J Biochem Tech (2018) 9(2): 32-41
ISSN: 0974-2328

Antimicrobial Activity Evaluation of Phytochemicals Derived from Some Plants of Indian Origin

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Received: 03 May 2018 / Received in revised form: 03 July 2018, Accepted:11 July 2018, Published online: 17 July 2018

Abstract

The present study includes evaluation of the antimicrobial activity of methanolic extracts of some plant parts of Indian origin against two gram-positive and two gram-negative bacterial strains with reference to *B. subtilis, S. aureus, P. Aeruginosa, E. coli* using the disk diffusion method. The methanolic plant extracts used in present study are derived from pomegranate peel, lemon peel, wheatgrass, *curcuma longa* leaves, sheesham leaves and papaya leaves. A systematic concentration variation method was used to evaluate antimicrobial potential of these plant extracts. Results showed that pomegranate peel extract, lemon peel extract, *curcuma longa* leaves extract and wheatgrass extract have higher antimicrobial activity, whereas sheesham leaves extract and papaya leaves extract have lower antimicrobial activity.

Keywords: Antimicrobial Activity, Methanolic Plant Extracts, Disk Diffusion Method, Gram Negative and Positive Bacteria.

Introduction

Antimicrobial susceptibility testing can be utilized for drug discovery, epidemiology and foresight of therapeutic outcome (Balouiri et al., 2016). Search for healing properties in plants is an age-old idea. Dating back prehistory people on all continents have long exploited plants for their medicinal properties. The evidence reveals that 60,000 years ago people from Iraq utilized plants such as hollyhock; these plants are still accepted in ethnomedicine worldwide (Thomson, 1978). After the revolution in the "golden era", when almost all groups of major antibiotics (tetracyclines, cephalosporins, aminoglycosides, and macrolides) were identified and the main problems of chemotherapy were resolved in the 1960s, the history repeats itself nowadays, and these interesting compounds are in the risk of losing their efficacy because of the increase in microbial resistance (Mayers et al., 2009). Currently, its impact is significant with treatment failures related to multidrug-resistant bacteria and it has become a global matter for public health (Guschin et al., 2015; Martin et al., 2015). Thus, the identification of new antibiotics is an exclusively important goal. Microbial and plant products occupy the main part of the antimicrobial compounds identified until now (Berdy and Antibiot, 2005).

Plants have been considered as a source of medicinal agents for centuries and a huge number of new drug components have been isolated from natural plant sources. Historically, the therapeutic results have been mixed. Sometimes the results show curative properties and sometimes a relief in symptom have been recorded. Many of these plants and their extracts were used in traditional medicine.

Medicinal plants play an important role in health care with about 80% of the world's populations depending on the use of traditional medicine which is mainly based on plants (Owolabi et al., 2007). According to WHO, medicinal plants would be the best source to obtain various drugs. Plant-derived medicines have made large contributions to human health (El-Astal et al., 2005). This is because of the notable healing power of the traditional medicinal systems (Adebolu et al., 2005). Medicinal plants are distributed all over the world but they are most abundant in tropical countries (Elvin-Lewis, 2001; Naqvi et al., 1991). Natural products from plants may provide new agents for antimicrobial application. A special feature of higher plants is their ability to produce a large number of organic chemicals of high structural variety (secondary metabolites) (Momani et al.,2007). Plants are rich in a variety of secondary metabolites with antimicrobial features, such as tannins, terpenoids, alkaloids, and flavonoids (Bisignano et al., 2000; Bouzada et al., 2009; Chakraborty, Brantner, 1999; Cowan et al., 1999; Sawer et al., 1997; Setzer et al., 2000; Sohail et al., 2011).

Mainstream medicine is increasingly receptive to the application of antimicrobials and other drugs isolated from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become inefficient and as new, particularly viral, diseases remain intractable to this type of drugs. Also there is an increased interest in plant antimicrobials due to the increased plant extinction rate (Stockwell, 1988). Most of the

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natural product chemists and microbiologists have acknowledged that the multitude of potentially useful phytochemical structure which could be synthesized chemically is at risk of being lost irretrievably (Lewis, Elvin-Lewis, 1995).

Plants have an almost limitless potential to produce aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Borris, 1996). Most are secondary metabolites, of which at least 12,000 have been isolated, a number predicted to be less than 10% of the total (Geissman, 1963). In many cases, these substances serve as plant defense mechanisms against microorganisms, insects, and herbivores. These substances are responsible for plant odor (such as terpenoids), pigment (such as quinones and tannins), and flavor (*e.g.* terpenoid capsaicin from chili peppers). Some of the same herbs and spices are used by humans to season food. Useful antimicrobial phytochemicals can be divided into several categories which majorly include phenolics, polyphenols, terpenoids, essential oils, alkaloids, lectins, polypeptide mixtures and other compounds.

Pomegranate juice has strong antibacterial effect against gram-positive and gram-negative bacteria (Schultes, 1978). *In vitro* microbiological tests revealed that the hydroalcoholic extracts of pomegranate juice and peel are capable of contrasting the main cariogenic bacteria involved in tooth decay (Dr. Abdullah A, 2014). Lemon essential oils had the highest inhibition effect against *S. carnosus*, *E. gergoviae*, and *E. amnigenus* (Ferrazzano et al., 2017). Methanolic extract of the peel of *Citrus limon* includes a high amount of phytochemicals. The methanolic extract of the plant was found to have promising antimicrobial activity when compared with the standards (Viuda-Martos et al., 2008). Methanolic extract of lemon peels showed the maximum zone of inhibition against *Pseudomonas aeruginosa* whereas hot water extract of lemon peels showed least zone of inhibition. Ethanolic extract of lemon seeds revealed maximum zone of inhibition against *Pseudomonas aeruginosa* whereas hot water extract had least zone of inhibition (Ali et al., 2017). The citric acid extracts were more potent against *E. coli*. But the methanolic extract of leaves of *Dalbrgia sissoo* was the second more potent drug against the bacterial test species (Pandey et al., 2011). *D. Sissoo* plant has antimicrobial activity (Singh Parmar, Johari, 2014). The extracts of wheat grass were found to possess antibacterial activity against some of the major pathogens (Bijauliya et al., 2017). There is evidence in the literature suggesting this supplement possess some efficacy towards microbial pathogens (Sundaresan et al., 2015). Wheatgrass extract as natural medicine can be extremely valuable for treating various sicknesses from minor scratches and blazes to genuine infections (Wakeham, 2013). Similarly, many studies have been conducted for *Carica papaya* and turmeric for antimicrobial activity. These are highly effective against bacteria and other pathogens. From the literature review it is evident that these plants have good antimicrobial properties. Present experiment is an effort to determine the antimicrobial activity of the pristine parts of the above-stated plants. Methanolic extracts of peel of pomegranate and lemon, leaves of *Dalbergia Sissoo*, *Carica papaya*, *C. longa* and wheatgrass have been taken as test samples for the determination of their antimicrobial activity so that these plant parts can be utilized as rich source of antimicrobial agents.

Methodology

The plant material was initially identified, washed, disinfected and shade dried for 5-6 days. These samples were then crushed and extracted. The samples were extracted by Soxhlet extraction method using hydroalcoholic solvent (methanol: water at 70:30 ratio). Different concentrations of plant extracts are then prepared and used for the experiment.

Agar disk-diffusion testing introduced in 1940, is the official method used in many clinical microbiology laboratories for regular antimicrobial susceptibility testing. Now, many accepted and recommended standards are published by the Clinical and Laboratory Standards Institute (CLSI) for testing bacteria and yeasts (Cockerill et al., 2012). Although not all fastidious bacteria can be tested precisely by this approach, the standardization has been made to test certain fastidious bacterial pathogens.

When a filter paper disc impregnated with a chemical is placed on agar, the chemical will disperse from the disc into the agar. This dispersion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will reveal the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is called a “zone of inhibition”. After incubation, zone diameter is measured to the nearest whole millimeter at the point wherein there is a prominent reduction of 80% growth. Previous studies suggest that *B. subtilis, S. aureus, P. Aeruginosa* and *E. coli* are two gram-positive and two gram-negative bacteria respectively used to evaluate agar diffusion method.

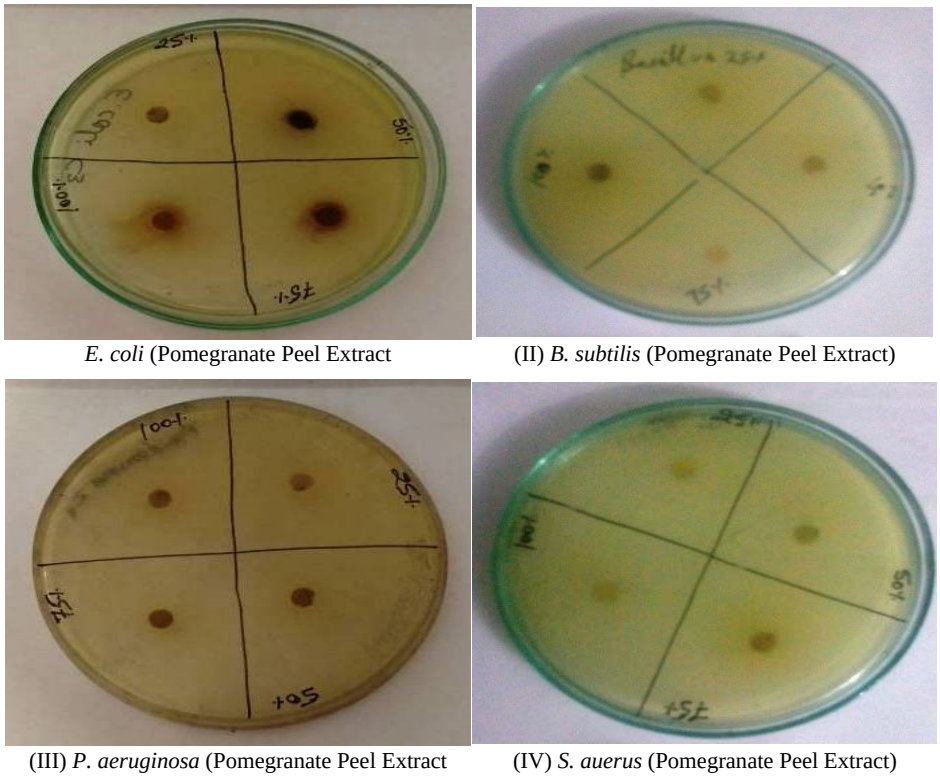
The cultures were grown overnight in liquid broth. The cultures were applied on NAM agar medium. A 6 mm Whatman filter paper disc of different concentrations of plant extracts of 75 mg/ml dilution (25, 50, 75 and 100 %) were placed on the medium, incubate at 37 ºC for 8 hrs, and the zone of inhibition was measured in mm by zonal scale (Hi-Media) (Dutta, Raja, 2016).

Results and Discussions

Antimicrobial Activity of Pomegranate Peel Extract

The methanolic pomegranate peel extract used against *B. subtilis* revealed almost similar zone of inhibition at various concentrations (figure 1.a. II). This observation suggests the dose-independent response of the *B subtilis* strain towards methanolic pomegranate peel extract. The maximum zone of inhibition of methanolic extract of pomegranate peel against *S. aureus* was found at 50 mg/ml and 100 mg/ml concentration, while the zone of inhibition was low in 25 and 75 mg/ml concentrations (Figure 1.a IV). The zone of inhibition observed by methanolic extract of pomegranate peel against *P. aeruginosa* was 14 mm and 12 mm with 25 mg/ml and 100 mg/ml concentrations, respectively (Figure 1.a. III). *E. coli* showed maximum zone of inhibition at 25 mg/ml

concentration followed by 75 mg/ml, 50 mg/ml and 100 mg/ml, respectively (Figure 1.a. I)



E. coli (Pomegranate Peel Extract (II) *B. subtilis* (Pomegranate Peel Extract)

(III) *P. aeruginosa* (Pomegranate Peel Extract (IV) *S. auerus* (Pomegranate Peel Extract)

Figure 1 a. Experimental outcome showing zone of inhibition produced from pomegranate peel extract against various microbial strains.

The results show that the maximum response of the methanolic pomegranate peel extract was against *E. coli*. A comparative analysis of responses produced by other microbial strains of gram-positive and gram-negative strains highlights the fact that pomegranate peel could act as a good antimicrobial agent (figure 1b).

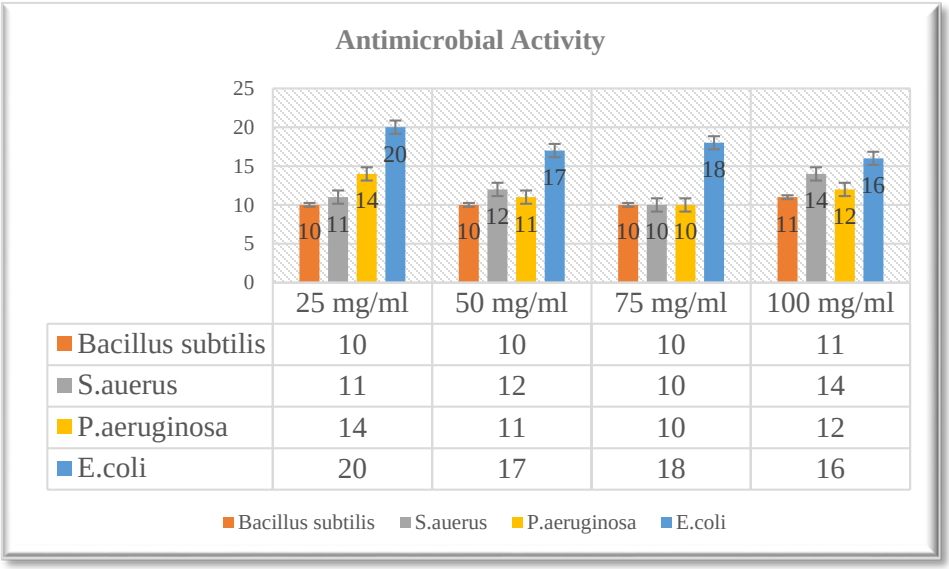
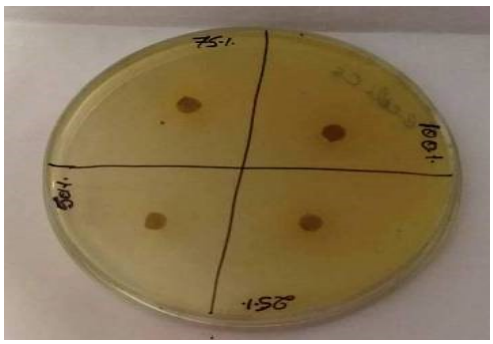


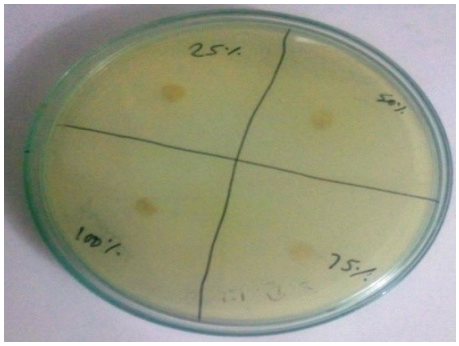
Figure 1 b: Graphical correlation of antimicrobial activity of pomegranate peel extract against two gram-positive and two gram-negative bacteria.

Antimicrobial Activity of Lemon Peel Extract

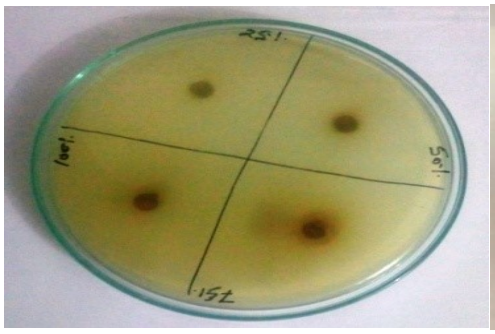
The zone of inhibition observed against *B. subtilis* was maximum at 25 mg/ml of 20 mm and a decreasing trend is observed. Figure 2.a.II shows that as the concentration increases, the zone of inhibition decreases. Similarly, the bacterial strain *E. coli* shows the same trend as *B. subtilis*. The maximum zone of inhibition is at 25 mg/ml which later on decreases as the concentration is increased to 100 mg/ml (Figure 2.a. I). The bacterial strains *S. aureus* and *P. aeruginosa* show the first decreasing trend from 25 mg/ml to 50 mg/ml which later on increases and decreases respectively (Figure 2.a III & IV).



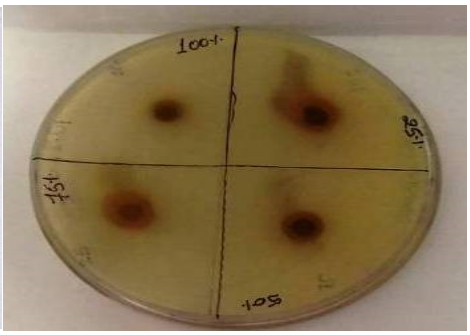
E. coli (Lemon Peel Extract)



(II) *B. subtilis* (Lemon Peel Extract)



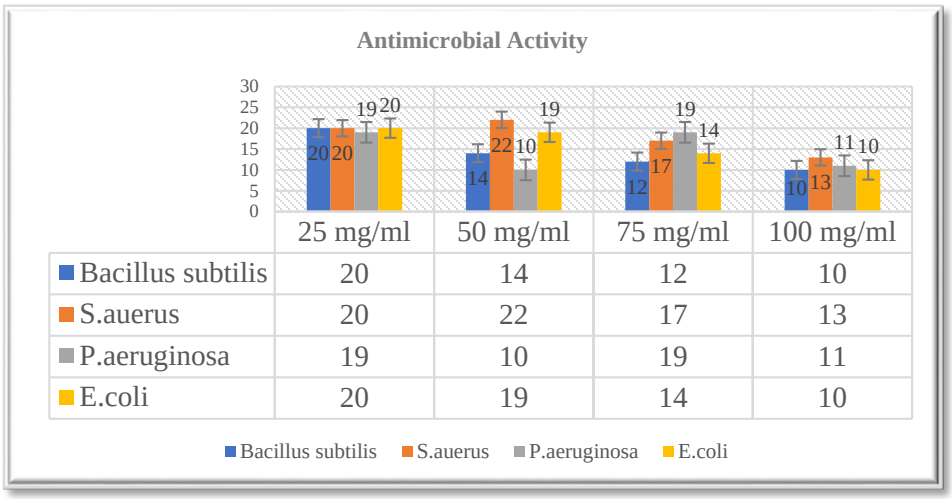
(III) *S. auerus* (Lemon Peel Extract)



(IV) *P. aeruginosa* (Lemon Peel Extract)

Figure 2 a. Experimental outcome showing zone of inhibition produced from lemon peel extract against various microbial strains.

A comparative graph (Figure 2.b) shows that *S. aureus* has maximum response against lemon peel methanolic extract followed by *P. aeruginosa, E. coli and B. subtilis* confirming that lemon peel can be a potent antimicrobial agent.

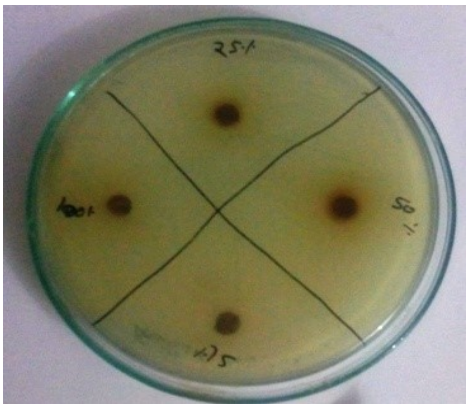


	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
Bacillus subtilis	20	14	12	10
S.auerus	20	22	17	13
P.aeruginosa	19	10	19	11
E.coli	20	19	14	10

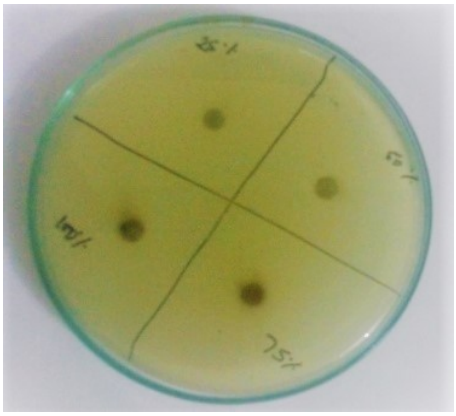
Figure 2 b: Graphical correlation of antimicrobial activity of lemon peel extract against two gram-positive and two gram-negative bacteria.

Antimicrobial Activity of Carica papaya Leaves Extract

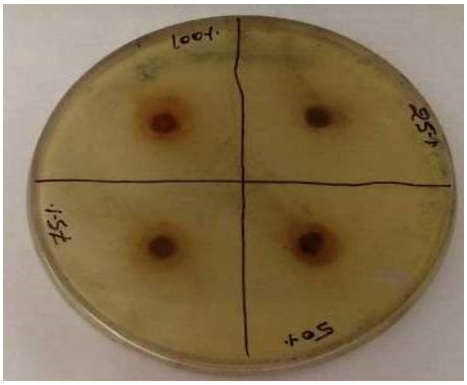
The zone of inhibition of *Carica papaya* leaf extract shows that the bacterial strain *B. subtilis* and *S. auerus* have same zone of inhibition. The zone of inhibition remains constant at 10 mm and changing concentration doesn't have any impact on it (Figure 3a I, II) *i.e* with the changing concentration (25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml) the zone of inhibition remains 10 mm displaying that the bacterial strains response doesn't depend on the concentration of the methanolic extract. The bacterial strain *P. aeruginosa* showed a slight increase in the zone of inhibition (Figure 3a III). At 25 mg/ml the zone of inhibition is 10 mm which later on increases to 11 mm and 12 mm at 75 mg/ml and 100 mg/ml concentrations, respectively. *E coli* responded against the methanolic extract of *C. papaya* (Figure 3a IV)



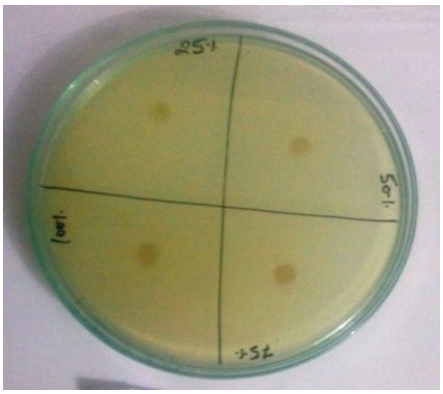
B. subtilis (Papaya Leaf Extract)



II) *S. auerus* (Papaya Leaf Extract)



(III) *P. aeruginosa* (Papaya Leaf Extract)



(IV) *E. coli* (Papaya Leaf Extract)

Figure 3 a. Experimental outcome showing zone of inhibition produced from papaya leaf extract against various microbial strains.

Figure 3.b shows the graphical correlation between the methanolic extract of *C. papaya* leaf extract and the zone of inhibition. It is evident from the graph that *P. aeruginosa* is slightly concentration-dependent and with the increasing concentration the zone of inhibition increases. The values of the other bacterial strains remain constant whereas no activity is recorded for *E. coli*. The graphical representation clearly indicates that *C. papaya* leaves have low antimicrobial activity (Figure 3b).

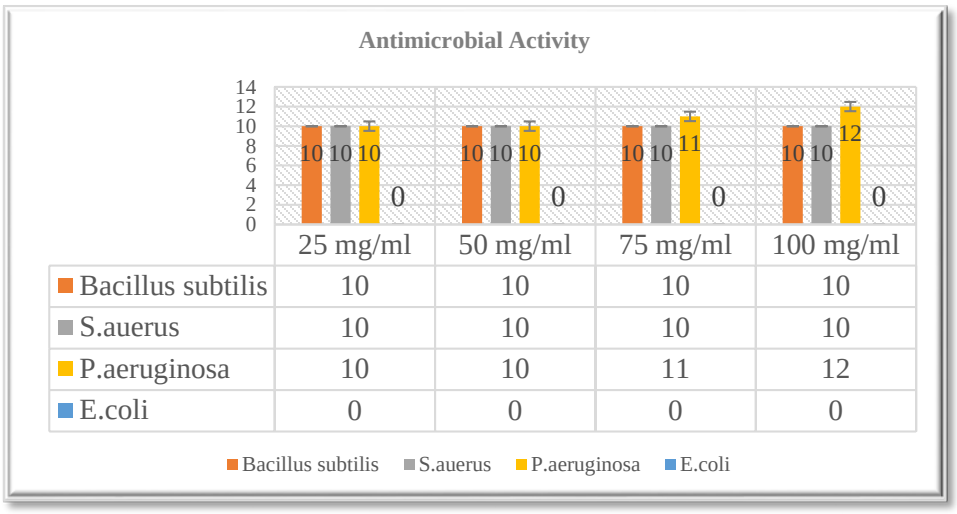
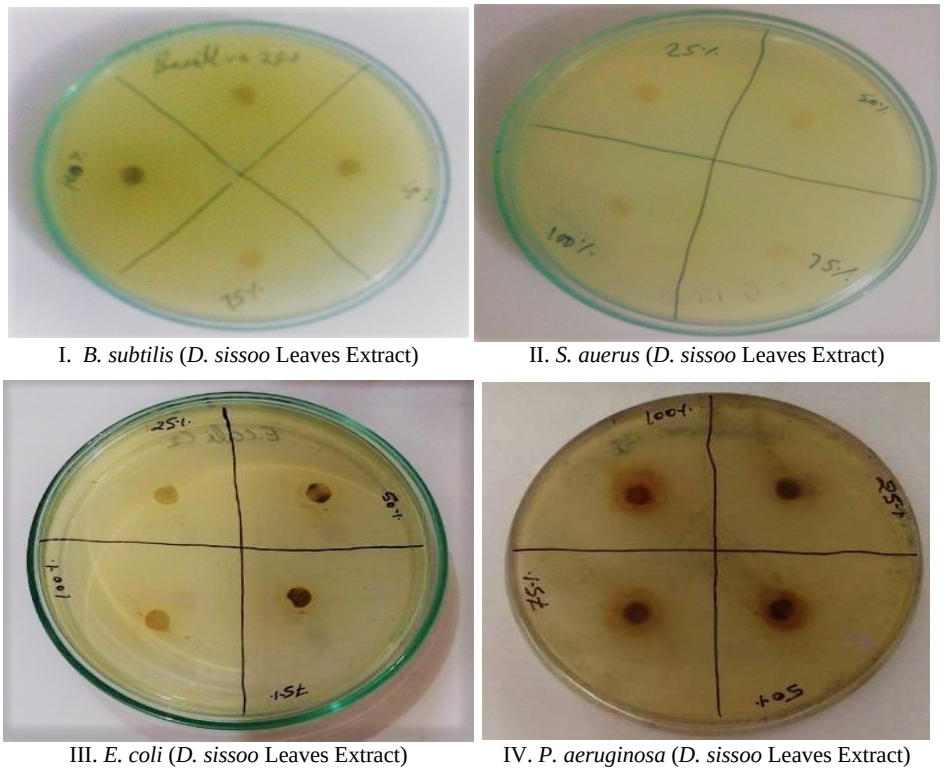


Figure 3 b: Graphical correlation of antimicrobial activity of *C. papaya* leaf extract against two gram-positive and two gram-negative bacteria.

Antimicrobial Activity of D. sissoo Leaves Extract

The zone of inhibition observed with bacterial strains *B. subtilis* and *S. aureus* were zero showing that the methanolic extract of *D. sissoo* leaves do not have any activity against these bacterial strains. The values remain nil for all the concentrations as shown by Figures 4.a I and II. The initial zone of inhibition of *P. aeruginosa* at 25 mg/ml shows 0 mm zone of inhibition but with increase in concentration to 50 mg/ml, 75 mg/ml and 100 mg/ml the zone of inhibition remains constant at 10 mg/ml as shown by Figure 4.a IV. Whereas with *E. coli* all the concentrations of the *D. sissoo* leaves methanolic extract the value of the zone of inhibition remains constant *i.e* 10 mm (Figure 4.a III) which shows that increasing concentration has no effect on the antimicrobial activity.



I. *B. subtilis* (*D. sissoo* Leaves Extract) II. *S. auerus* (*D. sissoo* Leaves Extract)

III. *E. coli* (*D. sissoo* Leaves Extract) IV. *P. aeruginosa* (*D. sissoo* Leaves Extract)

Figure 4 a. Experimental outcome showing zone of inhibition produced from *D. sissoo* leaf extract against various microbial strains.

A graphical correlation is shown in Figure 4.b which depicts that *E. coli* bacterial strain is the only active strain showing some positive results as compared to the other strains. Thus, confirming that *D. sissoo* leaves have low antimicrobial properties.

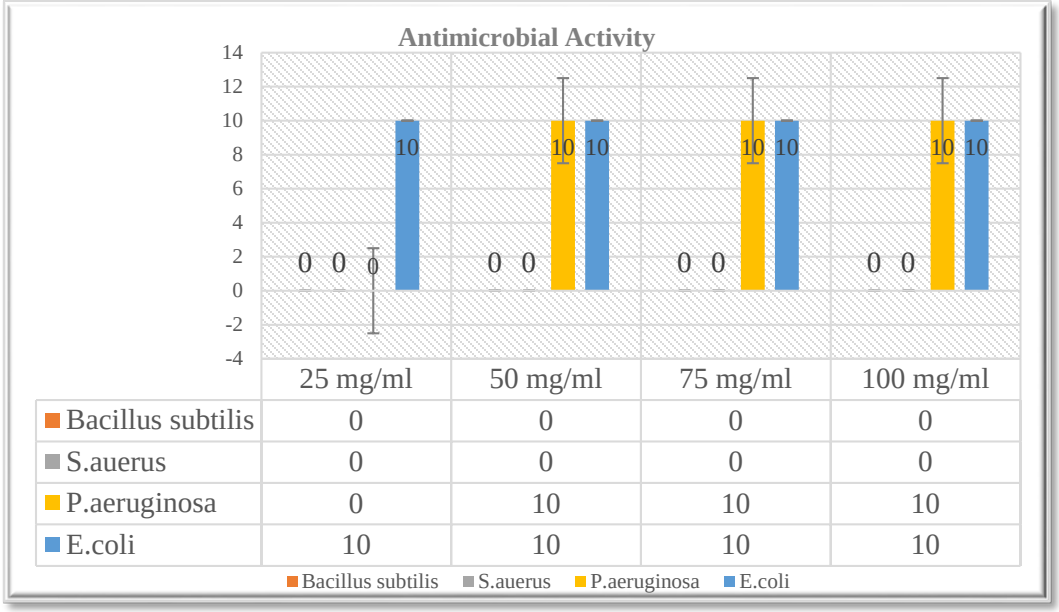
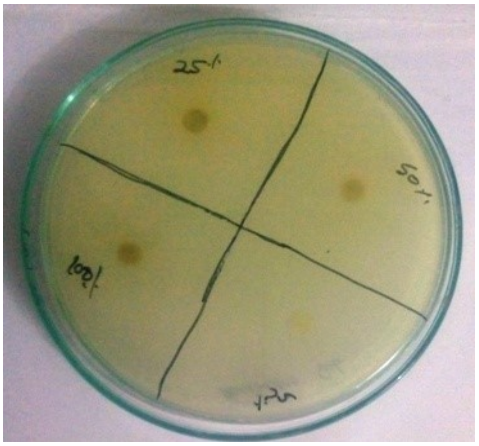


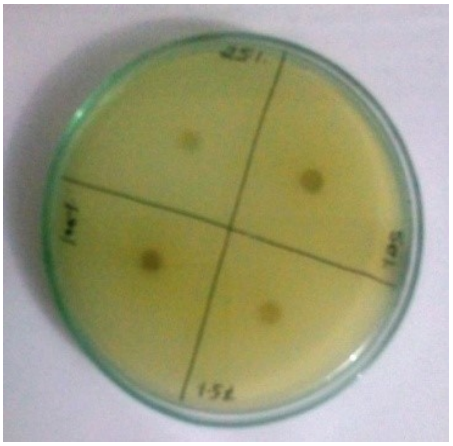
Figure 4 b: Graphical correlation of antimicrobial activity of *C. papaya* leaf extract against two gram-positive and two gram-negative bacteria.

Antimicrobial Activity of C. longa Leaves Extract

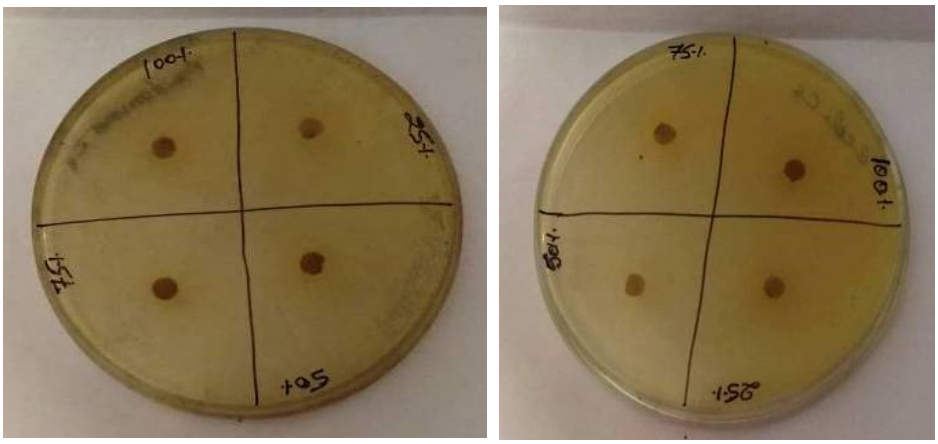
Figure 5.a III shows that the maximum zone of inhibition is related to P. *aeruginosa* at 75 mg/ml and 100 mg/ml. The zone of inhibitions is 14 and 17 mm respectively. The other values also show an increase at 25 and 75 mg/ml. The bacterial strain *E. coli* on the other hand shows an ascending and then descending trend. At 25 mg/ml the zone of inhibition is found to be 12 mm which increases to 14 mm at 50 mg/ml concentration whereas the values get constant at 75 and 100 mg/ml concentrations (Figure 5.a IV). Both *B. subtilis* and *S. aureus* have same decreasing trend as shown by Figures 5.a.I, II. Thus the present figures show that the maximum zone of inhibition is related to *P. aeruginosa* at 75 and 100 mg/ml followed by *E. coli*, *B. subtilis* and *S. auerus*.



B. subtilis (*C. longa* Leaf Extract)



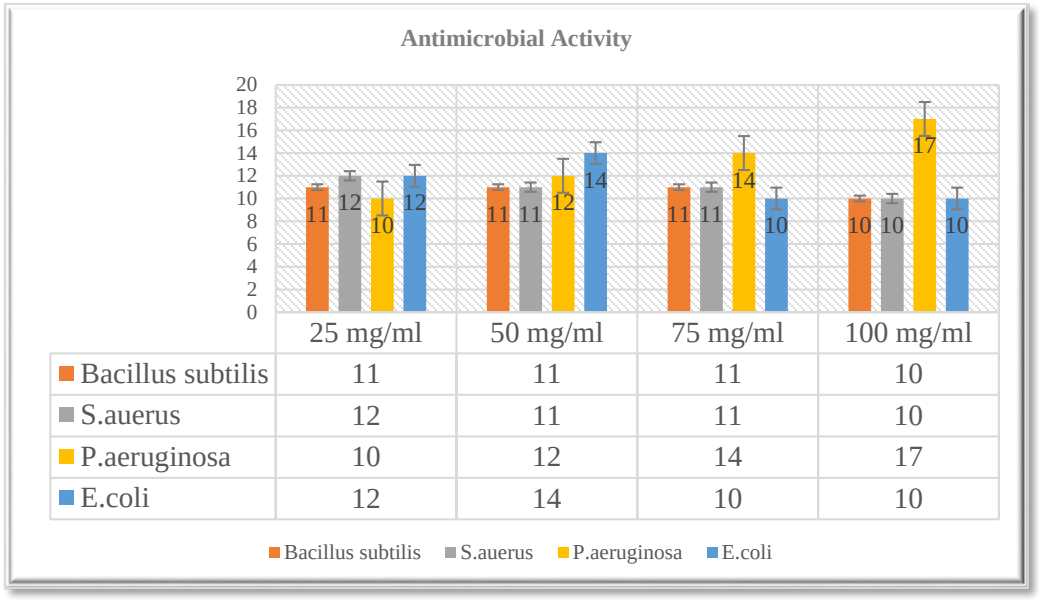
II. *S. auerus* (*C. longa* Leaf Extract)



III. *P. aeruginosa* (*C. longa* Leaf Extract) IV. *E. coli* (*C. longa* Leaf Extract)

Figure 5 a. Experimental outcome showing zone of inhibition produced from *C. longa* leaf extract against various microbial strains.

The comparative analysis of the four bacterial strains against *C. longa* leaves methanolic extract is shown in the figure 5.b. Various zones of inhibitions of different bacterial strain shows that *P. aeruginosa* had maximum response to the *C. longa* leaves extract. The results clearly show that *C. longa* leaves are potent antimicrobial agents.

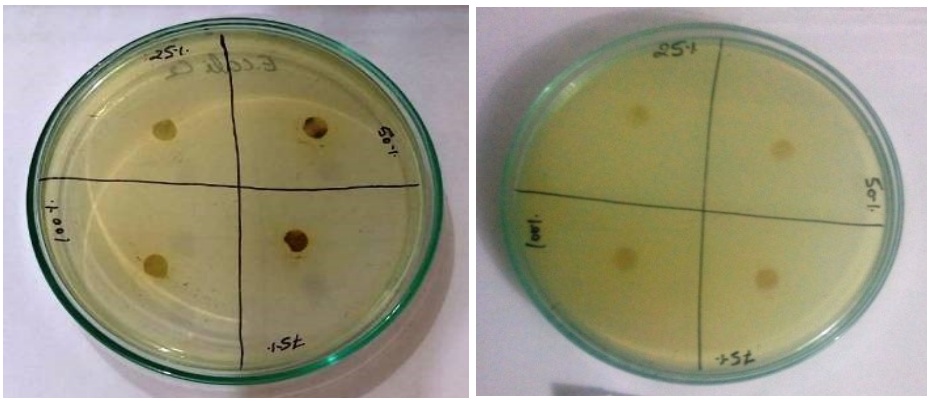


	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
Bacillus subtilis	11	11	11	10
S.auerus	12	11	11	10
P.aeruginosa	10	12	14	17
E.coli	12	14	10	10

Figure 5.b: Graphical correlation of antimicrobial activity of *C. longa* leaf extract against two gram-positive and two gram-negative bacteria.

Antimicrobial Activity of Wheatgrass Extract

B. subtilis shows maximum efficacy against methanolic extract of wheatgrass. The zone of inhibition increases with increase in concentration (Figure 6.a III). The zone of inhibition increases with 25 mg/ml to 75 mg/ml but decreases slightly at 100 mg/ml. *S. aureus* and *E. coli* show same zone of inhibition at all concentrations. Again, the trend in the zone of inhibition is the same as that of *B. subtilis* (Figure 6.a I, II). *P. aeruginosa* shows an increasing zone of inhibition. The zone of inhibition is 11 mm at 25 mg/ml, 14 mm at 50 and 75 mg/ml and it increases to 16 mm at 100 mg/ml as shown in figure 6.a IV. Thus, the maximum zone of inhibition is related to *B. subtilis* at 75 mg/ml as shown by figure 6.a III.



E. coli (Wheatgrass Extract) II. *S. auerus* (Wheatgrass Extract)

III. *B. subtilis* (Wheatgrass Extract) IV. *P. aeruginosa* (Wheatgrass Extract

Figure 6 a. Experimental outcome showing zone of inhibition produced from wheatgrass extract against various microbial strains.

The graphical representation of the antimicrobial activity of wheatgrass methanolic extract against different bacterial strain is shown in figure 6.b. The graph shows that the maximum zone of inhibition is related to *B. subtilis* at 75 mg/ml concentration followed by *S. aureus*, *E. coli* at 75 mg/ml and *P. aeruginosa* at 100 mg/ml. Thus, it is evident that the bacterial strains show maximum zone of inhibition at higher concentration depicting that wheatgrass is a potent antimicrobial agent.

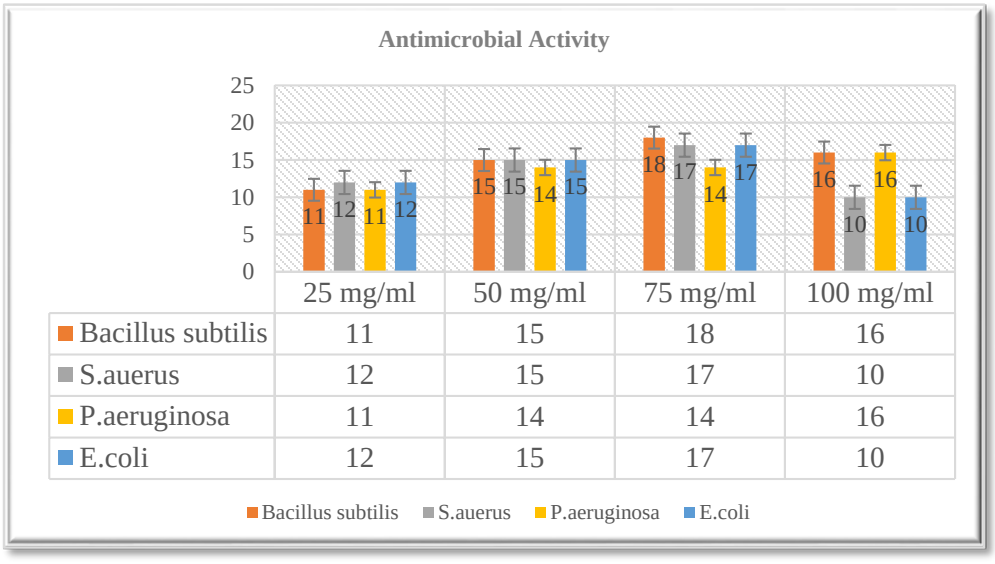


Figure 6.b Graphical correlation of antimicrobial activity of wheatgrass extract against two gram-positive and two gram-negative bacteria.

Conclusions

In the present study the hydroalcoholic extracts of various non-productive plant parts are evaluated for their antimicrobial activity against gram-positive and gram-negative bacteria which are common human pathogenic microorganisms. The Disk diffusion method has been employed for antimicrobial activity evaluation. A significant *E. coli* growth inhibition has been recorded when pomegranate peel extract was used against selected bacterial strains. Lemon peel extract shows promising results as antibacterial agent against *S. aureus*. Papaya leaves extract proved to be ineffective as an antimicrobial when screened against selected gram-positive and gram-negative bacteria. Similar ineffective results were observed when sheesham leaves extract was applied to these selected bacterial strains. Turmeric leaves extract was found effective in the inhibition of *P. aeruginosa* and *E. coli* strains. Wheatgrass extract was able to inhibit growth of *B. subtilis* and *P. aeruginosa* significantly.

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