Pharmacogn J. 2021; 13(6)Suppl: 1727-1733
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Antibacterial Activity of Papaya (*Carica papaya*) Leaf and Seed Extracts Against Some Selected Gram-Positive and Gram-Negative Bacteria

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History

- Submission Date: 12-05-2021;
- Review completed: 11-09-2021;
- Accepted Date: 03-11-2021.

DOI : 10.5530/pj.2021.13.223

Article Available online

http://www.phcogj.com/v13/i6

ABSTRACT

Background: Medicinal plants are well-known natural sources of remedies, used in the treatment of innumerable diseases since antiquity. Wide range of medicinal plant extracts are used to treat several infections as they have potential antimicrobial activity. **Objectives:** This study was aimed to investigate antibacterial activity of *Carica papaya* leaf and seed with different solvents against Gram negative and Gram positive bacteria. The leaves and seeds of Carica papaya were dried, powdered and extracted with 80% of Acetone, Ethanol, Methanol and water. Disc diffusion method was used for the antibacterial assay and measuring the zone of inhibition, and the MIC was determined by broth macro dilution method. **Results:** The highest percent yield of crude extract72.5% was obtained from Carica papaya leaf with Methanol, Ethanol and Acetone as solvents while, the lowest yield 24% was obtained from Carica papaya seed with Acetone extract. All Crude extracts from the different plant parts showed antibacterial activity. Accordingly, Carica papaya Seed with Ethanol extract exhibited the highest antibacterial activity 14.3±1.2 (mm) against *P. aeruginosa* which was lower than standard antibiotic disc Ciprofloxacin with inhibition zone of 25.9 ±0.00 (mm), while the lowest inhibition of 3.8±0.5 (mm) was recorded from Carica papaya leaf with Ethanol crude extract against *E. coli*. The MIC of 12.5mg/ml was observed from Ethanol crude extract of Carica papaya seed on *P. aeruginosa*. Crude seed extract of Ethanol showed the highest antibacterial activity. The results of the antibacterial assay of the crude extract of Carica papaya (leaf and seed) with four different solvents showed zone of inhibition against all tested bacteria. However, among the plant parts of Carica papaya included in the current study, the seed of Carica papaya were found to be the best source of antibacterial agents. **Conclusion:** In this study, limited (only leaf and seed parts) of Carica papaya was tested for its antimicrobial activity. So, the same work should be carried out on different parts of Carica papaya plant like; bark, root, flower and fruits on antibacterial activities so, as to have a clear picture of the spectrum of antibacterial activity of the plant.

Key words: Antibacterial activity, Bacterial pathogens, *Carica papaya*, Medicinal, MIC.

INTRODUCTION

Background of the study

Medicinal plants are well-known natural sources of remedies, used in the treatment of innumerable diseases since antiquity. Many plants from various Brazilian biomes, such as the Cerrado (savannah-like), the Atlantic (uplands) and the Amazon (lowlands) rain-forests, have been used as natural medicines by the local population in the treatment of tropical diseases, including leishmaniasis, malaria, *schistosomiasis*, fungal and bacterial infections.[1]

Medicinal plants are abundant source of antimicrobial molecules. Wide range of medicinal plant extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries.[2] Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. Experts turned their concentration back towards obtaining advantages from medicinal plants after observing more side effects of synthetic drugs compared to their benefits.[3]

It is estimated that about 35,000 to 70,000 plant species are used as medicinal plants out of 422127 reported worldwide plant species.[4] In Pakistan 80% of the population belonging to the rural areas depends on the traditional medicines.[5] In Nepal, the juice of *Artemisia indica* is used to treat stomachic, diarrhea, dysentery, abdominal pains and relieving burning sensation in conjunctives.[6]

Currently, studies on herbal medicines appear under different names, such as plant medicines, phytomedicines, and natural products and under pharmacognosy usually referring to products processed from living organisms: plants, animals, insects, microorganisms and marine organisms. A tropine, morphine, quinine, ephedrine, warfarin, salicin, digoxin, vincristine, taxol, and hyosine are some examples of extracts from traditional plants currently used in modern medicines. Findings from ethnobotanical and ethno medicinal studies have shown correlation between medicinal use and laboratory results. Natural sources are usually the starting points for most pharmacological agents.[7]

The Ethiopian flora is estimated to contain between 6,500 and 7,000 species of higher plants of which about 12% are endemic. Ethiopia is also a home for many languages, cultures and beliefs that have in turn contributed to the high diversity of traditional

Cite this article: Dagne E, Dobo B, Bedewi Z. Antibacterial Activity of Papaya (*Carica papaya*) Leaf and Seed Extracts Against Some Selected Gram-Positive and Gram-Negative Bacteria. Pharmacogn J. 2021;13(6)Suppl: 1727-1733.

knowledge and practice of the people, which among others include the use of medicinal plants. More than 95% of traditional preparations in the country are of plant origin.[8]

Besides bacteria, infectious diarrhoea can be caused by viruses and parasites. The prevalence of each pathogen varies with geographical region and population factors. Bacteria, for instance, are responsible for 10–55% of diarrhoeal episodes, with the highest number of episodes occurring in the developing world.[10]

Currently there are different types emerging diseases and other drug resistance diseases. It is very difficult to cure human and animal with the currently known drugs, therefore there is a need of other alternative drugs. Antibacterial effects against certain resistant infectious pathogens are profound and effective if combinations of different plant extracts are used.[11] Various studies have established that herbal medicines can be developed as safe, effective and less costly alternatives to the current medicines to the treat certain bacterial infections.[12]

Therefore, the objective of the present study is to investigate the antimicrobial activity of crude extracts of *Carica papaya* (leaf and seed) against six pathogenic bacterial strains (*B. subtilis, E. coli, K. pneumonia, S. aureus, P. aeruginosa* and *S. boydii)*.

MATERIALS AND METHODS

Description of the study area

Boricha district is found in Sidama Zone, SNNPR in Ethiopia. It is located between 6°46′ and 7°04′ N latitude and 38°04′ and 38°24'E longitude. The district has an estimated area of 588.05 km^2. Boricha district comprises of 42 *kebeles* (villages) of which 39 are rural and three are suburban towns. The district is known to be malarious as altitude falls below 2000m above sea level. Boricha has an estimated total population of 236,341 of which 118,566 are men and 117,775are women. Close to 96.0% of the population is estimated to be rural inhabitants while about 4.0% are urban dwellers. The majority of inhabitants in Boricha District belong to the Sidama ethnic group whose language (Sidamu Afoo) belongs to the Cushitic language family. Land use in the district is dominated by rain-fed agriculture with small holding farms where cropping and dairy farming are commonly practiced. Maize (*Zea mays*), *enset* (*Ensete ventricosum*), coffee (*Coffea arabica*), Khat (*Catha edulis*), potato, sugarcane, and sweet potato are the major products and are used for consumption and as a source of income. Although the vegetation in Boricha District and other surrounding localities has currently dwindled, the area was known to be covered by *Acacia* forest as recently as one generation ago.

There are 47 elementary schools and three high schools in Boricha District, in which a total of 57,098 students (53% males and 47% males) are attending their education. The district possesses six governmental health centers, one nongovernmental clinic, and thirty-nine health posts. In Boricha, the 10 leading diseases are malaria, intestinal parasites, diarrhea, upper respiratory tract diseases, urinary tract infection, rheumatism, skin diseases, and fever of unknown diseases, eye diseases, and anemia. In Boricha district besides modern medicine, traditional healers use different herbal medicines to cure these diseases. However efficacy, dosage and side effects of these herbal medicines are not well known.

Study design

For this laboratory based study, Papaya (*Carica papaya)* was selected. Two parts of Papaya (leaves and seeds) were obtained from Boricha woreda of Sidama zone and extracted using four extraction solvents (ethanol, acetone, methanol and water). Six microorganisms were used for the antimicrobial assays. Antimicrobial assays were carried out for each extract in triplicate and results were compared against the standard drug.

Collection of plant material

Approximately one kg of fresh leaves and seeds of *Carica papaya* were collected from the study area. Leaves of *Carica papaya* was put in polyethylene bag followed by sprinkling with water and piercing the bag at several points in order to allow air circulation. The seed samples of the test plant were placed in separate bags and transported to the laboratory in Hawassa University Main Campus. The leaf and seed samples were thoroughly washed three times by clean water followed by rinsing once with sterile distilled water. They were then placed on clean plastic plates and air dried at room temperature until their weight became constant. Then the dried samples were grounded to fine powder using electric grinder (FM100 model, China) and stored in sterile bottle at 4^{0}c for analysis.

Preparation of plant extracts

The extraction methods involved solvent extraction technology. The extracts of leaf and seed of *Carica papaya* were prepared by dissolving 20 gm of each fine plant powder separately in 200 ml of 80% of acetone, (80%) methanol, (80%) ethanol and water. The extracts were prepared in 250ml capacity conical flask by soaking 20 g of each plant powder separately in 200 ml of each solvent; methanol, ethanol, acetone and water for 72 hours until the soluble material became dissolved. Thereafter, each extract was filtered using *Whatman no.1* filter paper and the filtrate were dried by Rota vapor until the solvent from extracts further evaporated, The resulting extracts were packed into a vial and stored at 20°C until further investigation.

Determination of extraction yield

The yield is the amount of extract obtained from the plant powder. It is expressed as a percentage or without any unit. In practice, it is determined by the ratio of weight of the solids content after evaporation by the weight of the dry powder of the plant material used for the extraction, multiplied by 100.

The percentage yield of each extract was obtained using the formula:

$$\text{Percent Extracts} = \frac{W_2 - W_1}{W_0} \times 100$$

Where, W_2 is the weight of the dried *Carica papaya* leaf and seed extract and the container, W_1 the weight of the container alone and W_0 the weight of the dried *Carica papaya* leaf and seed.

Test microorganisms

The test Microorganisms were Gram-positive bacteria (*S. aureus)* and Gram-negative bacteria (*E. coli*, *K. pneumonia, B. subtilus, P. auruginosa and S. boydii*). All the test microorganisms were reference strains and collected from the Microbiology Laboratory of Ethiopian public health institute (EPHI), Addis Ababa. The test organisms were grown on nutrient agar at 4^{0}C and maintained in Muller Hinton agar medium according to.[13]

Preparation of media

The medium was prepared according to manufacturer's instructions. 38g Mueller Hinton Agar was added to a flask containing 1000 ml of distilled water and gently heated until the medium is completely dissolved. The medium was sterilized by autoclaving at 121°$_C$ for 15 min. After cooling to about 45-50°C, approximately 25ml of the sterilized medium was aseptically poured into 90 mm diameter sterilized Petri-dishes and allowed to dry until excess moisture from the surface of the agar is removed before use. The sterility of the prepared media was checked by incubation of randomly selected plates at 37°C for 24hours.

Inoculum preparation

This method assesses the antimicrobial activity of a bioactive compound by culturing bacteria in the presence of the compound/extract and measuring the zone of inhibition which corresponds to the area where no bacterial growth is observed under optimum conditions for bacterial growth. The higher the diameter (zone of inhibition) the more susceptible bacteria to the bioactive compounds/extracts of plant. The method was executed according to the procedures described by [13]. Accordingly, three to five colonies from pure cultures of each of the six selected bacterial species were transferred with the help of a sterile wire loop into a separately labeled test tube containing 5ml of nutrient broth and incubated to grow at a temperature of 37°$_C$ for two hours. The prepared culture was standardized to 0.5 McFarland turbidity standards using the spectrophotometer (optical density of 1.0 at 625 nm)by adding sterile nutrient broth to obtain the desired cell density of 1.5 X 10^8 (cells/ml).

Preparation of disc

Diffusion discs of 6mm diameter were prepared from absorbent filter paper (*Whatman no*.1) by using a paper Puncher and sterilized at 120°c for 1hour and dried in oven. Then after, sterilized discs were soaked aseptically by applying 30µl of each crude extract of plant at a concentration of 100 mg/ml using sterile digital micropipette and then allowed to dry at a room temperature for 15 minutes then, placed in sterile container and stored at 4°c until further use.

Disc diffusion test

The disc diffusion technique has been widely used to assay plant extract for antimicrobial activity.[14] A sterile cotton swabs were dipped into the adjusted standardized broth inoculums suspension by rotating the swab. The swab then evenly streaked over the entire surface of Muller Hinton agar plate. Streaking was repeated by rotating the plate approximately 60° each time to ensure an even distribution of inoculum. After inoculation, for each test bacterium, a sterilized discs which were soaked under 30µl of each crude extract of plant at a concentration of 100 mg/ml were applied while sterile, blank paper discs were soaked by each solvents ethanol, methanol, acetone and water served as negative control and standard antibiotic ciprofloxacin disc 30µg/disc was used as positive control.

Finally, the disc was applied on the inoculated 90 mm plates using flame sterilized forceps approximately equidistance to each other. Finally, all the plates were incubated at 37°$_C$ for 24 hours. The antibacterial activities of the plant extracts were evaluated by measuring the diameter of the inhibition zone in each of the plates at the end of the incubation period. The diameter of the inhibition zone, including the diameter of the disc was measured by using sliding digital micro caliper. The bacterial activity of the crude extracts on the test bacteria were compared with those of the negative and positive controls according to.[13]

Minimum inhibition concentration (MIC)

The minimum inhibitory concentration is defined as the lowest concentration which is able to inhibit any visible bacterial growth on the culture plates.[15] In this study Broth macro dilution assay were used. For each plant extract a stock solution of 200mg/ml was prepared. A 10ml of Muller Hinton broth was added into each sterilized test tubes. From a stock solution applying a double dilution method to bring 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml and 1.56mg/ml) were performed by using sterile digital micropipette.[13]

Finally, 20µl standard suspension of the test organism (which is adjusted to 0.5 McFarland standards) was added to each tube. Mixed by gently shaking the tubes and incubated the tubes at 37°$_C$ for 24 hours. After 24 hours incubation, the solution was further inoculated in agar plates. MIC was taken as the highest dilution of the extract that inhibited the growth of the bacteria. The lowest concentrations of the extracts, which inhibited the bacterial growth after a period of 24 hours of incubation at 37°$_C$, were recorded as MIC. Broth inoculated with test organism without extract solution was used as a positive control and only broth was used as a negative control. All testes were done in triplicates as of.[13]

Data analysis

For each assay, all the measurements were triplicate and the results were presented as mean ± SE. The statistical analyses were performed by one way ANOVA. Then Duncan`s multiple range test were used to compare means of antibacterial activity as compared to extraction solvents, plant parts and the difference in the sensitivity of the test microorganisms using the statistical package for social Sciences (SPSS) version 22 and P-values ≤ 0.05 were considered as statistically significant.

RESULT AND DISCUSSION

Percent extract yield

In this study the highest amount of extract yield 72.5% were obtained from 80% methanol, ethanol and acetone extracts of *Carica papaya* leaf, while the lowest amount of extract yield 59% was obtained from water extract of *Carica papaya* leaf (Table 1).

The highest amount of extract yield was observed from *Carica papaya* seed from water (49.5%) followed by 80% methanol (41.5%) and 80% ethanol (26.5%) of Carica *papaya* seed. However, this result showed better extract yield than that of reported by [16] on 80% ethanol (19.7%) and water (43.2%) crude extract. The lowest extract yield observed from *Carica papaya* seed (24%) from 80% acetone solvent.

Wed mixture of solvents' varying polarity used in the extraction had a significant effect on the extraction capacity. Selecting appropriate extraction solvent is crucial in the extraction process. It has reported that different solvents have different extraction capacities and spectrum of solubility for phyto-constituents.[16] Thus, the selective extraction of bioactive molecules from natural sources by appropriate solvents is important for obtaining compounds with high biological activities which can be used as preservative ingredients in the pharmaceutical industry [17] which is supportive for the finding of this study.

Antibacterial Assay

The antibacterial assay of the crude extract of all tested plant parts are shown in Table 2. The highest zone of inhibitions were observed among all tested plant parts on the ethanol crude extract of *Carica papaya* leaf (13.2±2.3mm) against *pseudomonas auruginosa* followed by acetone crude extract (10.9±1.04mm) of *Carica papaya* leaf against *Klebseilla pneumonia* and methanol crude extract of *Carica papaya* leaf (10.0±1.15mm) against *Pseudomonas auruginosa*, while the least zone

Table 1: Percent extract yield of *Carica papaya* leaf and seed extracted using different solvents.

Plant Type	Parts Use	Extraction Type	%Yield (w/w)
Carica papaya	Leaf	Methanol	72.5
		Ethanol	72.5
		Acetone	72.5
		Water	59.0
Carica papaya	Seed	Methanol	41.5
		Ethanol	26.5
		Acetone	24.0
		Water	49.5

of inhibition was observed from *Carica papaya* leaf with ethanol crude extract 3.8±0.5mm against *E.coli* shown in Table 2.

The results of the antibacterial assay of the crude extract of *Carica papaya* seed within four different solvents showed zone of inhibition against all tested bacteria. The highest zone of inhibition observed was 14.3±1.2mm on ethanol extract against *Pseudomonas aeruginosa* which was significantly different (P<0.05) from methanol (10.3±0.3mm), acetone (9.1±0.2) and water (5.1±0.3) crude extracts against *Pseudomonas aeruginosa*. The highest zone of inhibition observed was 10.6±2.6mm on methanol crude extract of *Carica papaya* seed *against S.aureus,* which was significantly different (P<0.05) from acetone crude extract (7.4±1.4mm) and Water crude extract (5.8±0.6). The highest zone of inhibition (14.3±1.2 mm) showed by *Psedomonas aeruginosa* with ethanol crude extract of *Carica papaya*, also *S.aurous* showed highest zone of inhibition (10.6±2.6mm) with methanol crude extract. This study demonstrated a comparable result obtained from [17] inhibition zone of the methanol crude extract of *Carica papaya* seed showed (12mm) against *E.coli*, followed by (9mm) against *S.aureus*,(10mm) against *S.typhimurium* and (8.6mm) against *P.aerugenosa*.

According to [18] the ethanol extract of leaf of *Carica papaya* scored highest antibacterial activity (12mm) against *P.aeruginosa* and (9.1mm) against *K.pneumonia* which was in agreement with the result obtained in this study. Ethanol crude extract of *Carica papaya* leaf showed the highest zone of inhibition 13.2±2.3 (mm) against *P.aeruginosa* and 12.6±1.5 (mm) against *K.pneumonia* which was better antibacterial activity than acetone crude extract (10.9mm) against *K.pneumonia* and (10.0mm) against *P.aeruginosa.* The least zone of inhibition regarding *Carica papaya* leaf was observed (3.8mm) with ethanol extract against *E.col.* The ethanol extract of *Carica papaya* seed shows the highest zone of inhibition 14.3±1.2 (mm) against *P.aeruginosa* which exhibited comparable activity to that of the standard antibiotic *Ciprofloxacin* (25.9 ±0.0mm), which was significantly different (P<0.05) with methanol (10.3±0.2 mm), acetone (9.1±0.2mm) and water (5.1±0.3mm) crude extract of *Carica papaya* seed against *S.aureus*. Also ethanol extract of *Carica papaya* seed showed highest zone of inhibition against *B. subtilis* (13.9±1.1mm) which was significantly different (P<0.05) with methanol (7.3±1.1mm), acetone (7.10±0.7mm) and water (5.7±0.4mm) crude extract of *Carica papaya* seed against *B. subtilis*.

In the current study there was a significant difference (p< 0.05) in antibacterial activities among the crude extracts of the two plant parts of *Carica papaya* (leaf and seed) depend on the extraction solvents against all tested bacteria. Comparing the mean inhibition zone of *Carica papaya* leaf, the crude extracts from leaf with ethanol as a solvent showed the highest inhibition zone (9.9mm) against all tested bacteria and significantly different (P<0.05) from the three solvents. The crude extract from the leaf of *Carica papaya* showed mean inhibition zone with acetone extract (8.4 mm) and (7.10 mm) with methanol extract had a better antibacterial activity and also had a significant difference (P<0.05) among them. The lowest antibacterial activity (6.3mm) showed from the crude extract of *Carica papaya* leaf with water as solvent. This indicated that the release of bioactive compounds on plant part depend on extraction solvent against all tested bacteria and comparing among *Carica papaya* leaf with different solvents, the ethanol extract of *Carica papaya* leaf was effective to treat those tested bacteria (Table 3).

Table 2: (Mean ±SE) of antibacterial activities of leaf and Seed of *Carica papaya* crude extracts obtained using different solvents against six bacterial species.

Plant parts		Zone of inhibition					
	Solvents	*B.subtilis*	*E.coli*	*K.pneumonia*	*S.aureus*	*P.aeruginosa*	*S.boydii*
Carica papaya leaf	Ethanol	7.5±0.9^{b}	3.8±0.5^{b}	12.6±1.5^{b}	12.0±0.35^{b}	13.2±2.3^{b}	10.6±2.8^{b}
	Methanol	8.2±1.03^{b}	4.5±0.6^{a}	7.4±1.4^{a}	8.3±1.8^{a}	10.0±1.15^{a}	9.4±0.6^{a}
	Acetone	7.2±0.3^{b}	4.5±0.3^{a}	10.9±1.04^{b}	8.1±1.4^{a}	10.0±0.7^{a}	9.7±0.5^{a}
	Water	5.5±0.2^{c}	7.2±1.02^{c}	5.5±0.4^{a}	6.5±0.5^{c}	6.6±0.0^{c}	6.3±0.6^{c}
Carica papaya seed	Ethanol	13.9±1.1^{a}	13.3±1.9^{c}	13.4±0.9^{b}	12.5±0.1^{c}	14.3±1.2^{b}	12.5±1.03^{b}
	Methanol	7.3±1.1^{a}	10.3±0.6^{b}	8.10±1.6^{a}	10.6±2.6^{b}	10.3±0.2^{a}	8.7±0.3
	Acetone	7.10±0.7^{a}	7.9±1.3^{a}	9.5±0.5^{a}	7.4±1.4^{a}	9.1±0.2^{a}	8.0±0.5^{a}
	Water	5.7±0.4^{c}	6.9±0.3^{a}	5.5±0.2^{c}	5.8±0.6^{a}	5.1±0.3^{c}	7.6±0.5^{a}
+ve control		23.9±0.0	25.3±0.0	21.1±0.0	25.2±0.0	25.9±0.0	24.4±0.0
-ve control		0.0±0.0	0.0±0.0	0.6±0.6	0.0±0.0	0.0±0.0	0.0±0.0

Table 3: Plant parts by extraction solvent interaction effect on antibacterial activity of *Carica papaya* plant.

			95% confidence interval for mean		
Plant parts	**Solvents**	**Mean**	**Standard Deviation**	**Lower bound**	**Upper bound**
Carica papaya leaf	Ethanol	9.9^{b}	4.2	7.8	12.0
	Methanol	7.10^{a}	2.5	6.7	9.2
	Acetone	8.4^{c}	2.5	7.2	9.6
	Water	6.3^{d}	1.0	5.8	6.8
Caricapapaya seed	Ethanol	13.3^{d}	1.8	12.4	14.2
	Methanol	9.4^{b}	2.3	8.2	10.5
	Acetone	8.3^{a}	1.5	7.6	9.1
	Water	6.1^{c}	1.1	5.6	6.4

Mean values under the same category that bear different superscript letters are significantly different (p<0.05)

Table 4: Plant parts of *Carica papaya* leaf and seed interaction with bacterial spp.

Plant parts	Mean	95% Confidence Interval for Mean		
		Standard Error	Lower bound	Upper bound
Carica papaya leaf	13.2^{a}	2.3	3.4	22.9
Carica papaya seed	14.3^{b}	1.2	9.7	19.4

Table 5: Minimum inhibitory concentrations (mg/ml) of *Carica papaya* leaf and seed with four different extraction solvents.

Plant parts	Extraction solvent	Bacterial species					
		B.subtilis	*E.coli*	*K.pneumonia*	*S.aureus*	*P.aeruginosa*	*S.boydii*
Carica papaya leaf	Ethanol	50	50	12.5	12.5	12.5	50
	Methanol	12.5	12.5	25	50	50	25
	Acetone	50	50	50	50	50	50
	Water	100	100	100	100	100	100
Carica papaya seed	Ethanol	25	25	25	25	12.5	100
	Methanol	50	50	100	100	100	100
	Acetone	50	100	100	100	50	25
	Water	100	100	100	100	100	100

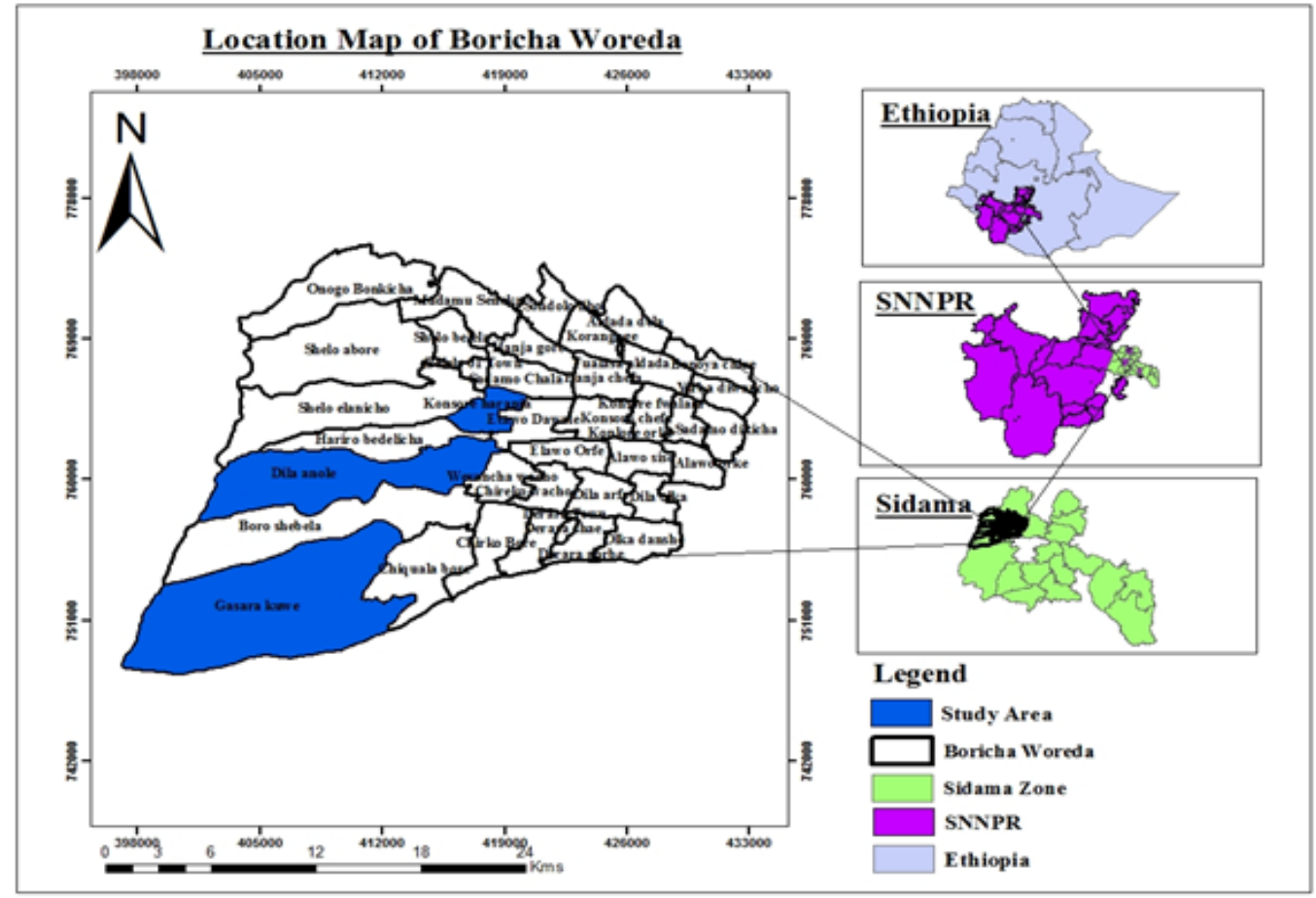

Figure 1: Map of Boricha woreda.

In the case of the seed solvent extract of *Carica papaya* the ethanol extract had the highest (13.3mm) antibacterial activity against all tested bacteria and significantly different ($p<0.05$) from the methanol (9.4mm), acetone (8.3mm) and water (6.1mm) crude extract of *Carica papaya* seed. There was no significance difference ($p>0.05$) among methanol (9.4mm) and acetone (8.3mm) crude extract of *Carica papaya* seed but significantly different ($p<0.05$) from water (6.1mm) and ethanol (13.3mm) crude extract in their zone of inhibition against all tested bacteria. The least zone of inhibition (6.1mm) was observed from water crude extract of *Carica papaya* seed against all tested bacteria. Comparing among *Carica papaya* seed with different solvents, the ethanol extract of *Carica papaya* seed was effective to treat those tested bacteria (Table 3).

Among all tested plant parts (*Carica papaya* leaf and *Carica papaya* seed), *Carica papaya* seed had a better antibacterial activity (14.3mm) and significantly different ($p<0.05$) from *Carica papaya* leaf (13.2mm) against all tested bacteria. This result indicated that *Carica papaya* seed had high antibacterial activity than the *Carica papaya* leaf against all tested bacteria (Table 4).

Minimum inhibitory concentration (MIC)

The MIC assay was also employed to evaluate the effectiveness of the extracts to inhibit the growth of the tested bacteria. The ethanol crude extract of *Carica papaya* leaf has inhibited *B.subtilis, E.coli* and *S.boydii* at a concentration of 50mg/ml and at 12.5mg/ml has been inhibited *K.pneumonia, S.aureus* and *P.aeruginosa*. Methanol extract inhibited *B.subtilis and E.coli* at a concentration of 12.5mg/ml; *K. pneumonia and S. boydii* at a concentration of 25mg/ml and *S.aureus* and *P.aeruginosa* at a concentration of 50mg/ml inhibited. Among acetone crude extracts of *Carica papaya* leaf the MIC was 50mg/ml which inhibited all tested

bacterial species. The water crude extracts of all tested plant parts were at 100mg/ml which inhibited all tested bacterial species. The MIC value of methanol extract of *Carica papaya* leaf against *E. coli* was 12.5mg/ml which was agreed with the result of.[19]

Among ethanol crude extract of *Carica papaya* seed the MIC was 12.5mg/ml for *P. aeruginosa,* 25mg/ml for *B. subtilis, E. coli, K .pneumonia* and *S. aureus.* At the MIC was 100mg/ml for *S. boydii.* In the case of methanol crude extracts of *Carica papaya* seed 50mg/ml for *B. subtilis* and *E.coli;* 100mg/ml for *K. pneumonia, P. aeruginosa, S. aureus* and *S. boydii*. The acetone crude extract MIC of *Carica papaya* seed was 50 mg/ml that inhibited *B. subtilis* and *P. aeruginosa.* Also 25 mg/ml inhibited *S. boydii and* 100 mg/ml inhibited *E. coli, K. pneumonia* and *S. aureus*. The water crude extracts of all tested plant parts were at 100mg/ml which inhibited all tested bacterial species.

The tested plant parts which were extracted with different solvents showed different MIC. The methanol crude extract of *Carica papaya* leaf had a potential to inhibit *B. subtilis* and *E. coli* at 12.5mg/ml. So, it had a potential to treat *B. subtilis and E. coli*. Also the ethanol crude extract of *Carica papaya* seed had a potential to inhibit *P. aeruginosa* at 12.5mg/ml. So, it had a potential for treating of *P. aeruginosa*.

CONCLUSION

The results of the antibacterial assay of the crude extract of *Carica papaya (leaf and* seed) with four different solvents showed zone of inhibition against all tested bacteria. However, among the plant parts of *Carica papaya* included in the current study, the seed of *Carica papaya* were found to be the best source of antibacterial agents. Likewise, among the extraction solvents employed for the current study, 80% ethanol, methanol and acetone were found to be the best extraction solvents. The MIC assay was also employed to evaluate the effectiveness of the extracts to inhibit the growth of the tested bacteria. Among ethanol crude extract of *Carica papaya* seed at 12.5mg/ml inhibited *P.aeruginosa.* At 25mg/ml inhibited *B.subtilis, E.coli, K.pneumonia* and *S.aureus* and at 100mg/ml inhibited *S. boydii. Carica papaya* seed with methanol crude extract exhibited high antibacterial activity against *S. aureus* at 100mg/ml this extract showed comparable antimicrobial activity with positive control *Ciprofloxacin* disc 30µg against *S. aureus* while, no growth inhibition was observed in the case of all the negative controls. This shows the potential of the plant for developing drugs for treating various illnesses in human beings. The susceptibility of the six bacteria species appeared to be influenced by the plant part and the extraction solvent used. MIC ranged from 3.125mg/ml to 200mg/ml with absolute water. This result confirmed that extract of medicinal plant showed a comparable result to standard antibiotics.

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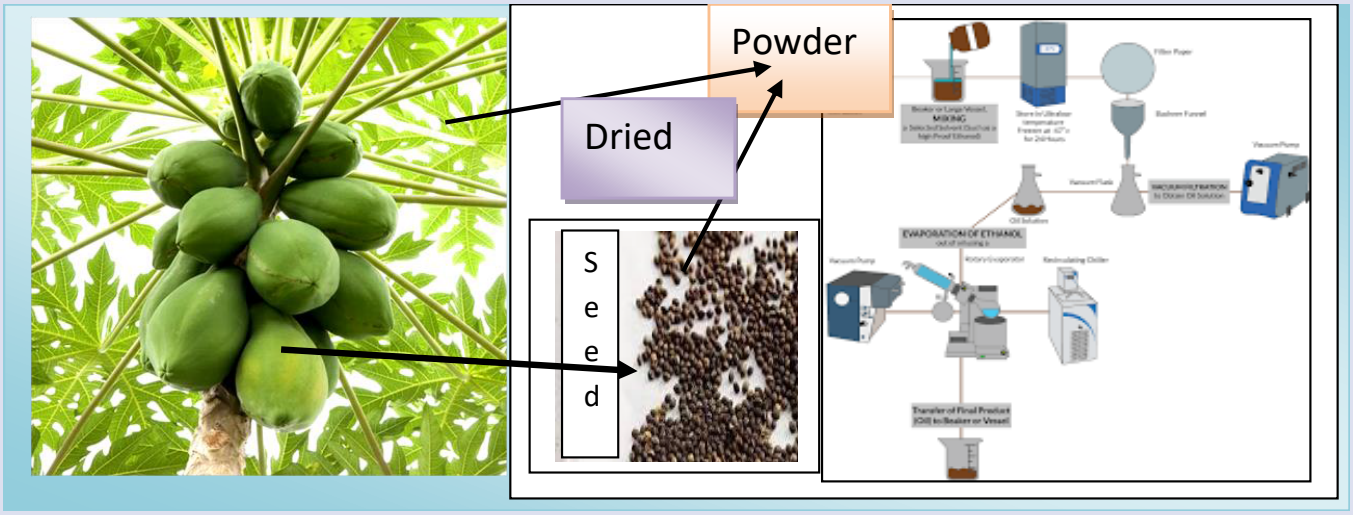
GRAPHICAL ABSTRACT



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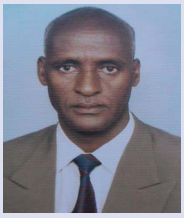

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Cite this article: Dagne E, Dobo B, Bedewi Z. Antibacterial Activity of Papaya (*Carica papaya*) Leaf and Seed Extracts Against Some Selected Gram-Positive and Gram-Negative Bacteria. Pharmacogn J. 2021;13(6)Suppl: 1727-1733.