Phytochemical Analysis, Antifungal and Antibacterial Screening of Artocarpus altilis: Guyana Flora Extracts

Comment [A1]: Phytochemical Analysis and Antimicrobial Screening of *Artocarpus altilis*: Guyana Flora Extracts

ABSTRACT

The plant material leaves of Artocarpus altilis (Breadfruit) were collected from university of Guyana road, Cummings lodge, Guyana. Leaves are dried in oven at 50-55 ºC for 72 h. The moisture content is calculated. The dried leaves were grounded and extracted in each acetone, ethanol and methanol solvents. Extracts were collected and evaporation of solvent was done on rotavapour. The respective solvent was added to viscous semi solid liquid extract to make up the desired volume of extract solution. The antimicrobial and antifungal activity of both plants were examined by well diffusion method, poison plate method, paper disc plate method and streak plate methods. In Artocarpus altilis leaves extract studies, maximum and minimum antimicrobial potential was observed for methanol and acetone solvent extracts, respectively. All plant extracts showed antimicrobial potential toward the organisms Staphylococcus aureus, Escherichia coli and Candida albicans. The phytochemical analysis of methanol leaves extract of breadfruit tested positive for flavonoids, terpinoids, phenol, phlobatannins, steroids and phytosteroids and negative for tannins, saponins , alkaloids and cardiac glycosides .

Comment [A2]: *Artocarpus altilis*

Comment [A3]: were oven dried

Comment [A4]: and the moisture content was calculated

Comment [A5]: pulverized

Comment [A6]: using different solvents such as acetone, ethanol and methanol

Comment [A7]: Rephrase sentence

Comment [A8]: The antimicrobial and antifungal activities

Comment [A9]: agar well diffusion method

Comment [A10]: *Artocarpus altilis*

Comment [A11]: were

Comment [A12]: was there no antimicrobial activity for ethanol extract

Comment [A13]: All plant extracts showed antimicrobial potentials toward the organisms; *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*

Keywords: Artocarpus altilis; Leaves extract; Phytochemical screening; Antifungal potential; Antimicrobial potential.

Comment [A14]: *Artocarpus altilis*

1. INTRODUCTION

According to DNA fingerprinting studies, the wild seeded ancestor of breadfruit is the breadfruit is the breadnut (Atrocarpus camansi) which is native to New Guinea, the Maluku Islands, and the Philippines. It was one of the canoe plants spread by

Comment [A15]: Recast or rewrite

Comment [A16]: *Atrocarpus camansi*

Austronesian voyagers around 3,000 years ago into Micronesia, Melanesia, and Polynesia, where it was not native [1,2]. Extent of the Austronesian expansion that carried crops like breadfruit, bananas and coconuts throughout the Indo-pacific Islands. Breadfruit trees growth to a height of 26 m (85 ft.). The large and thick leaves are deeply cut into pinnal lobes. All parts of tree yield latex [3] which is useful for boat caulking. Flower on an urban cheal axis is male while arising from single point is female. Fruit is cylindrical 9- 29 cm. long by 6 – 20 cm wide. They are normally green while yellow brown on ripe. Average weight of fruit is about 1 to 5 kg.

Comment [A17]: Rephrase

Breadfruit contains phytochemicals having potential as an insect repellent [4]. Sivagnana Sundaram and Karunanayeke [5] has investigated phytochemical screening and antimicrobial activity of Artocarpus heterophyllus and Artocarpus altilis leaf and stem hexane, dichloromethane and ethanol extracts. The local uses of paparahua (Artocarpus altilis) in Amazonia Ecquadore by phytochemical data review is reported by Luzuriaga- quichimbo et al. [6]. The plant is found to contain terpenoid, stelbenoid and different groups of flavonoids which is effective in a search of novel drugs. Riasari et al. [7] studied antibacterial and antifungal activities methanol extracts of green, fallen yellow, fallen dry breadfruit leaves against Escherichia coli, Staphylococcus epidermidis, propionbacterium acnes and candida albicans. Pradhan et al. [8] has explained phytochemical screening and comparative bioefficacy assessment of Artocarpus altilis petroleum ethane, methanol and ethylacetate leaf extract for antimicrobial activity. Mbaeyi-Nwaoha and Onwuku [9] has evaluate antimicrobial properties and phytochemical composition of ethanolic, hexane and watery leaf extract of Artocarpus altilis. The anti-nutrient analysis of leave extract also revealed the presence of phylate , oxalate, tannin and cyanide. Palupi et al. [10] studied anti-inflammatory, antioxidant and immunosuppressant

Comment [A18]: *Artocarpus heterophyllus* and *Artocarpus altilis*

Comment [A19]: *Artocarpus altilis*

Comment [A20]: *Escherichia coli, Staphylococcus epidermidis, Propionbacterium acnes* and *Candida albicans*

Comment [A21]: Explained the

Comment [A22]: *Artocarpus altilis*

Comment [A23]: also evaluated the

Comment [A24]: *Artocarpus altilis*

Comment [A25]: Remove

Comment [A26]: Phylate,

properties of ethanolic extract of leaf, fruit and bark from breadfruit (Artocarpus altilis [Park] Fosberg). This result indicated the A. altilis leaf extract significantly revealed anti-inflammatory, antioxidant and immunosuppressant activities at 200 mg/kg.

Gladys and Bukola [11] has reported that methanol extract of the leaf, root, stem, bark, flower, fruit and wood of A. altilis was tested against the fourth instar larvae of culex quinquefasciatus mosquito. Nisa et al. [12] has evaluated antifungal and antibacterial activity of ethanolic and distilled water extracts of Artocarpus altilis plant using broth micro-dilution methods. A altilis aqueous extract had the most significant antifungal activity against Penicillium sp with AFA value of 140.36 ± 3.76 %. Nochera and Ragone [13] has investigated to develop a pasta product using breadfruit flour, test the sensory qualities of the breadfruit pasta product by sensory evaluation, and evaluate the nutritional composition. Rante et al. [14] has studied ∝- glycosidase inhibitory activities of n- hexane and ethanolic, yellow and green breadfruit leaves extract ethanolic leaf extracts were found to have more ∝- glycosidase inhibitory activity than n-hexane leaf extract.

Nayagam et al. [15] has synthesized silver nanoparticles (AgNPs) from plant residue of breadfruit and analyzed for the antibacterial assay against human pathogens. The cyto-toxic activity AgNPs was analyzed with two human cancer cell lines namely MCF_7 lung cancer cell line and A594 breast cancer cell line. Marta et al. [16] has compared the effect of different thermal treatment viz heat moisture treatment (HNT), and osmotic pressure treatment (OPT) on physicochemical and pasting properties of breadfruit starch. Soifoini et al. [17] has described nutritional and nutraceutical traits of Artocarpus altilis (Parkinson) Fosberg by characterizing its main bioactive compounds, nutritional traits, and antioxidant properties in order to contribute to the development of traditional and innovative uses of this species as functional food. This anti-

Comment [A27]: *Artocarpus altilis*

Comment [A28]: *A. altilis*

Comment [A29]: *A. altilis*

Comment [A30]: *Artocarpus altilis*

Comment [A31]: *A altilis*

Comment [A32]: *Penicillium* sp

Comment [A33]: by sensory evaluation and evaluated the nutritional composition

Comment [A34]: cytotoxic

Comment [A35]: *Artocarpus altilis*

inflammatory activity between fermented and natural-dried breadfruit leaves extract were compared by Riasari et al. [18]. Fermented and dried breadfruit leaves were extracted with soxhlet devise using methanol solvent.

Comment [A36]: device

Comment [A37]: methanol.

Antioxident, totalphenolic content and FTIR analysis of breadfruit methanolic leaves extract, which are essential in management of diabetes was demonstrated by Leng et al. [19]. Total phenolic content in fresh breadfruit leaves (144.16 mg/g ± 17.98) was comparable to those of green tea. Acute toxicity, antiplasmodial and liver histopathological effects of aqueous extract of breadfruit leaves were explained by Udonkany et al. [20]. The aqueous extract of breadfruit leaves has antiplasmodial properties and is safer at lower doses. The anxiety – like effects of breadfruit methanolic leaves extracts in Swiss mice was explained by Ajah et al. [21]. Artocarpus altilis leaf extract was found to process antiolytic- like properties. The hepatoprotective effect of ethanolic extract of Indonesian breadfruit leaf in CCR_4 – induced liver injury Wistar rats by measuring Serum Glutamic Pyruvic Transminase (SGPT) and malondialdehyde (MDA) level was examined by Juliastuti et al. [22]. Ethanol-based breadfruit leaf extract was found to have effect on liver protection by decreased level of MDA and SGPT. The chemical composition, functional, mineral and anti-nutritional properties of composite flour produced from breadfruit and beniseed was discussed by Peters et al. [23]. The 70: 30 composite flour showed improved chemical composition and could be utilized for product development in different food system. A review on phytochemistry, bio-efficacy, medicinal and ethno-pharmaceutical importance of breadfruit was demonstrated by Mohanty and Pradhan [24].

Comment [A38]: Antioxidant, total phenolic

Comment [A39]: *Artocarpus altilis*

Comment [A40]: 70 : 30

The bioactive constituent and influence of ethanol concentration, time and temperature on the anti-oxidant and antibacterial activity of Artocarpus altilis leaves in order to find appropriate and effective condition for its extraction was investigated by Saraswaty et al. [25]. It was

Comment [A41]: *Artocarpus altilis*

recommended to heat the extraction process to 70^0 c in order to obtain both optimal antioxidant and antibacterial activity from ethanol extract of Artocarpus altilis leaves. Present work describes phytochemical analysis, antifungal and anti-bacterial screening of Artocarpus altilis,

Scientific Classification

Kingdom: *Plantae*

Clade: *Tracheophytes*

Clade: *Angiosperms*

Clade: *Eudicots*

Clade *Rosids*

Order: *Rosales*

Family: *Moraceas*

Genus: *Artrocarpus*

Species: *Artocarpus altilis*

2. METHODOLOGY

2.1 Collection of plant materials

The plant material leaves of Artocarpus altilis is collected from a yard on 5th Street, UG Road, Turkeyen, Georgetown. Guyana.

2.2 Preparation of plant materials:

The collected leaves sample of Artocarpus altilis is weighted on Citizen CTG 3000 E electronic balance. The leaves dried in oven (Gallenhamp Incubator Model IH-150) at

Comment [A42]: 70° C

Comment [A43]: *Artocarpus altilis*

Comment [A44]: This work investigated the phytochemical analysis and antimicrobial screening of *Artocarpus altilis* on selected organisms of interest.

Comment [A45]: **General comment on introduction.**
i. Reduce the length of your introduction by removing some irrelevant studies
ii. Avoid repetition of similar studies
iii. Work on your sentence composition and usage
iv. Stick to a particular style of writing your plant name i.e. after writing the first *Artocarpus altilis* others can be written in short form i.e. *A. altilis*
v. let all your paragraphs be aligned (uniform) or single tab paragraph.
vi. All in all let your introduction be brief
vii. **Ensure all your scientific names or botanical names are in italics except the journal requested otherwise**

Comment [A46]: Remove

Comment [A47]: *Artocarpus altilis*

Comment [A48]: was

Comment [A49]: yard on

Comment [A50]: what quantity of leaves was weighed in gram

Comment [A51]: *Artocarpus altilis* was

Comment [A52]: weighted on Citizen CTG 3000 E electronic balance

Comment [A53]: The leaves were dried in oven

50-55º C. The dried leaves were cooled at room temperature and weighted again on same citizen electronic balance. Weight of green leaves, dried leaves and value of percentage moisture content in various samples of Artocarpus altilis is given in Table 1.

The weight of ground leaves of Artocarpus altilis is found to be 561.0 grams.

2.3 Collection of test organism

Three micro-organisms (Escherichia coli, Staphylococcus aureus and Candidus albicans) were used for the study. All the tested strains are reference stains and were collected from the Microbiology Laboratory of Georgetown Public Hospital Corporation, Georgetown (GHPC). All cultures are maintained in nutrient broth (Himedia, M002) at 37ºC and maintained on nutrient agar (Himedia, MM012) slants at 4º C.

2..4 Extraction and preparation of test solutions

The grounded leaves of Artocarpus altilis was extracted in each acetone ethanol, and methanol solvents. At a time 20 g of dried pulverized leaves were soaked with 200 mL of solvent for 48 h. Solvent is decanted each time and residue again soaked with same solvent for 24 h. The total extract is combined and filtered. The evaporation of solvent was done on rotavapour (Buchi). The respective solvent was added to viscous semi solid liquid extract to make up the desired volume of extract solution.

3.5 Anti-Microbial Assay:

2.5.1 Materials

Comment [A54]: weighed

Comment [A55]: *Artocarpus altilis*

Comment [A56]: presented

Comment [A57]: The resultant weight of ground leaves of *Artocarpus altilis* was found to be 561.0 g.

Comment [A58]: *Collection of test organism*

Comment [A59]: Three (3) different microorganisms; *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were collected and used for the study.

Comment [A60]: All tested organisms are referenced stains obtained from the Microbiology Laboratory of Georgetown Public Hospital Corporation, Georgetown (GHPC).

Comment [A61]: All cultures were maintained in nutrient broth (Himedia, M002) at 37 °C and on nutrient agar (Himedia, MM012) slants at 4 °C.

Comment [A62]: *2.4*

Comment [A63]: *Extraction and preparation of test plant leaves*

Comment [A64]: The ground leaves of *Artocarpus altilis* was extracted using different solvents; acetone, ethanol and methanol at a proportion of 1 in 10 dilution.

Comment [A65]: Twenty (20) g of dried pulverized leaves was soaked in 200 mL of the corresponding solvent for 48 h

Comment [A66]: Solvent was decanted and the residue was again soaked in the same solvent to achieve a complete extraction of the bioactive components for 24 h. The total extract was combined and filtered. The evaporation of filtrate was done in rotavapour (Buchi). The respective solvent was added to viscous semi solid to liquid extract to make up the desired volume of extract solution.

Comment [A67]: *2.5*

Comment [A68]: *Antimicrobial Assay*:

Comment [A69]: Start from new page

Mueller Hinton Agar, Agar plates and microbial discs were purchased from the Caribbean medicals, parika in Guyana. Solvents acetone, ethanol, and methanol obtained from Aldrich. Scitilation vials 20 mL were obtained from Meditron Scientific Sales, Georgetown, Guyana.

2.5.2 *Aseptic chamber*

Aseptic chamber consist of a wooden box of L = 1 meter, B = 1 meter and D = 0.5 meter area. Chamber is cleaned with 70 % ethanol twice and irradiated with short wave UV light for 1 h.

2.5.3 Potato Dextrose *Agar (PDA) medium*

Potato Dextrose Agar medium prepared according to method reported by Talaro [26]. This is the medium on which cultured bacteria Escherichia coli and Staphylococcus aureus were grown. The 200 g potato was peeled finely chopped and boiled to a mash in distilled water, Each 12.5 g dextrose and 12.5 g Agar was placed in a 1 L measuring cylinder. Distilled water was added to make the solution to 500 m L. The content was stirred until the consistency of solution mixture. The stirred mixture poured into conical flasks, plugged with cotton wool and tightly wrapped by aluminum foil. The flasks were autoclaved at 121 ºC, 15 psi, for 15 minutes.

2.5.4 *Mother plates*

Mother plates were prepared by pouring potato dextrose Agar mixture into Petri dishes and to cool at room temperature, in the aseptic chamber.

2.5.5 Antimicrobial assay:

Comment [A71]: Mueller Hinton Agar (MHA), agar plates and microbial discs were purchased from the Caribbean medicals, Parika in Guyana. Solvents such as acetone, ethanol, and methanol were obtained from Aldrich. Scintillation vials (20 mL) were obtained from Meditron Scientific Sales, Georgetown, Guyana.

Comment [A72]: Aseptic chamber consisted of a wooden box of L = 1 meter, B = 1 meter and D = 0.5 meter area. Chamber was cleaned with 70 % ethanol twice and irradiated with short wave UV light for 1 h.

Comment [A73]: *Preparation of Potato Dextrose Agar (PDA) medium*

Comment [A74]: Potato Dextrose Agar (PDA) medium was prepared according to the method described by Talaro [26].

Comment [A75]: No! Please confirm your source. You must have copied wrongly. PDA is not used for the cultivation or growing of bacteria such as *E. coli* and *S. aureus* (maybe you wanted to report on nutrient agar)

Comment [A76]: Potato Dextrose Agar (PDA) is used for the cultivation of fungi like *C. albicans* and obviously not for bacteria

Comment [A77]: Two hundred (200) g of potato was peeled, finely chopped and boiled to a mash in distilled water. Each 12.5 g dextrose and 12.5 g agar was placed in a 1 L measuring cylinder. Distilled water was added to make up the solution to 500 m L.

Comment [A78]: The content was stirred until a uniform consistency of the solution was achieved. The stirred mixture was transferred into conical flasks, plugged with cotton wool and tightly wrapped with aluminum foil. The flasks were autoclaved at 121 °C (15 psi) for 15 minutes.

Comment [A79]: Mother plates were prepared by pouring approximately 20 ml Potato Dextrose Agar (PDA) mixture into Petri dishes and cooled at room temperature (RT) in the aseptic chamber.

Comment [A80]: Move to a new page

Comment [A81]: *2.5.5Antimicrobial assay*

Comment [A82]: What was the proportion of the diluted extract for reconstitution in the solvent to get the dilution series used (i.e. 25, 50, 75, 100 e.t.c.)

Antimicrobial assay was done by well diffusion method, poison plate method, paper disc method and streak plate methods. Which are as follows:

2.5.5.1 **Well diffusion method:**

In this method well are made using a sterilized the cork borer on the seeded nutrient agar in a petri dish to which the test compound (leaves extract) of different concentration (25, 50, 75, 100, 125 µLs) are added. The treated petri discs are incubated at room temperature for 24 hrs. The inhibition zone formed around each well indicates the antibacterial activity. The procedure was repeated in duplicate and inhibitory zone was measured by ruler in mm [27].

2.5.5.2 **Poison plate method:**

The test organism (S. aureus, E. coli, C. albicans) seeded into nutrient medium were poured into petri discs and allow to cool and solidify. A 9 cm sterile cork borer was used to make a disk on pathogen plate. Pathogen disc was taken from pathogen plate and kept at the center of test compound (leaves extract of 25, 50, 75, 100, 125 µL concentrations) seeded plate with the help of a sterile inoculum needle and was incubated for 2 to 3 days. The inoculum needle was sterilized with the alcohol and flame before each application. The experiment was done in duplicate and zone of inhibition was measured [28].

2.5.5.3 **Paper disc plate method:**

The circular discs of 6 mm diameter was prepared from whatman no.1 filter paper and sterilized ia a autoclave. These paper discs were impregnated with test compounds (leaves extract 25, 50, 75, 100, 125 µLs) in respective solvent (acetone, ethanol, methanol) for overnight and placed on nutrient agar plates seeded with test organism (S. aureus , E. coli, C. albicans) . The plates are incubated at room temperature for 12 hr. After 12 h zone of inhibition around each disc was measured by horizontal and vertical method and the diameter was recorded. A reference control was prepared using only the several (

Comment [A83]: Antimicrobial assay was carried out using agar well diffusion method, poison plate method, paper disc method and streak plate methods described below;

Comment [A84]: **Agar well diffusion method**

Comment [A85]: wells were

Comment [A86]: Sterilized cork borer

Comment [A87]: concentrations

Comment [A88]: µL

Comment [A89]: were

Comment [A90]: Petri

Comment [A91]: dishes were

Comment [A92]: 24 h (stick to a particular patter. Either hr or h, ensure uniformity)

Comment [A93]: inhibitory zones were

Comment [A94]: **Poison plate method (PPM): Ensure you understand the principle and concept of this procedure. PPM is not used for bacteria but mold and Candida is a yeast and not a mould**

Comment [A95]: **Poison plate method**

Comment [A96]: The test organisms (*S. aureus, E. coli* and *C. albicans*)

Comment [A97]: Please note; Susceptibility test may not be well observed on NA for *C. albicans* because NA is not the suitable medium for *C. albicans.*

Comment [A98]: Petri dishes

Comment [A99]: allowed to cool and solidified

Comment [A100]: A sterile cork borer of a diameter of 6 mm was used to make a disc

Comment [A101]: Rewrite in a clear and simple term describing what you did

Comment [A102]: inoculating needle

Comment [A103]: flamed

Comment [A104]: zones of inhibition were measured

Comment [A105]: **Paper disc plate method**

Comment [A106]: discs of 6 mm diameter were

Comment [A107]: Whatman

Comment [A108]: in an

Comment [A109]: µL

Comment [A110]: *S. aureus, E. coli and C. albicans.* (Please see comment A95R94 on the choice of plate for *C. albicans*)

Comment [A111]: The plates were incubated at room temperature for 12 h.

Comment [A112]: After 12 h, the zone of inhibition

acetone , ethanol, methanol) and kept for comparison . The test done in duplicate to ensure the reliability of the results [29].

2.5.5. 4 **Streak plate method:**

The molten agar medium (20 m L) and each leaves extract and each leave extract (25, 50, 75, 100, 125 µL) was poured into a sterile petri dish under aseptic condition. It was cooled at room temperature. After cooling the each bacterial culture was taken at 12, 24, 36 hour intervals and using the surface of agar medium in the form of parallel strokes (streaks). The test repeated in duplicates. The plates were incubated at room temperature for 24 hours and inhibitory zone was measured. Control plates without the plant extract were also maintained for the reference [30].

2.6 Phytochemical analysis of the plant extracts

2.6.1 Materials

Glacial acetic acid, thyonil chloride, dichloromethane, copper sulfate, lead acetate, diethyl ether, ferric chloride, acetic anhydride, antimony chloride, amyl chloride etc. obtained from Aldrich.

2.6.2 Method

Phytochemical analysis of all the aqueous plant extracts was carried out by suitable methodologies in search of active ingredient responsible for antimicrobial toxicity.

The phytochemicals include under study were saponins, terpenoids, alkaloid, cardiac glycoside, phenol, steroid phytosteroid , tannins, phlobatannins and flavonoids the analysis was carried out according to the methodologies of Edeoga et al. [31].

Comment [A113]: solvents (acetone, ethanol and methanol)

Comment [A114]: comparison.

Comment [A115]: The test was carried out in duplicate to ensure the reliability of the results [29].

Comment [A116]: You should use a standard antimicrobial drugs (antibacterial and antifungal) to compare the efficacy of the leaf extracts. This will serve a basis for both positive and negative comparison.

Comment [A117]: 2.5.5. 4

Comment [A118]: **Streak plate method**

Comment [A119]: (20 mL)

Comment [A120]: leaf

Comment [A121]: delete

Comment [A122]: Petri

Comment [A123]: Cooling, each

Comment [A124]: Recast

Comment [A125]: The test was carried out in duplicate

Comment [A126]: inhibitory zone were

Comment [A127]: chloride were obtained from Aldrich.

Comment [A128]: The phytochemicals under study include; saponins, terpenoids, alkaloid, cardiac glycoside, phenol, steroid phytosteroid, tannins, phlobatannins and flavonoids. The analysis was carried out according to the methodologies of Edeoga et al. [31].

Table 1: PERCENTAGE MOISTURE CONTENT FOR ARTOCARPUS ALTILIS LEAVES

(Oven temperature 50 – 55 ^{0} C)

Sample Number	Weight of green leaves (gram)	Weight of dry leaves at 10 am(grams)	Weight of dry leaves at 4 pm (grams)	Percentage moisture content %
1	283	96	96	66.07
2	438	128.5	128.5	70.66
3	389	115	115	70.44
4	409	125	125	69.44
5	370	127.5	127.5	65.254

$$\text{Percentage moisture content} = \frac{\text{Weight of green leaves} - \text{weight of dry leaves}}{\text{weight of green leaves}} \times 100$$

Comment [A129]: Use either sentence case or title case for your table title

Comment [A130]: **Percentage Moisture Content for *Artocarpus altilis* leaves**

Comment [A131]: °C

Comment [A132]: This table should be part of your result. Move table under result.

Comment [A133]: Use equation editor to insert formula to avoid lopsided arrangement

Comment [A134]: General comments
i. adopt a uniform writing style
ii. use a uniform font size
iii. use a uniform margin and paragraphing
iv. check your sentence structure.
v. do necessary adjustment
vi. it was observed that you did not talk about the preparation of NA, NB and MHA (you can do that in brief).
vi. Reference all your procedures

3. RESULTS

3.1 Well diffusion method

Antimicrobial activity of Artocarpus altilis leaves extract against E coli, S. aureus and C. albicans are summarized in **Tables 2-4** by well diffusion methods.

Table 2. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against E. coli.

Compared with control by well diffusion method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.10	0.16	0.20
	50	0.25	0.20	0.25
	75	0.30	0.26	0.30
	100	0.35	0.30	0.35
	125	0.35	0.32	0.39

- Duplicate

Comment [A135]: *Artocarpus altilis* leaves extract against *E coli*, *S. aureus* and *C. albicans*

Comment [A136]: Antimicrobial activities of crude leaf extracts of *Artocarpus altilis* in various solvents against *E. coli*.

Comment [A137]: Join together with the title above

Comment [A138]: Leaf

Comment [A139]: Diameter of the inhibitory zones

Comment [A140]: *Artocarpus altilis*

Comment [A141]: There is need for you to use a key to interpret what your control means.
Duplicate of what?
Then are your results reported as mean value or single value? If it is in mean or average value, add your standard error mean or standard deviation.

Table 3. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against S. aureus.

compared with control by well diffusion method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.21	0.22	0.28
	50	0.25	0.25	0.29
	75	0.35	0.30	0.30
	100	0.38	0.32	0.33
	125	0.42	0.34	0.35

*Duplicate

Table 4. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against C. albicans.

Compared with control by well diffusion method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.15	0.21	0.25
	50	0.20	0.25	0.29
	75	0.25	0.29	0.30
	100	0.30	0.32	0.42
	125	0.35	0.35	0.49

* Duplicate

Comment [A142]: *Artocarpus altilis*

Comment [A143]: *S. aureus*

Comment [A144]: Join together with the title above

Comment [A145]: Diameter of the inhibitory zones

Comment [A146]: *Artocarpus altilis*

Comment [A147]: See comment in Table 2. You may also need to consider doing statistical comparison (ANOVA or Students' t test) to justify if there is a significant input in your study.

Comment [A148]: Check previous comments on the tables above.
You can also represent some of your tables in Figures using Bar chart, pie chart, histogram or line graph to make your work unique

3.2 **Poison plate method**

Antimicrobial activity of Artocarpus altilis leaves extract against E coli, S. aureus and C. albicans are summarized in **Tables 5 -7** by poison plate methods.

Comment [A149]: *Artocarpus altilis* leaves extract against *E coli*, *S. aureus* and *C. albicans*

Table 5. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against E. coli.

Compared with control by poison plate method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.22	0.20	0.25
	50	0.31	0.22	0.27
	75	0.32	0.26	0.29
	100	0.44	0.30	0.33
	125	0.50	0.33	0.34

*duplicate

Comment [A150]: Follow comment raised in tables above

Table 6. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against S. aureus.

Compared with control by poison plate method.

Comment [A151]: Follow comment raised in tables above

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.19	0.28	0.29
	50	0.22	0.32	0.30
	75	0.26	0.33	0.32
	100	0.28	0.45	0.34
	125	0.30	0.52	0.35

*duplicate

Comment [A152]: Follow comment raised in tables above

Table 7. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against C. albicans.

Compared with control by poison plate method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.18	0.24	0.33
	50	0.20	0.25	0.34
	75	0.22	0.26	0.35
	100	0.24	0.28	0.46
	125	0.26	0.29	0.53

*duplicate

Comment [A153]: Follow comment raised in tables above

3.3 Paper disc method

Antimicrobial activity of Artocarpus altilis leaves extract against E coli, S. aureus and C. albicans are summarized in **Tables 8-10** by paper disc plate methods.

Comment [A154]: *Artocarpus altilis* leaf

Comment [A155]: *E coli*, *S. aureus* and *C. albicans*

Table 8 Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against E. coli.

Comment [A156]: Follow comment raised in tables above

Compared with control by paper disc plate method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.22	0.23	0.24
	50	0.31	0.25	0.25
	75	0.32	0.27	0.38
	100	0.44	0.29	0.30
	125	0.51	0.30	0.32

*duplicate

Comment [A157]: Follow comment raised in tables above

Table 9. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against S. aureus.

Compared with control by paper disc plate method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.15	0.26	0.23
	50	0.17	0.28	0.25
	75	0.19	0.29	0.28
	100	0.20	0.41	0.30
	125	0.21	0.48	0.33

*duplicate

Comment [A158]: Follow comment raised in tables above

Table 10. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against C. albicans.

Compared with control by paper disc plate method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.15	0.21	0.27
	50	0.30	0.25	0.31
	75	0.35	0.28	0.32
	100	0. 39	0.30	0.45
	125	0.35	0.34	0.51

*duplicate

Comment [A159]: Follow comment raised in tables above

3.4 Streak plate method

Antimicrobial activity of Artocarpus altilis leaves extract against E coli, S. aureus and C. albicans are summarized in **Tables 11-13** by Streak plate methods.

Comment [A160]: Activities

Comment [A161]: *Artocarpus altilis*

Comment [A162]: *E coli*, *S. aureus* and *C. albicans*

Table 11. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against E. coli.

Compared with control by streak plate method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.16	0.20	0.23
	50	0.18	0.22	0.24
	75	0.19	0.25	0.26
	100	0.20	0.27	0.28
	125	0.24	0.29	0.30

*duplicate

Comment [A163]: Follow comment raised in tables above

Table 12. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against S. aureus.

Compared with control by Streak plate method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.16	0.18	0.20
	50	0.17	0.19	0.22
	75	0.19	0.21	0.24
	100	0.21	0.23	0.29
	125	0.23	0.25	0.31

*duplicate

Comment [A164]: Follow comment raised in tables above

Table 13. Antimicrobial activity of crude leaves extracts of Artocarpus altilis in various solvents against C. albicans.

Compared with control by Streak plate method.

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory (mm)*		
		Acetone	Ethanol	Methanol
Artocarpus altilis (Breadfruit)	25 control	0	0	0
	25	0.20	0.22	0.23
	50	0.22	0.25	0.26
	75	0.25	0.29	0.29
	100	0.29	0.33	0.31
	125	0.31	0.35	0.36

*duplicate

3.5 **Phytochemical analysis:**

Phytochemical analysis **Table 14** of the Artocarpus altilis (breadfruit) leaves extracts revealed the presence of tannins, saponins, flavonoids, alkaloids, cardiac glycosides, terpenoids, phenol, phlobatannins, steroids and phytosteroids.

Table 14 :Phytochemical analysis breadfruit Artocarpus altilis leaves extracts

S. No.	Phytoconstituents	
1	Tannins	Absent
2	Saponins	Absent
3	Flavonoids	Present
4	Alkaloids	Absent
5	Cardiac glycosides	Absent
6	Terpenoids	Present
7	Phenol	Present
8	Steroids and phytosteroids	Present
9	Steroids and phytosteroids	Present

Comment [A165]: Follow comment raised in tables above

Comment [A166]: **Phytochemical analysis**

Comment [A167]: Phytochemical analysis **Table 14** of the *Artocarpus altilis* (breadfruit) leaf extracts revealed the presence and absence of tannins, saponins, flavonoids, alkaloids, cardiac glycosides, terpenoids, phenol, phlobatannins, steroids and phytosteroids.

Comment [A168]: Table 14: Phytochemical analysis breadfruit *Artocarpus altilis* leaf extracts

Comment [A169]: Be consistent in the usage of terms. Use phytochemicals instead of phytoconstituents

Comment [A170]: Use negative or minus (−) sign to represent absent

Comment [A171]: Use positive or plus (+) sign to represent present

Comment [A172]: Why repetition?

Comment [A173]: **General comments:**
i. Transform some tables into figures
ii. Interpret your tables briefly (make a general overview)
iii. compare some of your values in relation to the solvents used for extraction.
iv. interpret the signs with keys at the end of the table.
i.e. **Key**: − = absent and + = present.

4. DISCUSSION

It is observed from **Tables 2-4** that (i) In acetone solvent maximum (0.42 mm: 125 µL) inhibitory zone was observed for S. aureus and minimum inhibitory zone (0.10 mm: 25 µL) was observed for E. coli. (ii) In ethanol solvent maximum (0.35 mm: 125 µL) inhibitory zone was observed for C. albican and minimum inhibitory zone (0.16 mm: 25 µL) was observed for E.coli. (iii) In methanol solvent maximum (0.49 mm: 125 µL) inhibitory zone was observed for C. albicans and minimum inhibitory zone (0.20 mm: 25 µL) was observed for E. coli.

It is clear from **Tables 5-7** that (i) In acetone solvent maximum (0.50 mm: 125 µL) inhibitory zone was observed for E. coli and minimum inhibitory zone (0.18 mm: 25 µL) was observed for C. albicans. (ii) In ethanol solvent maximum (0.52 mm: 125 µL) inhibitory zone was observed for S. aureus and minimum inhibitory zone (0.20 mm: 25 µL) was observed for E. coli. (iii) In methanol solvent maximum (0.53 mm: 125 µL) inhibitory zone was observed for C. albicans and minimum inhibitory zone (0.25 mm: 25 µL) was observed for E. coli.

It is seen from **Tables 9-10** that (i) In acetone solvent maximum (0.51 mm: 125 µL) inhibitory zone was observed for E. coli and equal (0.15 mm: 25 µL) inhibitory zone was observed for S. aureus and C. albicans. (ii) In ethanol solvent maximum (0.48 mm: 125 µL) inhibitory zone was observed for S. aureus and minimum inhibitory zone (0.21 mm: 25 µL) was observed for C. albicans. (iii) In methanol solvent maximum (0.51 mm: 125 µL) inhibitory zone was observed for C. albicans and minimum inhibitory zone (0.23 mm: 25 µL) was observed for S. aureus.

The **Tables 11-13** indicated that (i) In acetone solvent maximum (0.31 mm: 125 µL) inhibitory zone was observed for C. albicans and equal (0.16 mm: 25 µL) inhibitory zone was observed for E. coli and S. aureus. (ii) In ethanol solvent maximum (0.35 mm: 125 µL) inhibitory zone was observed for C. albicans and minimum inhibitory zone (0.18 mm: 25 µL) was observed for S. aureus. (iii) In methanol solvent maximum (0.36 mm: 125 µL) inhibitory zone was observed for C. albicans and minimum inhibitory zone (0.20 mm: 25 µL) was observed for S. aureus.

In general it is clear from **Tables 2- 13** that (i) inhibitory zone follow the order : Methanol > ethanol > acetone in most of the assay studies. This may due to high polarity of methanol

Comment [A174]: Numbering is not acceptable in discussion

Comment [A175]: *S. aureus*

Comment [A176]: *E. coli*

Comment [A177]: *C. albicans*

solvent. (ii) In most of the essay studies inhibitory zones among the methods follow the order well diffusion method > poison plate method > paper disc method > streak plate method. (iii) It is seen from **Tables 2-13** that inhibitory zone or antimicrobial potential increases as the amount of leaves extract increases from 25 µL to 125 µL. this may be due to increase in amount of extracts. (iv) It is clear from **Tables 2-13** that all plant extract showed antimicrobial potential activity toward the gram positive bacteria staphylococcus aureus also gram negative bacteria Escherichia coli and fungus C. albicans.

The phytochemical analysis of methanol leaves extract of Artocarpus altilis (**Table 14**) tested positive for flavonoids, terpenoids, phenol, phlobatannins, steroids and phytosteroids, and negative for tannins, saponins, alkaloids and cardiac glycosides. Medical benefits of breadfruit extracts include, cardiovascular health, good for skin, reduce diabetes, dental health, energy booster, bone health, sleep better at night, resistance against infection etc. The wood of breadfruits one of the most valuable timbers in the construction of traditional houses. Breadfruit contains phytochemicals having potential as an insect repellent.

List 1 : Breadfruit (Artocarpus altilis), Nutritive Value per 100 g. (Source: USDA National Nutrient data base) are as follow

Principle	Nutrient Value	Percentage of RDA
Energy	103 Kcal	5 %
Carbohydrates	27.12 g	21 %
Protein	1.07 g	2 %
Total Fat	0.20 g	1 %
Cholesterol	0 mg	0 %
Dietary Fiber	4.9 g	13 %

Vitamins

Comment [A178]: Rewrite the whole of your discussion. It is just an interpretation of your results (findings).
In discussion you will need to compare your findings with other similar study and ascertain if your findings agree or disagree with the findings of the other studies and make references to such studies. Please Google search recent work of your research topic and use it as basis for you discussion while following the journal forma t and instruction guidelines for writing a publishable article

Comment [A179]: Remove

Folates	14 µg	3.5 %
Niacin	0.900 mg	6 %
Pyridoxine	0.100 mg	8 %
Riboflavin	0.030 mg	2 %
Thiamin	0.110 mg	9 %
Vitamin A	0 IU	0 %
Vitamin C	29 mg	48 %
Vitamin E	0.10 mg	1 %
Vitamin K	0.5& mug	<1 %

Electrolytes

Sodium	2 mg	0 %
Potassium	490 mg	10.5 %

Minerals

Calcium	17 mg	2 %
Copper	0.084 mg	9 %
Iron	0.54 mg	7 %
Magnesium	25 mg	6 %
Manganese	0.060 mg	2.5 %
Phosphorus	30 mg	4 %
Selenium	0.6 µg	1 %
Zinc	0.12 mg	1 %

Photo-nutrients

Carotene-B	0 µg	--
Crypto-xanthin-B	0 µg	--
Lutein-zeaxanthin	22 µg	--

Comment [A180]: Remove

Medical benefits include, cardiovascular health, good for skin, reduced diabetes, dental health, energy booster, bone health, sleep better at night, resistance against infections, etc.

The wood of the breadfruit was one of the most valuable timbers in the construction of traditional houses in Samoan architecture. The part of fruit that are discarded can be used to feed livestock. The leaves of the breadfruit trees can also browse by cattle. Breadfruit tree with fruit and leaves is shown in Figure 1.

Comment [A181]: Remove

5. CONCLUDING REMARKS

(i) It is found from present studies that inhibitory zone or antimicrobial potential of leaves extract increases as their amount increases.

(ii) In general among four methods used for antimicrobial studies well diffusion method and streak plate methods were found to have highest and lowest inhibitory zone, respectively.

(iii) In most of the essay studies maximum and minimum antimicrobial potential was observed for methanol and acetone solvent extract, respectively.

(i) The phytochemical flavonoids, terpenoids, phenol, phlobatannins, steroids and phytosteroids are found to present in methanol leaves attract of Artocarpus altilis.

Comment [A182]: Summarize in a single paragraph

(ii) The phytochemicals tannins, saponins, alkaloids and cardiac glycosides are found to be absent in methanol leaves attract of Artocarpus altilis (breadfruit).

(iii) It is observed from the present studies that leaves extract of Artocarpus altilis have antimicrobial property and its potential increases as their amount increases.

(iv) Present research work is very useful for the researchers of similar research interest.

Comment [A183]: Summarize in a single paragraph

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REFERENCES

1. M. Smith, A. Elizabeth," Tracking Austronesian expansion into the pacific via the paper mulberry Plant", *Proceedings of the national Academy of Sciences*, 112 (44) (2015) 13432 – 13433.

2. N.J.C. Zerega, D. Rogone, J. J. Moteley, “The complex origins of breadfruit (Artocarpus altilis), moraceae): Implications for human migration in oceanic”, *American Journal of Botany*, 91(5) (2004) 760 – 766.

3. J. F. Morton, “Breadfruit: In fruits of warm climates”, New Crop, the New Crop Resource Online Program, Center for New Crops and Plant Products, Department of Horticulture and Landscape Architecture, Purdue University, In., 1987, pp. 50 – 58.

4. M. P. Jones, J. A. Klun, C. L. Cantrell, D. Ragone, S. J. Murch, “Isolation and identification of mosquito (Aedes aegypti). Biting Detlerent Fatty Acids from Male Inflorescences of Breadfruit (Artocarpus altilis (Parkinson) Fasberg)” *Journal of Agricultural and Food Chemistry*, 60 (15) (2012) 3868 – 3873.

5. P. Sivagnanasundaram, K. L. C. Karunanayake, “Phytochemical screening and antimicrobial activity of Artocarpus heterophyllus and Artocarpus altilis leaf and stem bark extracts”, *OUSL Journal* , 9 (2015) 1 – 17.

6. C. X. Luzuriaga-Quichimbo, J. Blanca-Salas, C.E. Caron-Martinez, T. Ruiz-Tellez, “Providing added value to local uses of Paparahua (Artocarpus altilis) in amazonian equadore by phytochemical data review”, *Revista Brasileira de Farmacognosia*, 29 (2019) 62 – 68.

Comment [A184]: Use minimum of not less than10 years reference except in rare conditions.

Comment [A185]: *Aedes aegypti*

Comment [A186]: *Artocarpus heterophyllus*

7. H. Riasari, M. Ulfah, D. Prayugo and N. A. Komariah, Antibacterial and antifungal activities of various breadfruit leaves (Artocarpus altilis (Parkinson) Fosberg), *IJPSR*, 2 (13) (2017) 1066 – 1073.

8. C. Pradhau, M. Mohanty, A. Ront, "Phytochemical screening and comparative bioefficacy assessment of Artocarpus altilis leaf extracts for antimicrobial activity". *Frontiers in Life Science*, 6 (3-4) (2012) 71 – 76.

9. I. F. Mbaeyi-Nwaoha, C. P. Onwuka, Comparative evaluation of antimicrobial properties and phytochemical composition of Artocarpus altilis leaves using ethanol, n-hexane and water, *African Journal of Microbiology Research*, 8 (37) (2014) 3409– 3421.

10. D. K. S. Palaps, D. S. Retnonigrum, M. I. Iwo, A. A. Soemardji, Leaf extract of Artocarpus altilis [Park] has potential as anti-inflammatory antioxidant and immunosuppressant. *Rasayan Journal of Chemistry*, 13 (1) (2020) 636 – 646.

11. F. F. Gladys, K. E. Bukola, Larvicidal activity of Artocarpus altilis against culex Quinquefasciatus, *International Journal of Plant Studies*, 2 (1) (2019) 1 – 4.

12. K. Nisa, W. Apriyana, V. T. Rosyida, Antimicrobial activity of Indonesian plant extracts against food borne microorganisms, *Asian J. Agric. and Biol.*, 7 (2) (2019) 300– 306.

13. C. L. Nochera, D, Ragone, "Development of a breadfruit flour pasta product" *Foods*, 8 (110) (2019) 1– 8.

14. H. Rante, A. Alan, M. Irwan, ∝ - Glycosidase inhibitory activity of breadfruit leaf extract (Artocarpus altilis (Parkinson) Fosberg). *The 3rd International Conference on Science, Journal of Physics: Conference Series,* 1341 (2019) 1– 9.

15. V. Nayagam, K, Palamsamy, D. Thiraviadoss, "Cyto-toxicity and oligogynamic effect of bio-synthesized silver nanoparticles from plant residue of Artocarpus altilis and its spectroscopic analysis" *Asian Journal of Nanoscience and Materials*, 2 (2019) 301 – 313.

16. H. Marta, Y. Cohaya, M. R. Arifin, L. Khairani, "Comparing the effects of four different thermal modifications on physicochemical and pasting properties of breadfruit (Artocarpus altilis) starch, *International Food Research Journal*, 26 (1) (2019) 269 – 276.

17. T. Soifoini, D. Donno, V. Jeannoda, E. Rakotoniaina, S. Hamidou, S, M. Achmet, N. R. Solo, K. Afraitane, C. Giacoma, G. L. Beccaro, "Bioactive compounds, nutritional traits and antioxidant properties of Artocarpus altilis (Parkinson) fruits: Exploiting a potential functional food for good security on the Comoros Islands, *Hindawi, Journal of Food Security*, 2018(2018) 1 – 11. (Article ID 569792 B).

18. H. Riasari, S. N. Fitriansyah, O. Putra, " Comparison of anti-inflammatory activity between fermented and dried breadfruit leaves extract (Artocarpus altilis)", *Pharmacology and Clinical pharmacy Research*, 3 (3) (2018) 72 –75.

19. L. Y. Leng, N. B. Nadzri, K. C. Shaari, Antioxidant and total phenolic content of breadfruit (Artocarpus altilis) leaves, *MATEC Web of Conferences*,150 (06007) (2018) 1 – 4.

20. M. I. Vdonkany, M. A. Eluwa, B. K. Enun, P. C. Inyang-Etoh and I. T. Inyang, "Studies of antimalarial activity and liver histopathological changes of Artocarpus altilis on Plasmodium berghei-infected mice" *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*, (2018) 106 – 114.

21. A. A. Ajah, S. A. Onasanwo, N. G. Aitokhuehi, O. S. Faborode, Anxiolytic potentials of Artocarpus altilis (breadfruit) in Swiss mice, *Ann Depress Anxiety*, 4 (2) (2017) 1089 – 1092.

22. H. Juliastuti, A. T. Navianti, B.A. Fatawi, E. R. Yuslianti, "Ethanol-based breadfruit leaf (Artocarpus altilis) extract as hepatoprotective in carbon tetrachloride-induced liver injury", *J. Pharmacol. Toxicol.* , 12 (3) (2017) 136 – 141.

23. H. Peters, F. N. Gloria, C. E. Ikpome, Y. M. Akinkunmi, "Nutritional evaluation of breadfruit and beniseed composite flours", *MOJ Food Processing & Technology,* 2 (6) (2016) 194 – 199.

24. M. Mohanty, C. Pradhan, A review on phytochemistry, bio-efficacy, medicinal importance of Artocarpus altilis, *IJPPR,* 3 (1) (2015) 219 – 231.

25. V. Saraswaty, C. Risdian, R. A. A. Lelone, T. Mozef, "Influence of ethanol concentration and temperature on antioxidant and antibacterial activity from Artocarpus altilis (Parkinson) Fosberg leaves" Oxid Antioxid Med Sci. , 4 (2) (2015) 97 – 109.

26. K. P. Talaro, Foundation in microbiology, Wm. C. Brown Publisher, USA, 1993.

27. C. Perez, M. Pauli , P. Bazerque , An antibiotic assay by the agar well diffusion method , *Acta Biological et Medicine Experiments* , 15(1990) 113-115.

28. P.R. Murray, E.J. Barron, M.A. Pfuller, F.C. Tenover, R.H. Yolke . *Mannual of Clinical Microbiology*, 6th edition. Mosby Year Book, London (1995).

29. J. C. Marujella and P.A Henry, Antimicrobial activity of perfume oil, Journal of American Pharmaceutical Association, 28(1958) 471 .

30. G. Orzechowski , Antibiotica in loheren pflanzen , *Pharmazie in Unser Zeitt* , 1 (1972) 42-53.

31. H.O. Edioga, D. E. Okwu. R. O. Mbalble, Phytochemical constituents of some Nigerian medicinal Plants, *Afr. J. Biotechnol.* , 4 (7) (2005) 685 – 688.

Comment [A187]: Follow the editor's reference style and stick to a uniform style.
You either write the place of publication in full or short form.

Figure 1. Breadfruit tree with leaves and fruits

Comment [A188]: Move to the second page of your introduction where you were describing the plant.