*Available online at* www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2017, 7(5):27-34

ISSN : 2249-7412
CODEN (USA): AJPSKY

# Antibacterial and Antioxidant Activities of *Aegle marmelos and Cassia fistula* Leave Extract

**Kiran Abha Singh[1] and Anup Kumar[2*]**

[1]*Department of Life Sciences, Singhania University, Jhunjhunu, Rajasthan, India*
[2]*Central Institute of Medicine and Aromatic Plants (CIMAP), Lucknow, India*

## ABSTRACT

*In this study, we investigated antibacterial and antioxidant activities of ethyl acetate and methanol extracts obtained from leaves of Aegle marmelos and Cassia fistula. Bacteria used in this study were Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli and Bacillus subtilis. The antibacterial activity was evaluated based on the diameter of inhibition zone (in millimeters, mm) using disk diffusion method and well diffusion method. MIC values of the plant extracts against these bacteria were assessed using broth dilution method. Result shows that leave extracts of these plants have varying degrees of antibacterial activity on tested microorganisms. Plant leaf extract in methanol shows quite good antibacterial activity in compare to extract in ethyl acetate. Therefore methanol extract can be used as an antiseptic in infection caused by these bacteria. This is alternative treatment method when these bacteria have resistance against antibiotics. Next, we evaluated DPPH Radical scavenging activity, Nitric oxide radical scavenging activity and reducing power of the ethyl acetate extract prepared from leaves of Aegle marmelos and Cassia fistula. Plant leaf extract in ethyl acetate shows significant scavenging activity as DPPH, Reducing power and Nitric oxide scavenging in compare to reference. Reference standard used in this study was ascorbic acid, which also has scavenging activity in significantly increased manner. Result of qualitative analysis shows that plant leaf extract prepare in ethyl acetate has the high antioxidant activity. Different leaves extracts with strong antioxidant activity indicates its scope for utilization in food and biological systems.*

**Keywords:** Antibacterial, Antioxidant, *Aegle marmelos*, *Cassia fistula*, MIC

## INTRODUCTION

Microorganisms are closely associated with human health and welfare. Many of these microorganisms are beneficial and many are detrimental. Plants are use as medicines since time immemorial. Infectious diseases are the leading cause of death worldwide. Various studies have identified that compounds isolated from herbal plants are effective antibiotics. Many infectious diseases have been known to be treated with herbal remedies throughout the history of humankind. The herbal remedies of traditional healing systems around the world can be utilized as an important source for the discovery of new antibiotics [1]; some traditional remedies have already produced compounds that are effective against clinically important strains of bacteria. Plants possessing antibacterial activity for various diseases are being studied by various methods to evaluate their antibacterial property. An attempt has been made to assess the antibacterial properties of *Aegle marmelos* and *Cassia fistula* for potential antibacterial activity against *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*.

Reactive species play a dual role in animals, sometime behaves like a toxic compound and some time as beneficial compounds. They exert beneficial effects on cellular redox systems at low concentration. At high concentrations, they can produce oxidative stress. Oxidative stress is a harmful process, which can damage cell structures and functions. Natural antioxidants are generally produced from plants. They are found in almost all parts of plants and are called as secondary metabolites. Leaf extract of *Aegle marmelos* and *Cassia fistula* prepare in ethyl acetate were evaluated for their Nitric oxide radical scavenging, DPPH Radical scavenging and reducing power activities [2-6].

## MATERIALS AND METHODS

### Collection of plant materials

The fully mature *Aegle marmelos* and *Cassia fistula* leaves were collected in September and October 2015 from Sonbhadra district of Uttar Pradesh, India from a single tree. The leaves were identified and authenticated wide voucher specimen by department of botany, Singhania University, Jhunjhunu, Rajasthan, India. Dust particles adhering with plant samples were washed thoroughly to remove these with the help of running tap water. Leafs were dried for two weeks under the shades and grounded into powder form. An airtight container was used to store powder for the further investigations.

### Test microorganisms

Test microorganisms were obtained from database named "Microbial Type Culture Collection and gene bank" maintain by Institute of Microbial Technology, Chandigarh, India. Nutrient broth media was used to maintain microorganisms. In test microorganisms *Staphylococcus aureus* (MTCC-96) and *Bacillus subtilis* (MTCC 8561) are gram-positive bacteria. And *Salmonella typhi* (MTCC-733), *Pseudomonas aeruginosa* (MTCC-647), *Escherichia coli* (MTCC-729) are gram-negative bacteria.

### Culture medium and inoculum

Microorganism's stock cultures were maintained on Plate Count Agar slants at 4°C. A loop of bacterial cultures was suspended into 10 mL of nutrient broth to prepare inoculum and was incubated for 24 h at 37°C. After 24 h, in a flask Muller- Hinton agar media was taken and sterilized it. After sterilization it was cooled up to 45-52°C and was distributed 20 mL by pipette into sterile Petri dishes and swirled to distribute the medium homogeneously. About 0.1 mL of bacterial suspension was taken and poured uniformly into each petri plates containing media using the L-shaped sterile glass spreader.

### Plant extracts activity assay

#### *Paper disc method*

Paper disc diffusion method was used to determine the diameter of zone of bacterial inhibition. A swab of the bacteria suspension was spread on to media containing petri plates. Sterile filter paper discs having 6 mm in diameter were impregnated with 50 µl/ml extracts and were places on to petri plates. These petri plates were place in incubator for 24 h at 37°C. 50 µl/ml streptomycin loaded on filter paper discs were served as positive control and ethanol served as negative control. Presence of clear inhibition zone around the paper discs indicates antibacterial activity and were measured the diameter of inhibition [7,8].

#### *Well diffusion method*

The agar well diffusion method was used for the antimicrobial evaluations. According to Obeidat et al. [9], an inoculum suspension was swabbed uniformly to solidified 20 ml Mueller-Hinton Agar for bacteria and the inoculum was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using glass pasteur pipettes. Aliquot of 20 µL from each plant crude extract was added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion. The plates were incubated at 37°C overnight and examined for the zone of inhibition. The diameter of the inhibition zone was measured in millimeters (mm).

#### *Determination of MIC of plant extract by broth dilution assay*

MIC was defined as the lowest concentration of extract that inhibit visible growth. The minimum inhibitory concentration values were determined by broth dilution assay. Ethyl acetate and methanol extracts of plants will be determined for their MIC values using a standard protocol of Andrews [6]. Nutrient broth was used as the medium to culture bacteria. 50 µL of 10 mg/mL of plant extracts was prepared. 1 ml of this broth will be added to the numbered tubes 1-9. One ml of the stock culture will be added to tube 1 and serially diluted until tube number 7. The last 1 ml of tube 7 will be discarded. Tube number 8 will be used as a negative control and the tube 9 as a positive control. The bacterial inoculum will be cultured in nutrient broth and incubated overnight. All the tubes will be inoculated with 1 ml of the test bacteria media except tube number 8 and incubated for 24 h at 37°C.

#### *DPPH radical scavenging activity*

DPPH free radical scavenging method was used to detect antioxidant activity of leaf extract. The scavenging activity

of different extracts was measured in terms of hydrogen donating or radical scavenging ability using a stable radical DPPH (1, 1-diphenyl-2-picrylhydrazyl). Different concentrations of each extracts and standard were taken in different vials. 3 ml of DPPH solution (2 mg/ml) were rapidly mixed with plant extracts. The mixture was then vortex mixed vigorously and left for 30 min in dark at room temperature. The absorbance was recorded at 517 nm. Standard reference was ascorbic acid. The activity was expressed in terms of percent of inhibition and was calculated using the following formula.

$$RSA\% = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

***Reducing power assay***

Generally reducing properties are linked with the presence of reductones, which show the evidence of antioxidant activity by breaking the chain reactions by donating the hydrogen atoms [7]. The reducing power was determined by using procedure of Oyaizu [8]. 2.5 ml of methanolic extracts at different concentrations were added to 2.5 ml of 200 mmol/l sodium phosphate buffer at pH 6.6 and 2.5 ml of one percent potassium ferricyanide. The mixture was incubated for 20 min at 50°C. After that 2.5 ml of ten percent trichloroacetic acid (w/v) were added. Further obtain mixture was centrifuged at 650 rpm for 10 min. 5 ml from upper layer was mixed with 5 ml de-ionized water and 1 ml of 0.1% of ferric chloride. Absorbance was measured at 700 nm.

***Nitric oxide radical scavenging (NO) assay***

At physiological pH, sodium nitroprusside generated Nitric oxide in aqueous solution, which interacts with $O_2$ to produce stable products, nitrate and nitrite through intermediates, which were measured using the Griess reagent [9]. Nitric oxide plays important role in the pathogenesis of pain, immune response, neural signal transmission, inflammation, control vasodilatation and blood pressure [10]. In phosphate buffer 3.0 ml of 10 mM sodium nitroprusside was mixed with 2.0 ml of extract and reference compound in different concentrations (25-400 µg/ml). The resulting solutions were then incubated for 60 min at 25°C. A similar procedure is repeated with methanol as blank, which serves as control. 5.0 ml of Griess reagent was added with 5.0 ml of the incubated sample and absorbance of the chromophore formed is measured at 546 nm. % inhibition of the nitrite oxide generated was measured by comparing the absorbance values of control and test preparations.

## RESULTS AND DISCUSSION

Effects of ethyl acetate and methanol extracts of the *Aegle marmelos* and *Cassia fistula* using 50 µL of 10 mg/mL of plant extracts against the *Staphylococcus aureus*, *Salmonella typhi Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* using Disc diffusion and Well diffusion method were shown in Tables 1 and 2.

### Antibacterial activity from leaves of *Aegle marmelos*

The ethyl acetate and methanol extracts prepare from leaves of *Aegle marmelos were* tested for antibacterial activity and shown in Table 1. Antimicrobial activity was determined quantitatively by measuring inhibition zone around the disc impregnated with leaf extract over the lawn of bacterial. Extracts showed significant antimicrobial activity against all the bacterial strains tested. The *Aegle marmelos* was found to be the high zone of inhibition was observed in methanol extract in 10 mg/mL concentration was against *Bacillus subtilis* in both methods.

### Antibacterial activity from leaves of *Cassia fistula*

Ethyl acetate and methanol extracts from leaves of *Cassia fistula* showed pronounced antibacterial activity against

**Table 1:** Antibacterial activity from leaves of *Aegle marmelos*

| Microorganisms | Diameter of inhibition zones (mm/50 µL) | | | |
|---|---|---|---|---|
| | Disc diffusion method | | Well diffusion methods | |
| | Ethyl Acetate | Methanol | Ethyl Acetate | Methanol |
| *Staphylococcus aureus* | 13 | 13 | 12 | 13 |
| *Salmonella typhi* | 11 | 14 | 11 | 13 |
| *Pseudomonas aeruginosa* | 13 | 11 | 12 | 13 |
| *Escherichia coli* | 12 | 15 | 11 | 12 |
| *Bacillus subtilis* | 12 | 16 | 13 | 18 |

Used concentrations: 50 µL of 10 mg/mL of plant extracts

**Table 2:** Antibacterial activity from leaves of *Cassia fistula*

| Microorganisms | Diameter of inhibition zones (mm/50 µL) | | | |
|---|---|---|---|---|
| | Disc diffusion method | | Well diffusion methods | |
| | Ethyl Acetate | Methanol | Ethyl Acetate | Methanol |
| *Staphylococcus aureus* | 16 | 28 | 15 | 24 |
| *Salmonella typhi* | 12 | 14 | 12 | 16 |
| *Pseudomonas aeruginosa* | 20 | 24 | 21 | 26 |
| *Escherichia coli* | 14 | 18 | 15 | 21 |
| *Bacillus subtilis* | 11 | 13 | 12 | 15 |

Used concentrations: 50 µL of 10 mg/mL of plant extracts

all the microorganisms tested. Among the leaf extracts, methanol extract exhibited higher activity than ethyl acetate. Methanol (13-28 mm/50 µl inhibition zone) and ethyl acetate (11-21 mm/50 µl inhibition zone) extracts of the leaf exhibited marked activity against all the tested organisms in both the method is shown in Table 2.

Many naturally occurring compounds found in plants have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against pathogens. Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide. Therefore, the development of new antimicrobial agents for the treatment of bacterial infections is of increasing interest.

Ali et al. [2] performed *in vitro* antibacterial screening of ethyl acetate and methanol extracts of *Cassia fistula* against 14 pathogenic bacteria as similar as this work. In this study, Kanamycin was used as standard antibiotic in comparison of antibacterial tests. The antimicrobial activities were determined by measuring the diameter of the inhibitory zones in mm. Ethyl acetate extract of *Cassia fistula* leaves (400 µg $disc^{-1}$) shows strong activity against *Staphylococcus aureus* (6 mm), *Salmonella typhi* (10 mm), *Pseudomonas aeruginosa* (10 mm), *Escherichia coli* (12 mm) and *Bacillus subtilis* (10 mm). Methanol extract of *Cassia fistula* leaves (400 µg $disc^{-1}$) shows inactive against most of the tested bacteria such as *Staphylococcus aureus* (0 mm), *Escherichia coli* (0 mm) and *Bacillus subtilis* (0 mm). In Overall consideration, of methanol extract was not so enough as those of ethyl acetate extracts [2].

**Minimum inhibitory concentration (MIC)**

The results of MIC values for ethyl acetate and methanol extracts of the *Aegle marmelos* and *Cassia fistula* against the *Staphylococcus aureus*, *Salmonella typhi Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* were shown in Tables 3 and 4.

All plant showed a good inhibition against all the bacterial strains tested. The present study indicates that the photochemical of these plants have significant inhibition for all the bacterial strains tested and justified the medicinal use of these plant leaves and further study is required to find out the active component, which is of utmost medicinal value against this range of microorganisms. Similar study was performed by Duraipandiyan and Ignacimuthu [6], they were tested ethyl acetate and methanol extracts from the flower of *Cassia fistula* against bacteria. These extracts exhibited antibacterial activity against gram-positive organisms with minimum inhibitory concentrations between 0.078 and 2.5 mg/ml [6].

**DPPH radical scavenging activity**

The scavenging effects of ethyl acetate extract from leaves of *Aegle marmelos* and *Cassia fistula* on DPPH were examined at the different concentrations (20, 40, 60, 80, 100 µg/ml) and were shown in Graphs 1 and 2. Ascorbic acid was used as a reference standard. It was observed that the DPPH Radical scavenging activity of these plants were as comparable to that of standard ascorbic acid (Graphs 1 and 2).The Ethyl acetate extract of the plant showed promising free radical scavenging effect of DPPH in a concentration dependent manner up to a concentration of 100 µg/ml. The % of inhibition was increased with increasing concentration of the extract.

Concentration of the sample necessary to decrease the initial concentration of DPPH by 50% ($IC_{50}$) under the experimental condition can be calculated from the Graphs 1 and 2. A lower $IC_{50}$ value denoted a higher antioxidant activity. Ethyl acetate extract from leaves of *Aegle marmelos* ($IC_{50}$ value is not found) and *Cassia fistula* ($IC_{50}$ value is 82.4 µg/ml) showed promising free radical scavenging activity by DPPH method as compared the positive standard (ascorbic acid) for *Aegle marmelos* ($IC_{50}$ value is 84.0 µg/ml) and *Cassia fistula* ($IC_{50}$ value is 75.5 µg/ml).

**Reducing power assay**

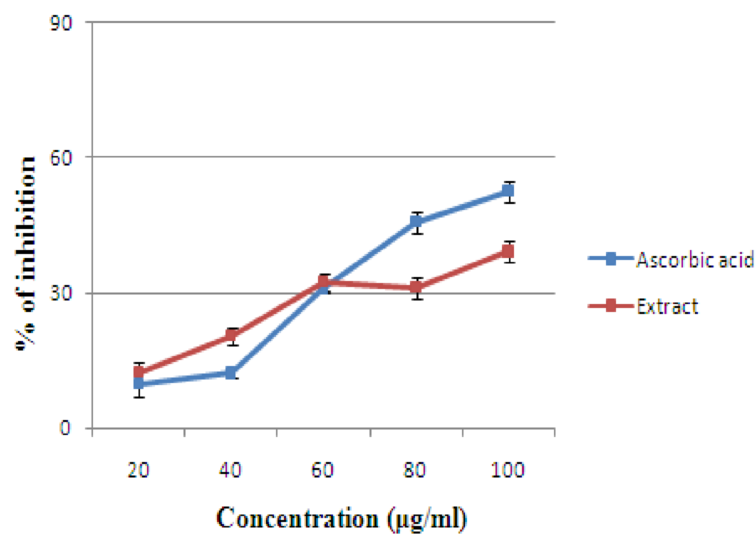
Ethyl acetate extract from leaves of *Aegle marmelos* and *Cassia fistula* on reducing power were examined at the

**Table 3:** MIC value of ethyl acetate extract from leaves of plant

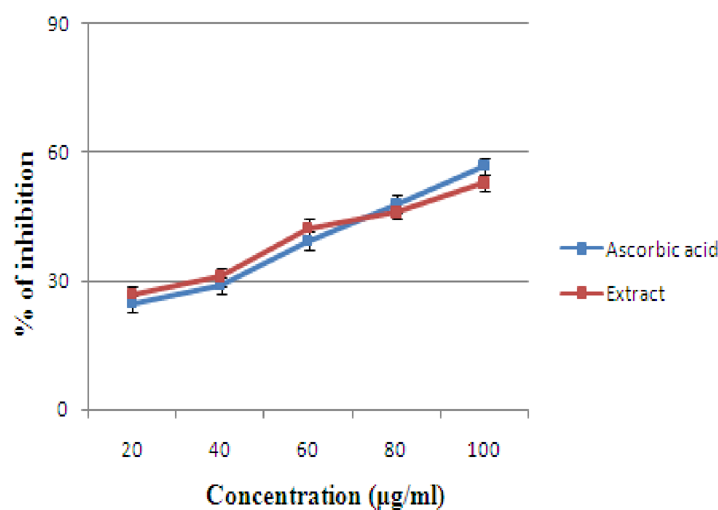
| Microorganisms | MIC (mg/mL) for ethyl acetate extract | |
|---|---|---|
| | ***Aegle marmelos*** | ***Cassia fistula*** |
| *S. aureus* | 53 | 55 |
| *S. typhi* | 58 | 39 |
| *P. aeruginosa* | 15 | 52 |
| *E. coli* | 46 | 59 |
| *B. subtilis* | 14 | 74 |

**Table 4:** MIC value of methanol extract from leaves of plant

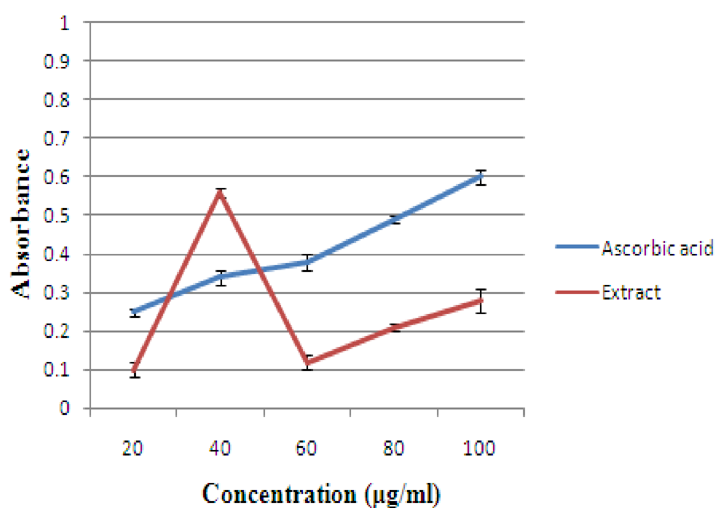
| Microorganisms | MIC (mg/mL) for methanolic extract | |
|---|---|---|
| | ***Aegle marmelos*** | ***Cassia fistula*** |
| *S. aureus* | 52 | 56 |
| *S. typhi* | 58 | 40 |
| *P. aeruginosa* | 10 | 54 |
| *E. coli* | 40 | 60 |
| *B. subtilis* | 10 | 76 |



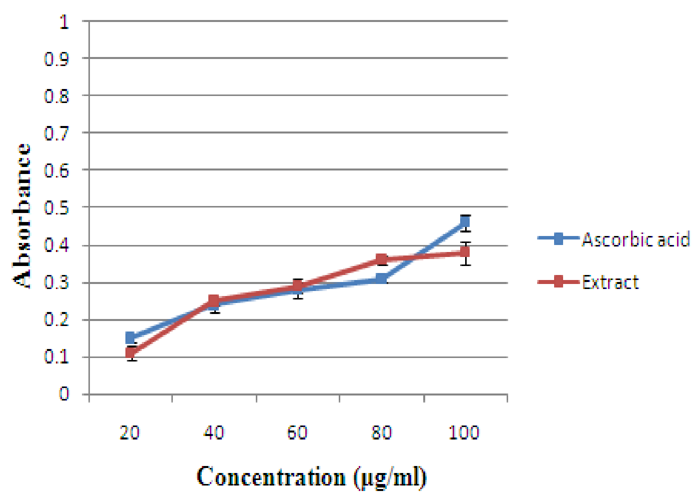

**Graph 1:** Graph displaying DPPH radical scavenging activity of ethyl acetate extract of *Aegle marmelos*



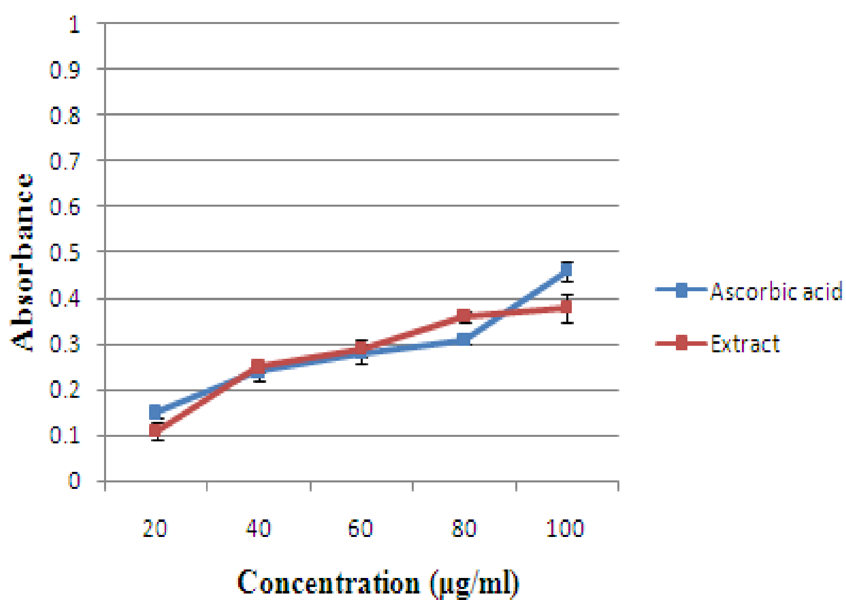

**Graph 2:** Graph displaying DPPH radical scavenging activity of ethyl acetate extract of *Cassia fistula*



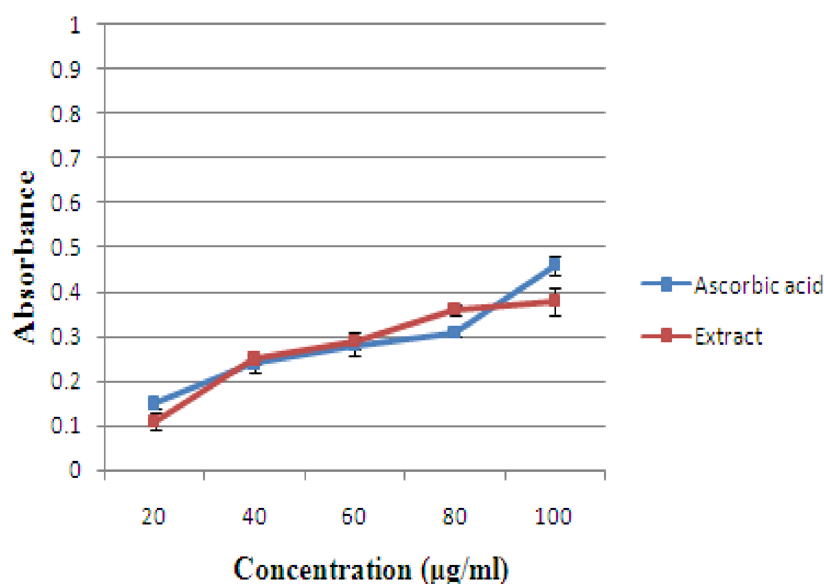

**Graph 3:** Graph displaying reducing power assay of ethyl acetate extract of *Aegle marmelos*




**Graph 4:** Graph displaying reducing power assay of ethyl acetate extract of *Cassia fistula*




**Graph 5:** Graph displaying nitric oxide scavenging activity of ethyl acetate extract of A*egle marmelos*




**Graph 6:** Graph displaying nitric oxide scavenging activity of ethyl acetate extract of *Cassia fistula*

different concentrations (20, 40, 60, 80, 100 µg/ml) and were shown in Graphs 3 and 4. Ascorbic acid was used as a reference standard.

**Nitric oxide radical scavenging activity**

Percent inhibition of the nitrite oxide generated is measured by comparing the absorbance values of control and test preparations and ascorbic acid was used as a positive control. The scavenging effects of ethyl acetate extract from leaves of *Aegle marmelos* and *Cassia fistula* on nitric oxide radical scavenging activity were examined at the different concentrations (20, 40, 60, 80, 100 µg/ml) and were shown in Graphs 5 and 6.

## CONCLUSION

The leaf extracts of *Aegle marmelos* and *Cassia fistula* showed varying degrees of antibacterial activity on the microorganisms tested as *Staphylococcus aureus*, *Salmonella typhi, Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*. The activity of leaf methanol extract was found to be quite good compare to ethyl acetate extract therefore they can be used as an external antiseptic in the prevention and treatment of bacterial infections caused by these pathogenic bacteria, which have developed resistance to antibiotics. This study demonstrated that the methanolic leaf extract of these plant are as effective as modern medicine to combat pathogenic microorganisms. It was observed that ethyl acetate has possessed prominent significant scavenging activity as DPPH, Reducing power and Nitric oxide scavenging when compared to the reference standard ascorbic acid. Ascorbic acid also has shown significantly increase in the scavenging activity [11,12].

## REFERENCES

[1] Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. *BMC Complement Altern Med*, **2008**, 8: 53

[2] Ali MA, Sayeed MA, Bhuiyan MSA, Sohel FI, Yeasmin MS. Antimicrobial screening of *Cassia fistula* and *Mesua ferrea*. *J Med Sci*, **2004**, 4: 24-29.

[3] Andrews JM. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother*, **2001**, 48: 5-16.

[4] Atata RF, Sani A, Ajewole SM. Effect of stem bark extracts of *Enantia chloranthaon* some clinical isolates. *Biokemistri,* **2003**, 15: 84-92.

[5] Divya BT, Mini S. *In vitro* radical scavenging activity of different extracts of *Butea Monosperma* bark. *Int J Curr Pharm Res*, **2011**, 3: 114-116.

[6] Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activity of *Cassia fistula* L.: An ethno medicinal plant. *J Ethnopharmacol,* **2007**, 112: 590-594.

[7] Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, et al. Analysis of nitrate, nitrite and nitrate in biological fluids. *Anal Biochem*, **1982**, 126: 131–138.

[8] Lai HY, Lim YY, Tan SP. Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. *Biosci Biotechnol Biochem*, **2009**, 73: 1362-1366.

[9] Obeidat M, Shatnawi M, Al-alawi M, Al-Zu`bi E, Al-Dmoor H, et al. Antimicrobial activity of crude extracts of some plant leaves. *Res J Microbiol*, **2012**, 7: 59-67.

[10] Okpekon T, Yolou S, Gleye C, Roblot F, Loiseau P, et al. Hocquemiller R. antiparasitic activities of medicinal plants used in ivory coast. *J Ethnopharmacol*, **2004**, 90: 91-97.

[11] Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Jpn J Nutr*, **1986**, 7: 307-315.

[12] Venkatachalam U, Muthukrishnan S. Free radical scavenging activity of ethanolic extract of *Desmodium gangeticum*. *J Acute Med*, **2012**, 2: 36-42.