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SCREENING OF SECONDARY METABOLITES, ANTIBACTERIAL AND ANTIOXIDATIVE CAPACITY OF *PAEDERIA FOETIDA* L. LEAF EXTRACTS

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ABSTRACT

Plant resources have been utilized since ancient times for the healing of various illnesses of humankind. This study investigated the secondary metabolites, antibacterial, and antioxidative capability of *Paederia foetida* L. (*P. foetida*) leaf extract. Qualitative tests were done to determine the presence of secondary metabolites. The disc diffusion method was used for the determination of the antibacterial activity of the extract. The DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, reducing power assay, total phenolic, and total flavonoid content methods were followed for the evaluation of antioxidative potentials of the leaf extract. The leaf extract was found to show significant ($p < 0.05$) antibacterial activity against Gram-positive bacteria compared to Gram-negative ones. In DPPH free radical scavenging assay, the inhibitory concentration (IC_{50}) value for methanol crude extract was found to be fairly significant (162.929 µg/mL) in comparison with that (1.57 µg/mL) of the reference standard ascorbic acid. The maximum absorbance for reducing power assay was 0.823 compared to 1.891 for standard ascorbic acid. The extract was found to have a total phenolic content of 8.03 ±1. 05 mg/g gallic acid equivalent and flavonoid content 134.74 mg/g quercetin equivalent. The results demonstrated a moderate antibacterial and promising antioxidative activity of *P. foetida* leaf extract, but it is necessary to further analyze prospective pharmaceutical preparation.

Keywords: *Paederia foetida*, Antioxidant, Reducing power, DPPH, IC_{50}

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INTRODUCTION

The research of medicinal plants for antioxidant activities is increasing day by day due to the positive impact on health. (Katalinic *et al*., 2006; Rafat *et al*., 2010). Many studies showed that some medicinal plants possessed more potent antioxidant activity than common dietary plants (Cai *et al*., 2004). In the case of free radicals, causing tissue ruptures is implicated in various diseases defined as any molecular species capable of independent existence that have an electron in an atomic orbital—the presence of electron results in certain common properties shared by most radicals. Most radicals are unstable and very sensitive to the reaction. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. So, free radical and antioxidants are now common for discussing the different disease mechanisms (Liu, *et al*., 2018; Lobo *et al*., 2010). Many researchers have reported on the food and vegetables containing flavonoids, terpenoids, and phenolic compounds with antioxidant activity (Brown and Rice-Evans 1998). However, the information on the biological effects of these plants is very few. As part of our continuous phytopharmacological work, we investigated secondary metabolites, antibacterial and antioxidant effects of *P. foetida* leaf extract. N-hexane, ethyl acetate, chloroform, and methanol fraction of *P. foetida* have significant antimicrobial activity (Morshed *et al*., 2012).

P. foetida L. commonly known as Gandhabhaduli, Chinese fever vine, Stink vine belonging to the family *Rubiaceae* of the order Gentianales and sub-kingdom *Viridiplantae* (Green plants) (Kirtikar *et al*., 1981, Nandkoni, 2002 and Khare, 2007) is an aggressive competitive vine with a fetid smell growing top into the canopy of trees in a variety of environments (Majumder *et al*., 2011). This is usually found in Bangladesh, India, Japan, Malaysia, Myanmar, Nepal, Thailand, Vietnam, Bhutan, Cambodia, and China (Christian, 1991). It occurs as a non-native species in Hawaii and Southwestern United States (Ewald, 2003, Pemberton 2002). In Bangladesh, *P. foetida* is mainly found in hilly areas of Chittagong and Sylhet (Ghani, 2003). The plant is rich in perennial triterpenoids, saponins, ursolic acid, epifriedelinol, and friedelin (Wong and Tan, 1994). The leaf and stem contain oxygen-containing monoterpenes, linalool, sulfur-containing compounds like dimethyl disulfide, hentriacontane, benzofuran. Iridoidglucosides like asperuloside, paederoside, scandoside, sitosterol, stigmasterol, and campesterol (Kirtikar and Basu., 1993). At the same time, the aerial parts of the plant contain Quinones like embelin and alkaloids like a-

paederine (Prieto *et al*., 1999).The leaves are cooked and taken as a remedy for indigestion and loose motion. Juice of the root is useful in piles, inflammation of the spleen, pain in the chest and liver (Yusuf *et al*., 2007). Ethanol extract of the whole plant possesses a wide range of good antibacterial and mild antifungal properties (Begum *et al*., 2007). Ethanolic extract of this plant has also been found to have analgesic and acute anti-inflammatory effects on experimental animal models (Das *et al*., 2012). It is reported that the fresh sample of *P. foetida* has higher antioxidant activity than the dried sample (Osman *et al*., 2009*),* and it is also found that the potential antidiarrhoeal properties when 90% ethanol extract was used against castor oil and magnesium sulfate-induced diarrhoea models in mice (Afroz *et al*., 2006).

In this present study, the effort was made to evaluate the potential antimicrobial and antioxidant activities of the methanolic extract of *P. foetida* leaf by *in vitro* approaches.

MATERIALS AND METHODS

Chemicals: Methanol (98%) was purchased from Sigma-Aldrich, USA. Mueller Hinton Agar Media from HiMedia, Mumbai, India, and tetracycline antibiotic disc from Thomas Scientific, USA, were procured. Standard ascorbic acid was purchased from BDH Laboratory Supplies, England. DPPH from Sigma-Aldrich, Germany. Ferric Chloride ($FeCl_3$) and Potassium ferricyanide [K_3Fe (CN) 6] were procured from Merck (Darmstadt, Germany) and May and Backer (Dagenham, UK), respectively. Quercetin and gallic acid were purchased from Sigma Chemicals Co., USA. All chemicals and reagents used in the study were of analytical grade.

Plant Collection and Taxonomical Identification

The fresh and fully matured leaves of *P. foetida* were collected from the Khagrachari hills (Chittagong Hill Tracts). Plant species were identified and taxonomically authenticated by Dr. Sheikh Bokhtear Uddin, Taxonomist and Professor, Department of Botany, University of Chittagong, Bangladesh. The voucher specimen of the plant has been deposited in the departmental Herbarium of the University of Chittagong with the accession number (AB-5726).

Preparation of extract

The collected fresh leaves of *P. foetida* were washed with distilled water and shade-dried for 14 days at room temperature. The leaves were chopped into small

pieces and ground into a coarse powder with a blender (Miyako, China) and stored in an airtight container. Dried 246 g powder was soaked into 750 ml 98% methanol for 7 days at room temperature (25 ±1)°C with occasional stirring. The methanol extract was filtered with Whatman No.1 filter paper after 7 days of digestion. The solvent was evaporated by Rotary evaporator (RE 200, Bibby Sterling, UK) with reduce pressure at 50°C. The collected concentrated extracts were allowed to air dry for complete evaporation of methanol. Blackish-green semisolid extracts (19.15 g, yield 7.78% w/w) were preserved at 4°C in the refrigerator until further use.

Preliminary phytochemical screening of extract

The preliminary screenings for qualitative phytochemicals (alkaloids, glycosides, cardiac glycosides, anthraquinone glycosides, flavonoids, terpenoids, tannins, steroid, saponin, and phlobatannins) were carried out with freshly prepared crude extract, which was determined by the presence of chemicals identified by using standard procedures (Sofowara 1993; Evans 1984; Ghani 1998).

Determination of total phenol content

The modified Folin-Ciocalteu method (Velioglu, 1998; Ren-You *et al*., 2010) was followed to determine the total phenolic content of the extract. A 0.5 ml of each extract (200 µg/mL) was mixed with 5 mL Folin-Ciocalteu reagent (1:10 v/v distilled water), and 4 mL (20 g/L) of sodium carbonate and the mixture was then vortexed for 15 seconds for the development of color then the mixture was incubated at 40°C for 30 min. Gallic acid solution was prepared by dissolving 5 mg Gallic acid in 50 ml distilled water (1:10 v/v). Then the absorbance was read at 765 nm using a UV-VIS spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan) against blank. All experiments were performed in triplicate. Total phenolic contents of the fractions were expressed as Gallic acid equivalents (GAE) after calculation using the following formula:

$$C = (c \times V)/m$$

Where, C = Total contents of phenolic compounds, mg/g plant extract in GAE; c = Concentration of Gallic acid obtained from calibration curve (mg/mL); V = the volume of the sample solution (ml) and m = weight of the sample (g).

Determination of total flavonoid content

By using the spectrophotometric method, the total flavonoid content of *P. foetida* was evaluated (Ganesan *et al*., 2008). One mL of sample (200 µg/mL) and standard (Quercetin) in different concentrations were taken in test tubes, and 3 mL of methanol was added into the test tubes. Then 200 µl of 10% aluminum chloride solution and 200 µL of 1 M potassium acetate solution was added to the mixtures into the test tubes and then diluted to 1.5 mL of distilled water. The suspension was incubated for 45 min at room temperature, and then the absorbance of the solution was measured at 760 nm using a UV-VIS spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan) against the blank. Methanol was used as the blank. All experiments were performed in triplicate. The percentage content of flavonoid, expressed as quercetin equivalent, the following equation was used for calculation:

$$C = (c \times V)/m$$

Where, C = Total contents of flavonoid compounds, mg/g plant extract in QE; c = Concentration of Quercetin obtained from calibration curve (mg/mL); V = the volume of the sample solution (ml) and m = weight of the sample (g).

Determination of the antioxidant activity of *P. foetida*

DPPH free radical scavenging activity of plant extract was determined on the basis of scavenging potential of stable DPPH free radical at 517 nm by the modified spectrophotometric method as described (Braca*et al*., 2001; Sanchez-Moreno *et al*., 1998). The radical scavenging potential of the sample was determined by measuring the decrease in absorbance due to $DPPH^{\cdot}$ at 517 nm, representing the formation of its reduced form, 1, 1-Diphenyl-1-2- picrylhydrazine (DPPH), which was yellow in color. Because of the odd electron, the purple-colored methanol solution showed a strong absorption band at 517 nm.

Preparation of ascorbic acid, plant extract and DPPH solution

Hundred mg of plant extract was dissolved in 20 mL methanol to give the final concentration of 5 mg/mL. In the same way, 100 mgascorbic acid was dissolved in 20 mL methanol. A 0.002% DPPH or 0.002g DPPH was dissolved in 100 mL methanol.

Procedure

The plant extract and ascorbic acid were usedin seven different concentrations (10, 20, 40, 80, 160, 320, and 640 µg/mL) for this study. For each concentration, 1 mL of DPPH solution was mixed with 1 mL of sample solution and standard. For blank, 1 mL of DPPH solution was mixed with 1 ml of methanol to see whether the solvent used has any antioxidant activity or not. These suspensions were shaken vigorously and incubatedin the dark for 30 min at room temperature. After 30 min absorbance of the resulting solution was measured at 517 nm using a UV-VIS spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan). The percentage DPPH radical scavenging activities (% SCV) were calculated using the following formula.

$$\text{Percent (\%) inhibition of DPPH} = \frac{A_0 - A_1}{A_0} \times 100$$

, where, A_0 = Absorbance of the control and A_1 = Absorbance of the test (extracts / standard).

Reducing power assay

The reducing power of the methanolic extract of *P. foetida* leaves was assayed, followedby themethod of Dhu *et al*., 1999 with slight modification (Hemayet *et al*., 2012). In this method, the yellow color of the test solution was changed to numerous shades of green and blue that depending on the reducing power of the antioxidant of samples. The availability of antioxidant substances in the samples causes the reduction of the Fe^{3+}-ferricyanide complex to the ferrous form by donating an electron. The Fe^{2+}complex was monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu 1985).

$$Fe^{3+} + \text{Potassium ferricyanide} + e^- \longrightarrow Fe^{2+} + \text{Potassium ferrocyanide}$$

Different concentrations of the extract (10-160 µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The suspension was then incubated at 50°C for 20 min, and 2.5 mL of TCA (10%) was added to it. Later, it was centrifuged at 3000 rpm for 10 min. The supernatant of a mixture (2.5 mL) was collected and mixed with 2.5 mL of distilled water, 0.5 mL of 0.1% $FeCl_3$. Finally, the absorbance of the mixture was measured at 700 nm with the same spectrophotometer. An increase in absorbance of the sample with concentrations indicates a high reducing potential of the samples. The experiment was carried out thrice, and the results were expressed as mean ± standard deviation (SD). Ascorbic acid was used as the standard reference compound in this study.

Antibacterial effects of the extracts

Bacterial strains used in the study

For the present study, we have selected four Gram-positive and six Gram-negative bacteria (Table 1). All the test bacterial strains were collected from the Department of Microbiology, University of Chittagong, Chittagong- 4331, Bangladesh.

TABLE 1: LIST OF GRAM (+) VE AND GRAM (-) VE BACTERIA USED FOR THE STUDY.

Type of bacterial strain	
Gram (+) ve	Gram (-) ve
Bacillus cereus	*Vibrio cholera*
Bacillus subtilis	*Shigellasonnei*
Bacillus megaterium	*Shigelladysenteriae*
Staphylococcus aureus	*Escherchia coli*
	Salmonella typhae
	Pseudomonas sp.

Preparation of plant extract solution

A 1.0 g of *P. foetida* extract was accurately weighed and dissolved in 10 mL of DMSO to give a solution of known concentration (100µg/mL). DMSO was chosen as a solvent because, in addition to its ability to dissolve crude extract, it has no inhibitory effect on the cultures.

Assay of in vitro antibacterial test by disc diffusion method

The disc diffusion method was performed for the antibacterial activity of plant extracts (Dekkers *et al*., 1996). In this experiment, 38 g of Mueller Hinton Agar Media was dissolved in 1000 mL of distilled water, and the final pH: 7.3 ±0.1. The discs of about 4 mm in diameter were prepared by punching machine from Whatman's No.1 filter paper. The sterilized discs were taken in a Petri dish, and 0.1 mL of bacterial culture was inoculated through the spread plate technique. The previously marked zone was used for placing the sample discs, standard antibiotic discs (Tetracycline), and the other control (DMSO) discs were used side by side on the agar plates where 30 µg/disc of Tetracycline and 1% DMSO served as positive and negative control respectively. The plates were then allowed to be kept in a refrigerator at 4°C for 2 hr to permit the sufficient diffusion of the

materials around the discs on the agar medium. The plates were incubated at 37°C for 24 hr. The experiment was carried out thrice.

Statistical analysis

Data were expressed as Mean ± SD. Statistical calculations were performed with Microsoft Excel (Microsoft office 2007) followed by a linear regression model.

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening

Qualitative phytochemical screening of the crude methanol leaf extract of *P. foetida* revealed alkaloids, glycosides, cardiac glycosides, terpenoids, flavonoids, and steroids, tannins, and saponins. Results for the phytochemical characters of *P. foetida* are summarized in Table 2. The characterization of these metabolites is indispensable in potentiating the plant sources for therapeutic approaches because huge numbers of plants have long been used to treat numerous diseases.

TABLE 2: RESULTS OF PRELIMINARY PHYTOCHEMICAL SCREENING.

Alkaloids	Glycosides	Cardiac Glycosides	Anthraquinone glycoside	Terpenoids	Flavonoids	Steroids	Tannins	Saponins	Phlobatanins
+	++	++	-	+++	++	+++	+++	+++	-

Here, (-) = Absence, (+) = Present, (++) = Moderately present, (+++) = Appreciable amount

Antioxidant activity of *P. foetida*

Total phenolic content

Phenols are essential components of plants. Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers. Therefore, it was reasonable to determine the plant extract's total phenolic content (Bauer *et al*., 1996). Plant extracts with high phenolic contents also show high flavonoid content reported for other plant species (Kumbhare *et al*., 2012). Reactive oxygen species (ROS) have been considered to cause harm to the body of living organisms and thus play a significant role in many human diseases such as atherosclerosis, arthritis, myocardial infarction, diabetes mellitus, and cancer (Elzaawely and Tawata, 2012; Gupta *et al.*, 2007). The amount of total phenolic content was calculated as quite significant in the methanol crude extract of *P. foetida* (8.0314 ±1.05 mg/g) of gallic acid equivalent (Table 3). Previous investigations showed that *P. foetida* was low in total phenolic content than other fruit and leaf samples of several plants (Oyedemi and Afolayan 2011). The test extract's total phenolic content was calculated using the standard curve of gallic

acid (y= 0.012x + 0.403, R2=0.987) (Figure 1). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily through a diet rich in fruits and vegetables (Lim *et al*., 2007).

TABLE 3: DETERMINATION OF PHENOL CONTENT OF *P. FOETIDA* METHANOL LEAF EXTRACT.

Extract	Amount of sample (µg/ml)	Avg. absorbance of sample	Total phenolic content
			mg of gallic acid equivalent (GAE) per g of dry extract
Methanol extract of *P. foetida* leaves	200	0.15±0.003	8.031 ±1.052

Each value is mean ± SD of three independent measurements

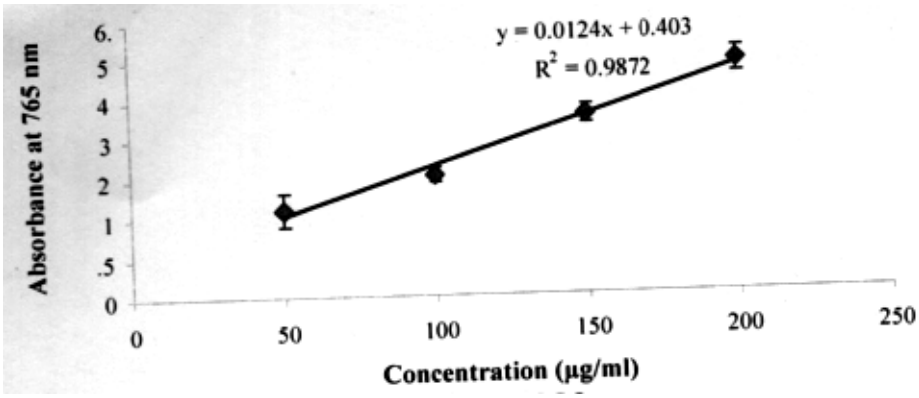

FIGURE 1: REGRESSION LINE OF GALLIC ACID (STANDARD) FOR DETERMINING TOTAL PHENOLIC CONTENT.

Total flavonoid content determination

The total flavonoid content was calculated using the standard curve of Quercetin (y= 0.009x- 0.039, R^2=0.992) (Table 4 and Figure 2) and was expressed as Quercetin equivalents (QE) per gram of the plant extract. Many researchers are showing interest in screened flavonoids due to the high potential of antioxidant activity and medicinal plants' positive health benefits. Accordingly, these studies showed that *P. foetida* possesses more potent flavonoids, which consequently contribute to antioxidant activity and antibacterial activity.

TABLE 4: DETERMINATION OF FLAVONOID CONTENT OF *P. FOETIDA* METHANOL LEAF EXTRACT.

Extract	Amount of sample (µg/ml)	Avg. absorbance of sample	Total flavonoid content
			mg of quercetinequivalent (QE) per g of dry extract
Methanolic extract of *P. foetida* leaves	200	0.22±0.003	134.74±0.89

Each value is mean ± SD of three independent measurements

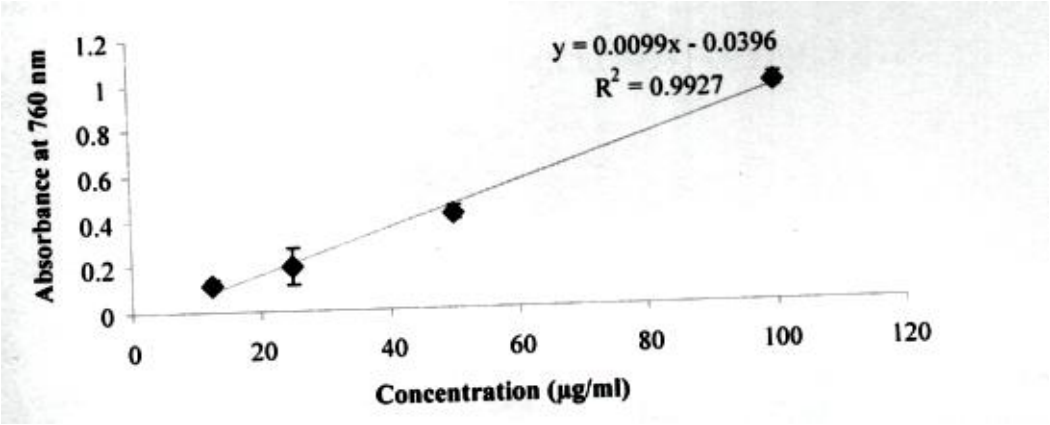

FIGURE 2: REGRESSION LINE OF QUERCETIN (STANDARD) FOR DETERMINING TOTAL FLAVONOID CONTENT.

DPPH radical scavenging assay

The radical scavenging activity of DPPH was evaluated by the degree of color change from purple to yellow and by the decrease in its absorbance at 517 nm. Among the seven different concentrations used in the study (10, 20, 40, 80, 160, 320 and 640 µg/mL), ascorbic acid showed 60.88%, 66.59%, 72.94%, 78.61%, 80.21%, 85.41% and 89.29% scavenging activity where the highest scavenging activity was 89.29% at concentration 640 µg/mL. On the other hand, *P. foetida* methanol leaf extract showed 19.73%, 26.04%, 37.74%, 43.86%, 51.45%, 55.78%, and 63.18% scavenging activity at the above mentioned seven different concentrations where the highest scavenging activity of *P. foetida* methanol extract was 63.18% at concentration 640 µg/mL. Percent of scavenging activity or percent of inhibition was plotted against log concentration, and from the graph, IC_{50} value was calculated by linear regression analysis. The IC_{50} value of ascorbic

acid and *P. foetida* methanol extract was 1.57 µg/mL and 162.92 µg/mL, respectively (Figure 3); it can be used to cut the generation of reactive oxygen species in the patient. The scavenging activity has many therapeutic uses to protect against the harmful biological effects of free radicals, especially against different humans' diseases (Lobo *et al*., 2010).

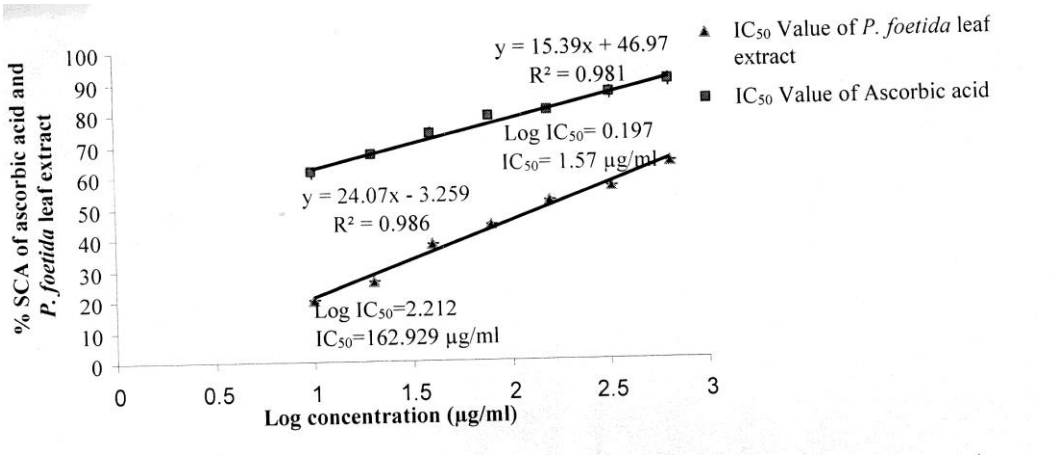

FIGURE 3: COMPARISON OF ANTIOXIDANT ACTIVITY OF ASCORBIC ACID (STANDARD) AND *P. FOETIDA* LEAF EXTRACT. DATA WERE EXPRESSED AS MEAN ± SEM AND WERE ANALYZED BY MICROSOFT EXCEL (MICROSOFT OFFICE 2007).

Reducing power assay

Ascorbic acid was used as a positive control for the determination of reducing power of methanolic leaf extract of *P. foetida*. Among the five different concentrations used in the study (10, 20, 40, 80, 160µg/mL), ascorbic acid was found to show absorbance of 0.94, 1.43, 1.51, 1.64, and 1.89 nm, whereas the plant extract was showed 0.32, 0.11, 0.41, 0.54, and 0.82 nm respectively. The maximum absorbance for methanolic extract was found to be 0.82 nm at a 160 µg/mL concentration. On the other hand, standard ascorbic acid was shown 1.89 nm, at the same concentration (Figure 4). So it is revealed that the increase of absorbance of both samples and standard simultaneously with their concentrations accordingly. A direct correlation between reducing power and antioxidant capacity of certain plant extracts has already been reported (Bors,*et al*., 1992).

Thus, the absorbance of *P. foetida* and ascorbic acid (standard) was calculated at five different concentrations Figure 4. Data reported three replications. Values are expressed as mean ± SD.

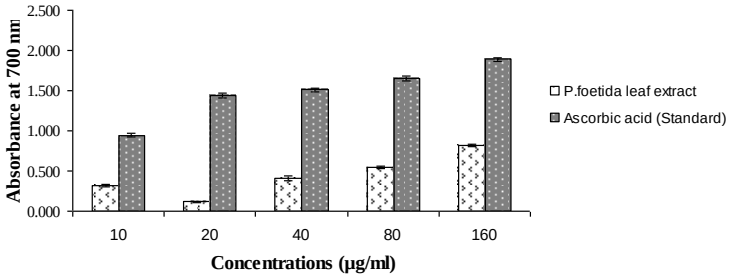

FIGURE 4: COMPARATIVE REDUCING POWER ASSAY OF ASCORBIC ACID (STANDARD) AND *P. FOETIDA* LEAF EXTRACT. DATA WERE EXPRESSED AS MEAN ± SEM AND WERE ANALYZED BY MICROSOFT EXCEL (MICROSOFT OFFICE 2007).

***In vitro* antibacterial screening of *P. foetida*methanol extract**

Antibacterial activity of *P. foetida* methanol extract was studied against four Gram-positive and six Gram-negative bacteria by the disc diffusion method compared with the standard antibiotic disc tetracycline (30 µg/disc). Antibacterial activities of leaf extract were measured at the concentration of 1, 2, and 3 mg/disc. All three concentrations of plant extract showed dose-dependent antibacterial activity. The zone of inhibition was also increased with the increase in extract concentration. More specifically, leaf extract showed 6 mm, and 5 mm zone of inhibition against four tested Gram-positive bacteria like *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*)**,** *Bacillus cereus* (*B. cereus*), *Bacillus megaterium* (*B. megaterium*), respectively, and 5.5 mm and 5 mm zone of inhibition was observed against six tested Gram-negative bacteria like *Salmonella typhae* (*S. typhae*) and *Shigella dysenteriae* (*S. dysenteriae*), *Escherichia. coli* (*E. coli*), *Vibrio cholera* (*V. cholera*), *Shigella sonnei* (*S. sonnei*), and *Pseudomonas sp.* respectively. On the other hand, standard antibiotic tetracycline (30 µg/disc) showed significant antibacterial activity against all the tested Gram-positive and Gram-negative bacteria (Figure 5).

The *P. foetida* methanol extract with 2 mg/disc showed 5 mm in diameter of zone

of inhibition against two bacteria like *V. cholerae, S. sonnei*, 5.5 mm zone of inhibition against five bacteria like *B. subtilis, B. megaterium, S. dysenteriae, E. coli*, and *S. typhae,* 6.5 mm zone of inhibition for *S. aureus, B. cereus*, 7.5 mm in diameter of zone of inhibition was found against *Pseudomonas sp*. Furthermore, the *P. foetida* extract with 3 mg/disc exhibited a moderate zone of inhibition 10 mm in diameter against two bacteria like *S. aureus and Pseudomonas sp.* Again it was recorded that slightly less inhibition zone (9.5 mm and 8.5 mm) against *B. cereus and B. subtilis*. The standard antibiotic tetracycline (30 µg/disc) showed significant antibacterial activity against all tested Gram-positive and Gram-negative bacteria. A comparative study of bacterial sensitivity towards plant extract and the standard antibiotic is shown in Figure 5.

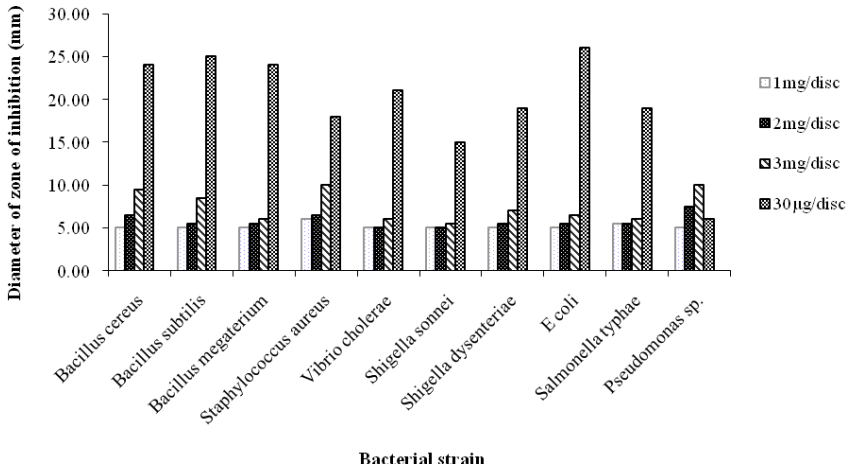

FIGURE 5: COMPARATIVE ANTIBACTERIAL ACTIVITY OF *P. FOETIDA* LEAF EXTRACT AT DIFFERENT CONCENTRATIONS.

CONCLUSION

Finally, it is concluded that the methanolic leaf extract of *P. foetida* was more effective against Gram-positive bacteria than Gram-negative bacteria, whereas antioxidant activity was promising. It is maybe the effectiveness of plant phytochemicals like flavonoids and phenolic compounds. Additionally, the

potential antioxidant values make it a good possibility for cosmetics, foods, and drugs against free radicals-induced damages. Hereafter, we attempt to identify and isolate the novel pharmaceutical compound responsible for these therapeutic properties utilizing sophisticated instruments by HPLC, GC-MS, and NMR.

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