs because of its stigmasterol and other phytochemicals that have been documented in several studies to contain significant antimicrobial activities, through some mechanisms like intercalation of DNA, damages on the cell membrane, inactivation of microbial adherence and obstruction of subsequent enzymatic activities [2]. In recent times, different kind of edible wild plants serves as food for human consumption [3]. The infection spread among patients and across facilities due to antimicrobial resistance which occurs via changes in a microbial specie [4]. The US Centre for Disease Control and Prevention estimated that the inpatient population in 2020 was very different from the pre-pandemic population hospitals around the Globe which saw higher numbers of sicker patients leading to hospitalization that could not be avoided with an extended length of stay [1,5,6]. Antibiotics resistance microbes have emerged as a major public health threat of the 21st century attributed to the natural occurrence as a result of frequent or inappropriate use of conventional antimicrobial agents [7]. This has increased the risk for resistant Nosocomial infections contraction [8].

Preventing infections is one of the greatest tools for combating antimicrobial resistance and saving lives by continues building of national capacity for infection prevention and control to ensure that these practices are implemented into action consistently. Increases in cases of antifungal-resistant Candida in 2020 were potential because of overcrowding patients, increased number of sicker patients, and staff shortages, which negatively impacted infection control and antifungal use. Candida species are a common cause of life-threatening bloodstream infections in hospitals and can also cause infections in the mouth, skin, and vagina. Only three classes of antifungal are available to treat severe Candida infections.

Many clinical laboratories cannot test Candida for drug resistance, limiting the ability to guide treatment and track resistance. There were 22% fewer overall Salmonella infections (susceptible and resistant) reported during 2020 compared to the average annual incidence from 2017 through 2019 on post coronavirus (Covid-19) outbreak [1,9]. In remote areas, rural farmers' production of a small quantity of millet and other grains give rise to a scarcity of food leading to nutritional deficiency [10]. This has increased the rate at which reliance on wild plant food for a supplement to their diet which addresses the concern of insufficient production of farm products [4,11]. Traditionally, edible wild plant fruits can play a role in combating infections caused by the microbial attack of biofilms [2]. Fighting food insecurity especially the hidden hunger caused by micronutrients, vitamins and minerals deficiencies [12,13].

Neocarya macrophylla, a well-known tropical fruit plant with edible roots, stems, leaves, flowers, fruits, and seeds, is one of the underutilized food crops in Nigeria due to its long-lasting and nutritious food supply. Most people eat it raw, but few realize that it has enormous potential as food for combating malnutrition (both macro- and micronutrient deficiencies) and even some forms of infection. Experts in the field of nutrition have shown that by tapping into the economic and nutritional potential of regional food sources, the world's most pressing nutritional crisis can be avoided. However, the gingerbread plum is one of Nigeria's underutilized food crops.

There is no record of a successful gingerbread product on the Nigerian market, but 15 products have been shown to provide a significant benefit that consumers can appreciate [2,14]. Numerous national and international forums have addressed the pressing need to boost the production and utilization of locally available food and antimicrobial resources. One of the underutilized food crops readily available in Nigeria is the gingerbread plum fruit, the utilization of which has the potential to contribute to the resolution of major antibiotic resistance and nutritional problems. The findings of this research will serve as a starting point for future analyses of gingerbread plum consumption. In the long run, this will help ensure food security by increasing its production on both the household and national levels through a wider variety of uses. Furthermore, it is anticipated that gingerbread plum fruit may be utilized in the prevention and treatment of some non-communicable diseases such as asthma, skin, and communicable diseases such as

bacterial and fungal infections, treatment of wounds, pulmonary infections, dysentery, inflammation, and it are also used for the treatment of eye and ear infections [14]. Through an understanding of its composition [2,15]. The primary goal of this investigation was to examine the effects of the *Neocarya macrophylla* (Gingerbread) plum on microorganisms through photochemical screening and mineral element analysis [16,17].

MATERIALS AND METHODS

Sample collection and processing

Fresh *Neocarya macrophylla* fruits were obtained from Birnin Kebbi central market in Kebbi State, Nigeria. The fruit was transported to the Department of Biochemistry at Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria. Where the fruit of *N. macrophylla* was transformed and crushed through stigmasterol extraction with 70% of methanol.

Antimicrobial activity of *Neocarya macrophylla* (Gingerbread) Plum

The antimicrobial activity of Stigmasterol extracted from *N. macrophylla* was assessed after using the selected pathogenic microorganisms. These organisms were obtained through sample collection from wounds among people living in BirninKebbi, Kebbi State, Nigeria. The isolated bacterial pathogens were examined for sterility and cultured in blood agar and fungi were cultured in Potato Dextrose Agar (PDA). Microorganisms isolated include *Staphylococcus aureus*, *Streptococcus faecalis*, *Pseudomonas aeruginous*, *Salmonella*Typhimurium, *Candida albicans and candida kriusei*.

Susceptibility pattern

The compound's antimicrobial activity was evaluated at a fixed concentration of g/mL Mueller Hinton agar is used as the growth medium for the bacterial cultures. The autoclave was used to sterilise the medium at 120 °C for 15 min, following the manufacturer's instructions. The medium was poured into sterilized Petri dishes, where it was allowed to cool and solidify before being used. The surface of the medium was inoculated with 0.1 mL of the standard inoculum of the test microbes. Each inoculated medium had a 6mm diameter well bored into its center using a standard sterile cork borer. After letting 0.1 mL of the compound solution sit in the well for an hour, we measured its concentration. Overnight incubation at 37 °C for bacteria and 25 °C for fungi was followed by observation of the medium for the zone of inhibition of growth. The zone of inhibition was measured using a ruler, and the test was performed twice [18].

Minimum inhibitory concentrations

The broth dilution technique was utilized to analyze the compound's minimum inhibitory concentration (MIC) [18]. Two-fold serial dilutions of the compound in sterile broth yielded concentrations of 100 g/mL, 50 g/mL 20 g/mL, 10.5 g/mL, 5.65 g/mL, and 3.05 g/mL 0.1 mL aliquot of a standard inoculum of the test microbe was inoculated into each of the compound's concentrations. The turbidity of the plates was measured after the tubes were incubated at 37 °C for 24 h and at 25 °C for 48 h for bacteria and fungi, respectively. Maximum inhibitory concentration (MIC) was defined as the concentration at which the growth of microorganisms was visibly inhibited.

Minimum bactericidal and /or fungicidal concentrations

Bactericidal and fungicidal minimum concentration tests were performed to establish whether or not the test microbes were killed or if their growth was merely slowed. To prevent contamination, the Mueller Hinton agar broth was sterilized at 121 °C for 15 min before being poured into sterile Petri dishes to set. Plates were incubated at 37 °C for 24 h with the MIC serial dilution contents subcultured into the medium and checked for colony growth. The plate with the lowest concentration of the compound in the serial dilution without colony growth was designated as the MBC/MFC [18].

Proximate Analysis

Moisture determination

This is based on the principle that a known weight of biologically material is exposed to heat under controlled conditions. This is achieved by placing the sample in an oven at 105 °C for 24 h. The water from the material evaporates leaving behind dry matter [16]. The difference in weight after heating gives the moisture content of the material. Clean Petri-dishes were dried in an oven at 80 °C for about 30 min, cooled in a desiccator and weighed (W_1). 2 g of each of the samples was taken into the petri –dish and weighed (W_2). The sample with the container was dried in an oven at 150 °C for 24h. It is then transferred into a desiccator to cool and weighed (W_3) with a minimum of exposure to the atmosphere. The moisture content was gotten as

% Moisture= <u>loss in weight to drying</u> X 100 Weight of sample

Ash determination

About 2 g of the sample was placed in a clean pre-weight expressed as a percentage. The crucible was transferred into a muffle furnace at 600 °C for 2 h [16]. Thereafter, the crucible was placed in a desiccator, cooled and weighed. The percentage ash content was calculated using the formula;

% Ash = weight of crucible + Ash – weight of empty crucible X 100 Weight of sample

Determination of crude protein

The three steps involved in crude protein determination were employed in this analysis which include digestion, distillation and titration. 2.0 g of the dried (grounded) sample was transferred into a micro-Kjeldahl flask and digestion tablets were added. The 15 cm³ of conc. H₂SO₄ was added to the sample mixtures of the micro-Kjeldahl flask and heated using a digestion block (heater) in a fumed cupboard continuously until the nitrogen present in the sample reduces to ammonium sulphate. Organic matter $(\text{sample}) + \text{H}_2\text{SO}_4 \rightarrow (\text{NH}_4)_2\text{SO}_4 + \text{CO}_2$, the digest was diluted to 50 cm³ with distilled water. 10 cm³ of the sample aliquot, 40 cm³ of the distilled water and 20 cm3 of 40% NaOH were transferred into a micro Kjeldahl flask. The distillate was collected into a flask containing 10 cm³ of boric acid and a few drops of methyl orange indicator which gives a green colour distillate. The distillate content in the flask was titrated against 0.01 M HCL and the colour changed from green to purple at the endpoint. The titre values were recorded, and the average titre value was calculated. This was used to determine the % of nitrogen [19].

Lipid determination

A 250 mL extraction flask was dried in an oven at 105-110 °C. It was then allowed to cool in a desiccator. The empty extractor flask was weight as (W1). Two grams of the sample were weighed into a labelled thimble. The porous thimble mouth was covered with cotton wool. 200 mL of n-hexane was then added to the dried 250 mL extractor flask. The covered porous thimble was placed in a condenser and the apparatus was assembled. It was extracted for about 5-6 h. The thimble containing the sample was oven dried at 105-110 °C for one hour, it was cool in a desiccator and the weight is taken [20].

Fibre determination

2 g of the sample was introduced into a conical flask. 100 mL of distilled water and 20 mL of 10% H₂ SO₄ were added and then fixed on a heater to heat for 30 min. The sample was filtered in a Muslim cloth, rinsed with water and a spatula was used to scrape the sample into the flask. It was then heated again for 30 min, filtered in a muslin cloth and rinsed with ethanol and allowed to drain. The residue was scraped into a pre-weighed crucible (W1). It was transferred into a muffle furnace to Ash for 2h at 600 °C and allowed in a desiccator and weighed (W_2) the percentage was then calculated.

% fibre = W_1 - W_2 /2 X 100 Where W_1 = dried weight W_2 = Ash weight

Carbohydrate estimation by the difference

The total portion of the carbohydrate in the sample was estimated with the calculation by difference. This is by subtracting all the other calculated nutrients like % of ash, crude protein, % of lipid and % of moisture from 100% [20]. The reminder account for the total percentage of Carbohydrate in the sample. This is known as calculation by difference. = 100% - (% protein + %lipid + %moisture)

Minerals Analysis

Mineralization of sample for the conversion of solid to liquid, a wet glass digestion technique was used. 1g of the finely powdered sample was placed in a beaker for digestion. The content of the beaker was treated with a mixture of nitric and perchloric acid in the ratio of 9:2. Determinations of minerals phosphorus and potassium were carried out by flame photometry [21]. In the flame Photometer, a flame was used to atomise a particular element in question. The amount of atomization was proportional to the quantity of the element in the feeding solution or extract, the intensity was measured with a photocell in a selected wave range corresponding to the given element. The quantity of atomization (emission) in the extract was compared with the known quantity of the element to be determined i.e. a standard curve was prepared.

Phosphorus

Sodium chloride salt (2.5 g) was weighed and dissolved in distilled water and made up to 1 litre, giving a stock solution. A serial dilution of 0-10 mg% solution was made from this stock solution and the readings were taken using a flame Photometer. A graph was plotted using these values. Distilled water was used to adjust the flame photometer to zero while 1.0 mg% was used to obtain the highest percentage transmittance. A standard curve transmittance reading against the concentration of sodium standard was plotted. The reading of the sample was extrapolated from the standard curve.

Potassium

Potassium chloride (1.9 g) was weighed dissolved in distilled water and further diluted to 1 litre solution after changing the filter from sodium to potassium. Serial dilution was made from this to give 0-10 mg% solutions. The readings were taken using a flame photometer. A graph was plotted with transmittance against the readings obtained. The readings of the sample were also extrapolated from the standard curve.

RESULTS AND DISCUSSION

Microbiological and biochemical studies were provided that the extracts of the fruit plant contain some antimicrobial actions use for the treatment of various kind of infections and diseases such as antiviral, anti-inflammatory, antimalarial, antibiotics resistance infections, anticancer, antidiabetic, hypolipidemic, hepatoprotective and nephroprotective properties. The compound, stigmasterol, indicated varying actions of antimicrobial activity against the microbes tested. The susceptibility test result showed inhibition ranging from 23 mm to 30 mm against all the organisms, such as *S. aureus* (24 mm), *Salmonella* Typhimurium (23 mm), *P. aeruginosa* (26 mm), *E. coli* (28 mm), *Streptococcus pyogenes* (25 mm), *B. subitilis* (23 mm), *A. niger* (29 mm), *C. albicans* (24 mm), *and C. kruseii* (23 mm). Creating a zone of inhibition of 32 mm–41 mm, while the standard antifungal drug, ketoconazole (5 μ g/mL), exhibited similar effects as stigmasterol activity against the two fungi species tested with an inhibition range of 24 mm–29 mm (**Table 1**).

Table 1. Susceptibility pattern of Microorganisms examined.

Strains of microbes	Diameter	Extract	Zone of inhibition (mm)
S. aureus	5/10(+++)	SEF	24
Salmonella Typhimurium	4/10(+++)	SEF	23
P. aeruginosa	7/10(+)	SEF	26
E. coli	5/10 (++)	SEF	28
Streptococcus pyogenes	4/10 (+++)	SEF	25
B. subitilis	4/10 (-)	SEF	23
A. niger	6/10 ()	SEF	29
C. albicans	3/10 (++)	SEF	24
C. Kruseii	6/10 (++)	SEF	23
Keys; No inhibition (-) 5-22 mm;	Zone of inhibition	n (+) 23-30 n	nm.

(SEF) Stigmasterol Extract Fruit

This study represents the first antimicrobial evaluation efficacy of stigmasterol derived from *N. macrophylla* stem bark extract and therefore highlights an important natural source of bioactive stigmasterol in Nigeria. The result obtained indicate that the nutritional richness of the mineral and carbohydrate composition of the fruit pulp of *Neocarya macrophylla* has a pharmaceutical and medical significance that are useful in combating antibiotics-related contagion, particularly in the treatment of infections resistant to antibiotics [21,22]. Stigmasterol showed variable antimicrobial activity against the bacteria and yeast used in the experiments. *S. aureus* (24 mm), *S. typhimurium* (23 mm), *Pseudomonas aeruginosa* (26 mm), *Bacillus subtilis* (23 mm), *Aspergillus niger* (29 mm), *Candida albicans*(24 mm), and *C. kruseii* (23 mm).

While the standard antifungal drug, ketoconazole (5 μ g/mL), showed similar effects as stigmasterol activity against the two fungi species tested (inhibition range of 24 mm–29 mm), stigmasterol created a zone of inhibition of 32 mm–41 mm. Thus, it is very heartening to see that the isolated stigmasterol has potent antibacterial activity against *E. coli* (24 mm zone of inhibition). The use of herbal medicine and supplements as a form of alternative medicine has been on the rise recently, especially in developing countries where a sizable percentage of the population relies on these remedies. Accordingly, *N. macrophylla* has been widely used in West Africa to treat a variety of conditions, such as dysentery, diarrhoea, and skin, ear, and eye infections [23].

Stem bark preparations of *N. macrophylla* have been shown to be effective in the treatment of these infections, and this study confirmed the broad-spectrum antimicrobial activity of stigmasterol isolated from *N. macrophylla*. While *N. macrophylla* shows promise, more research is needed to determine whether or not it poses any safety or toxicity risks. It would be useful to compare the stigmasterol yields from the stem bark of *N. macrophylla* grown in various climates and times of the year. This could lead to the quantification of stigmasterol content in crude extracts, which could help inform the posology of herbal preparations derived from this plant. The extract of *Neocarya macrophylla* fruits pulp shows the strongest inhibitory effect to *S. aureus* with the lowest MIC value (**Table 2**).

 Table 2. Minimum Inhibitory Concentrations (MIC) and minimum bactericidal concentrations of the extract of *Neocarya macrophylla* fruits pulp.

Test microbes	Fractions	MIC (mg/mL)	MBC (mg/mL)
S. aureus	SEF	2.82	5.01
S. Typhimurium	SEF	3.50	14.36
P. aeruginosa	SEF	3.62	14.32
E. coli	SEF	23.00	45.00
Streptococcus	SEF	32.01	67.01
B. subitilis	SEF	15.23	17.20
C. albicans	SEF	3.55	6.021
C. kruseii	SEF	3.05	6.042

Proximate analysis was carried out on dried pulp of Neocarva macrophylla to determine its nutritional compositions. The result of proximate composition revealed that the sample had an average moisture content of 14.33±0.099 (Table 3). This is lower when compared to the moisture content of Maerua crassifolia leaves reported by [24,25]. The freshness of seeds, fruits, and vegetables can be gauged from their moisture content. Seeds, fruits, and vegetables with high moisture content are more vulnerable to microbial attack and spoilage [22,26]. Since this is the case, drying the fruits thoroughly is a prerequisite for longterm storage. The crude fibre content was found to be 10.15±0.57. The value obtained is higher than 9.06±0.67 reported for dried Shea fruit pulp by [27,28]. Fibre helps in the maintenance of human health and has been known to reduce cholesterol levels, aid digestion and delay the emptiness of the stomach [29]. Thus, the fruit is a good source of dietary fibre and has the potential of providing body requirements of fibre,

Table 3. Proximate composition of the pulp of Neocarya macrophylla.

Parameter	Level of	
	parameter in	
	sample	
Moisture (%)	14.23%±0.10	
Ash (%)	$6.70\% \pm 0.05$	
Lipids (%)	11.00%±0.50	
Fiber (%)	$10.15\%{\pm}0.57$	
Crude protein (%)	1.015%±0.127	
Available Carbohydrate (%)	51.33%±1.025	

The ash content was 6.70 ± 0.05 . The result showed that the sample contained a good amount of inorganic matter which is confirmed by the mineral analysis result (Table 4). According to [30,31], the ash content in vegetables and other samples may be an index of the number of mineral elements present in the vegetables. The result indicates that the fruit could supplement the body with some of the macro and micro elements required. The crude protein was found to be 1.015±0.127. The crude protein content was lower when compared to that of 4.73% in Irvingia wombolu fruit pulp reported by [32]. This is an indication that the pulp contains low protein which is known for the growth and repair of worn-out tissues [33]. Also reported low level of protein in the pulp of Neocarya macrophylla [33,34]. Momordi cadioica fruit lipid content was found to be 11.00±0.50, which is higher than the 4.7±0.50 percent previously reported [3,35]. However, due to the fruit's high crude lipid content, it has the potential to be a useful source of edible vegetable oil and could supplement existing options.

Table 4. Mineral contents of the pulp of Neocarya macrophylla.

Minerals	Amount (mg/g)		
Ca	37.297± 0.486		
Mg	2.843 ± 0.025		
Cu	0.0087 ± 0.002		
Zn	0.0024 ± 0.001		
Fe	0.0856 ± 0.002		
Mn	0.0122±0.048		
K	89.740±1.629		
Р	89.5733 ±0.689		
Values are presented	as mean + standard deviation (n=3)		

Lipids aid in the intestinal absorption and transport of fatsoluble vitamins, and their contribution to energy production is roughly twice that of protein and carbohydrates. Research conducted by [36,37]. suggests that the available carbohydrate content of the pulp was found to be $51.33\%\pm1.025$, hence is lesser compared to 52.28%. The fruit has high carbohydrate content. Its consumption could provide the body with fuel and energy that is required for daily activities and exercise [24,38]. Adequate carbohydrate is also needed for optimum function of the brain, heart, nervous, digestive and immune system while carbohydrate deficiency causes depletion of these body tissue. This research showed the presence of some important minerals that play is essential for vital life processes. Potassium was the most abundant ($89.7\pm1.62mg$) element followed by phosphorus (89.573 ± 0.689 mg) and then calcium (37.297 ± 0.486 mg) [24].

Potassium is essential for protein synthesis, maintaining fluid balance, nerve and muscle function, as well as the uptake of glucose and glycogen, and the maintenance of normal blood pressure [27]. Calcium, along with phosphorus, is largely responsible for the hardness of bones and teeth, but it is essential for all tissues. It plays a crucial role in blood clotting, nerve sensitivity, and the body's acid-base balance [21,39]. Calcium is essential for bone health, and it also helps with blood clotting, nerve transmissions, and even vitamin B12 absorption. Osteoporosis, a condition characterized by brittle bones and heightened susceptibility to fractures caused by a lack of calcium, can be caused by a deficiency in this mineral. Phosphorus plays an important role in cell structure and many biochemical reactions such as energy metabolism [40].

The pulp also contains low concentrations of magnesium (2.843 ± 0.025) and very low concentrations of iron $(0.0122 \pm 0.048),$ $(0.0856 \pm 0.002),$ manganese copper (0.0087±0.002), and zinc (0.0024±0.001), which are important micro elements required by body for proper functioning. For example, Iron is responsible for the ability of hemoglobin to transfer oxygen throughout body tissues for internal respiration to occur [41,42]. Iron has been reported as an essential trace metal that plays numerous biochemical roles in the body, including oxygen-binding haemoglobin and acts as an important catalytic center in many enzymes for example, cytochrome [26]. Manganese is a part of pyruvate carboxylase and superoxide dismutase. It helps in the metabolism of protein [43].

Zinc is present in the entire tissues of the body and it is a part of more than 50 enzymes. It also plays an important role in cell growth, cell division, healing of a wound and the breakdown of carbohydrates [44]. The deficiency of copper causes cardiovascular disorders as well as anaemia and disorders of the bones and neuro systems [36,45]. Magnesium plays a significant role in carbohydrate metabolism, nucleic acids and binding agents of the cell walls [46]. Copper is required for osteogenesis and the activity of osteoblast [47]. The presence of these minerals contributes to its medicinal [49,50].

CONCLUSION

The result obtained indicate that Neocarva macrophylla fruit pulp is of pharmaceutical and medical significance that are useful in Combating antibiotics resistance infections, nutritionally rich in terms of minerals and carbohydrate composition. It is also suggested that the fruit if consumed in sufficient amount could help to overcome antibiotics resistance, and nutrient deficiencies disorders that are prevalent in vulnerable groups, Neocarva macrophylla will make a good nutrient source for the body. Extracts of stigmasterol from the fruit of N. macrophylla demonstrated broad-spectrum antimicrobial activity, suggesting its potential as a candidate in the development of novel antimicrobial drugs. When the medical significance of the microorganisms tested in this study is considered, the overall result can be seen as promising for the discovery of novel drugs from plant sources. This research confirms that N. macrophylla stem bark preparation is effective against both bacterial and fungal infections.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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