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In Vitro Antibacterial Activities of Methanol and Aqueous Extracts of Leaves of *Carica papaya* and *Moringa oleifera* Against Selected Human Pathogenic Bacteria

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Abstract: A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes. In developing countries all over the world, large numbers of people die daily of preventable or curable diseases because of the lack of even simple health care. So the study was interested entitled "In vitro antibacterial activities of methanol and aqueous extracts of leaves of Carica papaya and Moringa oleifera against selected human pathogenic bacteria". Moringa oleifera and Carica papaya is medicinal plants which have medicinal values for the treatment of various infectious illness were interested for investigation of their antibacterial activities against E. coli and H. pylore. Two solvent types (methanol and distilled water) were used for crude extraction. The vulnerability of the pathogen to the antibacterial substances was determined using the disc diffusion method. Minimum Inhibitory Concentration was determined by the broth dilution method. The results of the antibacterial activities revealed that both methanol and aqueous leaf extracts had inhibitory activities against the selected gram-positive and gram-negative test pathogens. Methanol extract of Carica papaya had the highest antibacterial activity (13.3 mm) against H. pylore, while Moringa oleifera indica exhibited the least zone of inhibition (8.2 mm) at a concentration of 150 mg/mL. The Antibacterial activities of heat treated crude extracts against the test pathogens were also determined at varying temperature (45-55°C) for a period of 30 and 60 minutes. The results revealed that at higher temperature and exposure time, there was a decrease in the zone of inhibitions. The Minimum Inhibitory Concentrations of the methanol extracts ranged from 1.25 mg/ml - 5 mg/mL; whereas, for aqueous extracts ranged from 2.5 mg/mL -10 mg/mL. In general, this study provides base line information for further work on the search for specific active compounds from the selected plant leaf extracts against human pathogenic bacteria.

Keywords: Antibacterial Activity, *Carica papaya*, *E. coli*, Disc Diffusion, Heat Treatment, *H. pylore*, *Moringa oleifera*

1. Introduction

Plants have been used for the treatment of different diseases all over the world before the advent of modern clinical drugs. They are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs [14]. Higher plants and various preparations from them are used to treat different human and animal infections from long period of time. Thus, the discovery of medicinal plants as source of antimicrobial agents is useful in expanding the wide variety of antibiotics available [20]. The majority of these herbal plants contain substances, which are precursors for the synthesis of conventional drugs, or substances that can be used for therapeutic purposes. According to WHO (2008), about 80% of individuals from developing countries meet their primary health care needs through the use of traditional medicine that includes one herb or another as the main therapeutic agent. Herbal medicines are basis of therapeutic use in the developing countries, but of recently, herbal medicines used has been an increase in the developed world too [5]. There are new diseases that we did not meet in the past. Against these, new medicines (active ingredients) will be needed and it may obtained from bioactive ingredients compounds from

plant sources [19].

Plants could be utilized as sources of active ingredients against new diseases and used as sources new and improved drugs [13]. Plants have been used traditionally for centuries and modern scientific studies have shown the existence of good correlation between the traditional or folkloric application and modern drugs against a variety of infectious diseases. Thus, the existence of such correlation strengthens the search for pharmacological active components from plants [6].

The discovery of antibiotics in the mid-twentieth century revolutionized the management and treatment of infectious diseases caused by bacteria. Infections that would normally have been fatal were made curable. Since then, antimicrobial agents (antibiotics and related medicinal drugs acting on bacteria, viruses, fungi and parasites) have saved the lives and eased the sufferings of millions of people. However, antibiotic resistance has increased substantially in the recent years and is posing an ever-increasing therapeutic problem. The growing prevalence of antibiotic resistance amongst clinically important pathogens necessitates the search for potential healing powers in herbal plants, as a way of avoiding the menace. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants [2]. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. Medical uses of plants range from the administration of roots, barks, stems, leaves and seeds to the use of extracts and decoction from the plants [3].

Carica papaya is an important ethno-medicinal plant belonging to the family of *Caricaceae*, and several species of *Caricaceae* have been used as remedy against a variety of diseases [1]. *Carica papaya* is a neutraceutical plant having a wide range of pharmacological activities. Papaya is a powerhouse of nutrients and is available throughout the year. Papayas are edible and have a sharp, spicy taste. *Moringa oleifera* are sometimes ground and used as a substitute for black pepper. It is important ethno-medicinal plant belonging to the family of *Moringaceae and it* has been used extensively in traditional medicine for the treatment of several ailments, promotes digestion, skin diseases, diarrhea, as stimulant in paralytic afflictions, epilepsy and hysteria [8]. So that; this research is aimed to *in vitro* evaluating of the antibacterial activity of the aqueous and methanol extracts of *Carica papaya* and *Moringa oleifera* leaves on two human pathogenic bacteria i.e *E. coli* and *H. pylore* bacteria isolates to provide a guide or direction on the concentration of the leave extract active against these organisms to the populace who use them to treat various diseases caused by the bacteria isolates.

The general objective of this study was, therefore, to determine the antibacterial activities of methanol and aqueous leaf extracts of *Carica papaya* and *Moringa oleifera* against *E. coli* and *H. pylore* bacteria.

The specific objectives were:

a) To determine antibacterial activities of crude methanol extracts of *Carica papaya* and *Moringa oleifera* against *E. coli* and *H. pylore* bacteria.
b) To determine antibacterial activities of crude aqueous extracts of of *Carica papaya* and *Moringa oleifera* against *E. coli* and *H. pylore* bacteria.

2. Materials and Methods

2.1. Description of the Study

The experiment was conducted in the laboratory of the College of natural and computational Sciences and Biotechnology departments Wachemo University, Ethiopia.

2.2. Experimental Design

Completely Randomized Design with three replications was used. Three sets of experiments were carried out. In the first experimental setup, sensitivity of *E. coli* and *H. pylore* to untreated crude extracts of *Carica papaya* and *Moringa oleifera* were investigated. In the second experimental setup, the sensitivity of the same test pathogens to heat treated (at temperatures 45, 50 and 55°C for 30 and 60 min) crude extracts of *Carica papaya* and *Moringa oleifera* were investigated. In the third experimental setup, the MIC of untreated plant material was investigated.

2.3. Collection of Plant Materials

Fresh branches of *Moringa oleifera* was collected from Dire Dawa town East Hararge Oromia region and *Carica papaya* was collected from durame framers fram Kambata Zone SNNPS region in clean bags. The plants were identified and authenticated properly at the Herbarium of Wachemo University. The plants were thoroughly washed in tap water and then the healthy leaves were separated. The leaves were again rinsed in water and shade-dried over a period of 2-3 weeks at room temperature. Then from the dried leaves, the fine powder was carefully prepared using electrical grinder in the laboratory (Wachemo university biotechnology department Laboratory) and stored in airtight plastic bottles. The bottles were labeled and stored in the refrigerator at 4°C for further use.

2.4. Preparation of Extracts

Plant extracts were prepared in accordance with the methods described in Basri and Fan (2005) with minor modifications. About 50 g of the powder was separately soaked in 200 ml of methanol and distilled water, in a 500 ml stoppered reagent bottle and the mixture was shaken for 72 hrs using an electrical shaker. The resulting mixture was first filtered with cheesecloth, then with Whatman No 1 filter paper. The filtrates were then separately concentrated in vacuum using Rotary Evaporator at 37°C - 40°C. The methanol extracts were transferred carefully to labeled vials and allowed to permit evaporation of residual solvents at room temperature for 3-4 days while the distilled water

extracts were dried using Lyophilizer to prepare crude powder. Then the dried extracts were stored in sterile bottles and kept in refrigerator until further use.

Heat treatment of plant crude extracts was done using the method described by [14, 16, 17]. Dried methanol and distilled water extracts of plant materials were kept under 45, 50, and 55°C for both 30 and 60 min. Then the samples were cooled at room temperature and stored in a refrigerator at 4°C until further use. Afterwards, all the three plant extracts were tested against *E. coli* and *H. pylore* pathogenic bacterial strains using the disc diffusion method [4, 10].

2.5. Test Pathogens and Preparation of Inoculum

The test bacterial pathogens, i.e. *E. coli* and *H. pylore*, were obtained from Ethiopian Public Health Institution (EPHI), Addis Ababa, Ethiopia. These bacteria were maintained on nutrient agar and stored in the refrigerator at 4°C [9]. Fresh bacterial cultures were prepared by sub-culturing stock bacterial cultures into freshly prepared nutrient agar and incubating at 37°C for 24 hours. The colonies formed were picked up with a sterile inoculating loop and transferred into a test tube containing sterile normal saline and vortexed thoroughly. This was repeated until the turbidity of each bacterial suspension matched the turbidity of the 0.5 McFarland Standards as described by the National Laboratory Standard Institute [11]. The resulting suspension was then used as inoculum for the test pathogen used in the antibacterial susceptibility test.

2.6. Preparation of Culture Media

Mueller Hinton agar was used for direct sensitivity testing. The media was prepared and treated according to manufacturer's guidelines. Thirty eight (38 g MHA) or twenty eight grams (28 g NA) of medium was mixed with one liter of distilled water in volumetric flask and autoclaved at 121°C for 15 minutes and allowed to cool to about 45°C before dispensing into petri dishes. The medium was later dispensed into 90 mm sterile petri dishes and left to set. The petri dishes were incubated for 24 hours at 37°C to confirm their sterility. When no growth occurred after 24 hours, the plates were considered sterile.

2.7. Preparation of Stock Solution and Serial Dilution

Different concentrations of plant extracts were prepared using methanol and water as solvent. Stock solutions of 200 mg/ml were prepared by reconstituting 1 g of each of the dried crude powder in 5 ml of respective solvent. From this stock solution, 150 mg/mL, 125 mg/mL and 100 mg/mL working solutions were prepared; and from the stock solution of the heat treated crude samples in hot air oven, working solutions of 150 mg/mL concentration was prepared and used for the antibacterial testing using the disc diffusion method. Another stock solution of crude extract 20 mg/mL was prepared by reconstituting 0.1 g of each of the dried crude powder in 5 ml of methanol and aqueous solution. Five sterile test tubes were arranged on a test tube rack and 1 m of sterile methanol and aqueous solution was dispensed into them. From the stock solution, 1 ml of extract was transferred into the first test tube and then successive two fold serial dilutions of the extracts were carried out. The resultant concentrations in the test tubes were 10, 5, 2.5, 1.25, 0.625 mg/ml [7, 15]. They were used, along with the stock, for determination of minimum inhibitory concentration.

2.8. Determination of the Antimicrobial Activities of the Crude Extracts

The antimicrobial activities of the plant extracts were determined using the disc diffusion method. Muller Hinton agar was sterilized using an autoclave at 121°C for 15 minutes. It was then poured on to 90 mm petri dishes and allowed to solidify. Standardized inoculums of each test organism were spread on the petri dishes to achieve a confluent growth.

2.8.1. Preparation of Sterile Paper Discs

Whatman No. 1 filter paper was punched into 6 mm diameter discs with the aid of paper punch and the resulting discs were sterilized. Each sterile disc was impregnated individually with 3 mg of 150 mg/ml, 125 mg/ml and 100 mg/ml concentration using a micropipette and they were allowed to dry in air.

2.8.2. Determination of Antimicrobial Activity Using the Disc Diffusion Method

The sterilized Muller Hinton Agar was poured onto sterile petri dishes. Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. Then, the dried surface of Mueller Hinton agar Petri dish was inoculated by spreading the swab over the entire sterile agar surface from the center to the rim of the Petri dish. This procedure was repeated by spreading two more times to ensure an even distribution of the inoculum. In order to expel the excess moisture from the inoculated Petri dish the lid was left ajar for less than 15 minutes. Then discs impregnated with concentrations of untreated and heat treated plant extracts were placed on the spread plated Muller Hinton agar surface using sterile forceps. Each disc was pressed down to ensure complete contact with the agar surface. Disc impregnated with Amoxicillin (5 µg/ml) was used as positive control while a disc soaked with pure solvent (distilled water/methanol) was used as a negative control. The Petri dishes were then incubated at 37°C for 24 hrs. After incubation, the diameters of the zone of inhibitions around each disc were measured by using transparent ruler and the mean values of three readings were recorded.

2.9. Determination of MIC

MIC was evaluated for methanol and aqueous extracts of untreated plant material. The minimum inhibitory concentration (MIC) of each extract was determined by using different concentrations (10, 5, 2.5, 1.25 and 0.625 mg/ml) of the plants extracts (*Carica papaya* and *Moringa oleifera)* using the broth dilution method described in the National

Committee for Clinical Laboratory Standards [11].

Four milliliter (4 ml) of the Nutrient broth was pipetted into each of six test tubes. 0.1 ml of the prepared successive two fold serial dilutions of the crude extract concentrations of each plant species was mixed with the nutrient broth. Thereafter, of 0.1 ml of the standardized inoculum of the test each pathogen was dispensed into each of the test tubes containing the suspension of Nutrient broth and the crude extract. Then, all test tubes were properly corked and incubated at 37°C for 24 hrs. The tube with lowest concentration of the extract showing no growth after incubation was taken as the MIC [11].

2.9.1. Percentage Residual Antibacterial Activity

The percentage residual antibacterial activity (%RAA) was calculated using the following formula:

$$\%RAA = \frac{ZIBHT - ZIAHT}{ZIBHT} \quad (1)$$

Where; % RAA = Percentage residual antibacterial activity
ZIAHT = Mean zone of inhibition after heat treatment and
ZIBHT = Mean Zone of inhibition before heat treatment

2.9.2. Percentage Increase in Antibacterial Activities

The percentage increase in the antibacterial activity of the crude extracts as a result of using high yielding solvent (i.e. methanol) was calculated using the following formula:

$$\%\ IAA = \frac{ZIME - ZIAE}{ZIME} \quad (2)$$

Where, % IAA = Percentage increase in antibacterial activity
ZIME = Mean Zone of inhibition caused by methanol extract and
ZIAE = Mean Zone of inhibition caused by the aqueous extract.

2.10. Data Analysis

Data was entered into Microsoft Excel spread sheet and analyzed using SAS software. The diameters of the zone of inhibitions on all test pathogens were expressed as mean ± standard deviation (SD). The mean diameters of inhibition zones between different antimicrobials were compared using analysis of variance (ANOVA). The statistical significance was fixed at $p \leq 0.05$.

3. Results and Discussion

3.1. Antibacterial Activities of Untreated Crude Extracts

3.1.1. Antibacterial Activity of Methanol Crude Extracts

The antimicrobial activities of the methanol extracts are shown in Table 1. The results generally revealed that all methanol extracts of the plant has various effective in prohibiting the growth of test pathogens. *Carica papaya* was the most effective extract retarding microbial growth of *H. pylore* and *E. coli* at all concentrations while extract *M. oleifera* had low inhibitory activity against them when compared with *Carica papaya* at all concentration. Methanol leaf extract of *Carica papaya* showed the highest (17.3) antibacterial activity against *H. pylore*. This agree what before the study of was showed [12, 21] by research investigated on antibacterial activity of the methanol extract of *Carica papaya* using the well diffusion technique and reported that the most significant activity of this plant's extract was seen against *H. pylore*. According to present study, preparing an extract with methanol was shown to provide a better antibacterial activity as in the results obtained by Nair *et al*. (2005). The results of the present study also agree with previous studies, which showed that methanol was a better solvent for more consistent extraction of antimicrobial substances from medicinal plants as compared to other solvents such as water and ethanol [1, 22]. Similarly, *M. oleifera* extract showed variable antimicrobial activities at all concentrations against all test pathogens, even though the inhibitory activity was not as high as that of *Carica papaya* extract. There were significant differences among the inhibitory activities of the methanol extracts of the plants against the test pathogens at both 150 mg/ml and 125 mg/ml ($p < 0.05$) against *E. coli* and *H. pylore*. The lowest inhibition zone at a concentration of 150 mg/ml was 9.65 mm and this was shown by *M. oleifera* leaves extract against *H. pylore*. All plant materials also showed inhibition zone at a concentration of 125 mg/mL, but good zone of inhibition was resulted by *Carica papaya* against *E. coli* and *H. pylore* (12.5 mm and 17.3 mm) respectively.

Generally, zone of inhibition was very low at a concentration of 100 mg/ml. However, the inactivity of a plant extract does not indicate the absence of bioactive constituents, nor that the plant is inactive. According to Taylor *et al.* (2001), active compounds may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed. This implies that the activity was dose dependent. As shown in Figure Table 1, the results also show that *Carica papaya* extracts were the most effective extracts, which showed a strong antibacterial activity against *E. coli* and *H. pylore* at all concentrations used.

Table 1. *The antibacterial activities of methanol leaf extracts on pathogenic bacteria.*

Antibacterial agent	Conc.	Zone of Inhibition (ZI) in mm	
		H. pylore	*E. coli*
Carica papaya	150 mg/mL	$17.3 \pm .10^{Aa}$	12.5 ± 1.0^{Ab}
M. oliefera		$9.6 \pm .67^{Ba}$	$11.67 \pm .58^{Aa}$
Carica papaya	125 mg/mL	$13.53 \pm .265^{Aa}$	$8.02 \pm .58^{Ab}$
M. oliefera		$7.9 \pm .252^{Ba}$	8.42 ± 1.0^{Ab}
Carica papaya	100 mg/mL	$7.3 \pm .58^{Aa}$	$8.0 \pm .00^{Ab}$
M. oliefera		$6.2 \pm .551^{Ba}$	$6.97 \pm .503^{Ab}$
MeOH (N. C)	3 mg /disc	0	0
Amoxicillin (P. C)	5 µg/disc	30.0 ± 3.61^{a}	29.3 ± 3.5^{a}

Capital letter superscripts compare between means in column, while Small letter superscript compares between means in row. Means with different letters show significant difference at $P<0.05$.

3.1.2. Antibacterial Activity of Aqueous Crude Extracts

The aqueous crude extracts of the plants also showed antibacterial activity on test pathogens Table 2. However, the activities were not as much as those of the methanol extracts were. This study revealed that the aqueous extracts exhibited significantly lower antibacterial activities against *E. coli* and *H. pylore*. Generally the aqueous extracts of plant extracts did not show antibacterial activity against tested bacterial species at all tested concentration. This is because water is polar and only miscible in itself; thus, it cannot dissolve non-polar compounds, which may have activity against test pathogens. Many researchers have used aqueous solvent as a solvent of extraction and most of their results indicated that water extracts had little or no inhibitory activity [15, 18]. According to [16, 22], the effect of an antimicrobial agent varies with target species.

Aqueous extract of *Carica papaya* showed the highest antibacterial activity against *H. pylore bacteraia*. The diameters of zone of inhibition caused by aqueous extracts of *Carica papaya* at 150 mg/ml concentration were 9.6 mm and 8.2 mm for *H. pylore* and *E. coli*, respectively; and these values were higher than the values obtained for other aqueous plant extracts at the same concentration (i.e. 150 mg/ml) of *Moringa oleifera* that resulted in (7.8 mm and 8.5 mm for *H. pylore* and *E. coli*) zone of inhibition respectively, and aqueous extracts of *Carica papaya* resulted in highest zone of inhibition (9.6 mm) against *H. pylore.* Relatively no activities were observed for aqueous extracts against the test pathogenic bacteria except *Carica papaya show very low inhibition zone (2.1 mm)* against *H. pylore* bacteria at a concentration of 100 mg/ml.

Table 2. *The antibacterial activities of aqueous leaf extracts on pathogenic bacteria.*

Antibacterial agent	Conc.	Zone of Inhibition (ZI) in mm	
		H. pylore	***E. coli***
Carica papaya	150 mg/mL	$9.6\pm.289^{Aa}$	$8.2\pm.58^{Ab}$
M. oliefera		$7.8\pm.153^{Ba}$	8.5 ± 1.155^{Aa}
Carica papaya	125 mg/mL	7.0 ± 1.0^{Aa}	4.5 ± 1.155^{Aa}
M. oliefera		$4.3\pm.643^{Ba}$	5.3 ± 0.577^{Aa}
Carica papaya	100 mg/mL	$2.10\pm.954^{Aa}$	0
M. oliefera		0	0
DW (N. C)	3 mg /disc	0	0
Amoxicillin (P. C)	5 µg/disc	30.0 ± 3.61^{a}	29.3 ± 3.5^{a}

Capital letter superscripts compare between means in column, while Small letter superscript compares between means in row. Means with different letters show significant difference at $P<0.05$.

Table 3. *The MIC of leaf extracts against four pathogenic bacteria.*

Source of crude Extract	Solvent Used	MIC of the methanol and aqueous leaf extracts (mg/ml) against selected bacterial pathogens	
		H. pylore	***E. coli***
Carica papaya	MeOH	1.25	2.5
	Aqs.	5.0	5.0
M. oleifera	MeOH	5.0	5.0
	Aqs.	NCI	10

MIC= minimum inhibitory concentration, MeOH= Methanol, Aqs= Aqueous. NCI= None of the concentrations was inhibitory.

3.2. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) against *E. coli* and *H. pylore* ranged from 0.625 to 10 mg/ml for both aqueous and methanol extracts. The Minimum inhibitory concentration (MIC) can be determined by culturing test pathogen microorganisms in lowest concentration of plant extract. In the present study, the efficacy of the different methanol and aqueous extracts as a potent antimicrobial agent was tested by determining their MIC. It is the best parameter to examine the potential of a given extract in the field of antimicrobial activity and the results is reveled Table 3. Minimum inhibitory concentration of methanol extract is from 1.25 mg/ml -5 mg/ml and Minimum inhibitory concentration aqueous extracts values ranged from 2.5 mg/ml -10 mg/ml. Thus, results show that from aqueous and methanol extraction; the methanol extracts are generally active at lower concentrations against the selected pathogens. According to Wendakoon *et al*. (2012), the broth dilution method requires preparing various dilutions of the compound under test in a suitable solvent and, as a result, the extracts had to be dried and re-dissolved. However, since water frequently does not adequately dissolve the intermediate polar or non-polar components of the dried extract, using water miscible solvents such as methanol or other organic solvents would be an alternative in serial dilution assay to increase the solubility as well as the effectiveness of the crude extract.

The methanol extract of *Carica papaya* had the MIC at 1.25 mg/ml against *H. pylore* and 2.5 mm MIC against *E. coli* While, the methanol extract of *M. oleifera* had a MIC of 5 mg/ml against both *E. coli* and *H. pylore*. In addition, the MIC of the aqueous extract of *Carica papaya* against both *E. coli* and *H. pylore* were 5 mg/ml and *M. oleifera* was much higher 10 mg/ml MIC in aqueous extract against *E. coli* bacteria and none of concentration was inhibitory against *H. pylore* bactera. The higher minimum inhibitory concentration reveled that, the lower potential of preventing growth of test pathogenic bacteria's; whereas, the lower the values of MIC the greater the potentials of the extract in the control of the growth of the pathogens.

4. Summary, Conclusion and Recommendations

4.1. Summary and Conclusions

In today world different disease are imarged day by day

and they may caused by risistante species of different pathogenic microbes. This buring issues make toword looking for antimicrobial compuonds from different plants species. As the attempt for the production of herbal medicines is in progress around the world, the current study will provide baseline information in the development of plant extracts with effective solvent system and antibacterial activities against several pathogenic bacteria. This study was conducted with the aim of determining the antibacterial activities of the methanol and aqueous extracts of leaves of two plants commonly used in traditional medicine (i.e. *Carica papaya* and *Moringa oleifera*) against *E. coli* and *H. pylore*. To attain these objective fresh plant leaves were collected; healthy leaves were separated, and/then washed, dried and ground. Then from dried leaves the fine powder were prepared using Electrical grinder and the resulting dry powder was extracted by maceration using methanol and distilled water as solvent. The crude powder of methanol extracts by evaporation of solvents at room temperature while distilled water extracts were concentrated using Lyophilizer and In *vitro* Anti selected bacterial pathogen evaluated.

In addition, the MIC of the crude extracts against each pathogen was determined. Amoxicillin and distilled water or methanol was used as positive and negative controls, respectively. The results indicated that both of the plants tested in this study (*Carica papaya* and *Moringa oleifera*) inhibited the growth of the test pathogenic bacteria. The methanol leaf extracts exhibited greater antimicrobial activities than the aqueous leaf extracts. The methanol leaf extracts proved to possess antimicrobial activity against all tested pathogenic bacteria at all concentrations of crude extracts used; whereas, the aqueous extract exhibited a moderate inhibitory activity against the test pathogens at all concentrations. The percentage increase in antibacterial activity was calculated and the results consistently showed that the methanol extracts showed from 5-38% increase in activity over those of the aqueous extracts. Therefore, these experimental results provide additional information regarding the effective solvent that must be used for the extraction of antibacterial agents from the selected plants.

The crude powders of the respective plant leaves were exposed to three different temperatures (45°C, 50°C and 55°C) for two time intervals (30 and 60 minutes) using a hot air oven. Stocks of treated samples (200 mg/ml concentration) were prepared in methanol and aqueous. In the study, the effect of temperature on the activity of the plant extracts was investigated. The results indicated that heat treatment resulted in a decrease in the activity of the crude extract and that the reduction in activity increased with increase in temperature and time of exposure. Of the two extracts, methanol extracts showed higher residual antibacterial activity than aqueous extracts. Generally, a residual antibacterial activity ranging from 6.7-38% was observed for both the aqueous and the methanol extracts after heat treatment.

The Minimum inhibitory concentration (MIC) of the leaf extracts of *Carica papaya* and *Moringa oleifera* against tested pathogens was determined using the broth dilution method. The MIC of the methanol extract of *M. oleifera* against *E. coli* was 1.25 mg/ml and that of *L.* against *H. pylore* was 1.25 mg/ml. The aqueous extract of *Carica papaya* leaves against *E. coli* and *H. pylore* was 5 mg/ml.

In conclusion, this study analyzed the activity of the two plant leaf extracts and found out that both extracts had degrees of antibacterial activities against the two pathogenic bacteria depending on their concentration, the nature of the plant and the type of solvent used for extraction. Methanol is better than water for extraction of potent antimicrobial substances from the selected plants. Heat treatment of the crude extracts at 45°C significantly reduces the antibacterial activities and therefore such plants may have little or no antimicrobial activity when used directly as food after cooking as claimed by folklore. Thus, on the basis of the findings of this research, the crude extracts from the two plants deserve further purification and identification of bioactive compounds that are potentially useful for development of new antibacterial drugs.

4.2. Recommendations

Based on the findings and limitations of the present study, the following recommendations are made:

a) Further studies are needed to isolate and separate the bioactive compounds responsible for these antibacterial activities using advanced tools and scientific techniques.
b) Evaluation of the effectiveness of the crude extracts and their bioactive compounds against a variety of pathogenic bacteria associated with various human diseases is suggested to promote the economic importance of the plants.
c) The results of the study indicated that methanol extracts resulted in better antibacterial activities as compared to aqueous extracts. Hence, methanol is recommended for use as the preferred solvent for extraction of crude substances from plant materials in place of water.
d) Further studies be made to determine the Minimum Lethal Concentration (MLC) against the test pathogens.

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