# EVALUATION OF THE BIOLOGICAL ACTIVITIES OF FRUIT OF *Diospyros malabrica (*KOSTEL).

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**ABSTRACT:** The current study encompassed the extraction of phytochemicals from the fruit of a well-known traditional medicinal tree, viz. *Diospyros malabarica* with different solvents and evaluation of their *in-vitro* antimicrobial and antioxidant activities using standard methods and standard drugs. Preliminary phytochemical investigation demonstrated the presence of some fascinating secondary metabolites like flavonoids, terpenoids, alkaloids, saponins and phenolic compounds. Also, the solvent extracts exhibited promising antimicrobial activity (60-80 mm, inhibition zone) against *Staphylococcus saprophyticus*, *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus parasiticus* and *Rhizopus oryzae*. The fruit extract showed scavenging antioxidant potential (>80%), total antioxidant content (>0.4) and total phenolic contents (>0.18 mg of gallic acid equivalents). This indicated that their antioxidant potential is even better than those of many standard antioxidants *i.e.* α-tochopherol, vitamin E and BHT. Based on the results, it was therefore proposed that the organic extracts of *D. malabarica* fruit should be propagated as modern and least harmful antimicrobial drug and antioxidant to combat the infectious diseases.

**Keywords:** *Diospyros malabarica* fruit, Antimicrobial activity, Total antioxidant content, Total phenolic content.

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## INTRODUCTION

Presently, the use of herbal plants as remedies is getting more prominent significance. Worldwide, various medicines are made using plant extracts, showing lesser contra-indicative symptoms as compared to compound-based medicines. According to World Health Organization, 65 percent of the world population depends on herbal medicines as main source of treatment for their illness. (Fabricant and Farnsworth, 2001). It is assumed that about 25% of the chemical drugs are derivatives of herbal medicines (Verpoorte, 2010). Examples of these include: analgesics (morphine), cardiotonics (digoxin), antioxidants (paclitaxels) and antimalarials (quinine) various secondary metabolites of medicinally importance including flavonoids, tannins, tarpenoids and alkaloids are also produced by plants. Various plants also shown anticancer activity for instance roots of *Peganum harmala* (Ayoob *et al.,* 2017) showed anticancer activity against breast carcinoma, *Curcuma longa* exhibited anticancer activity against colon cancer (Ooko *et al.,* 2017). However, the medicinal characteristics of many plant species still need to be explored.

Other Secondary metabolites are important for plants in defense mechanisms against tissue damage and pathogen attack *i.e.* glucosinolates and cyanogenic glycoside, are usually present as inactive precursor and stimulated as the reaction to tissue damage or pathogen attack. This type of stimulated production of compounds often comprises of plant enzymes, which are excreted as outcomes of disturbance in cellular environment (Van Etten *et al*., 1994).

*Diospyros malabarica* Kostel (Gaub) is known as a medicinal plant which belongs to the family *Ebenaceae.* It is an evergreen tree having leaves spreading in the patty crown. The leaves are glossy and of longitudinal shape. The flowers are creamish in color having a hard texture; these are arranged in clusters of 3-6. The fruit of this plant is thought to contain antimicrobial, antioxidant, blood purifying, anticancer and neuroprotective bioactive compounds (Ravikumar *et al*., 2014). Influenced by the pre-detailed restorative data of *D. malabarica* and its utilization in various local remedies, we focused our investigations on phytochemical constituents and therapeutic properties of this plant.

Present study aims to profile and probe the phytochemicals from *D. malabarica using* extracted with nonpolar and polar organic and aqueous solvents (chloroform, petroleum ether, distilled water and methanol) and to further subjected for in vitro antimicrobial properties.

## MATERIALS AND METHODS

**Collection of Plant material and extraction of phytochemicals:** *Diospyros. malabarica* fruit was obtained from the Jinnah Garden Lahore, Pakistan

throughout the summer season. The fruits were identified by Flora of Pakistan. Voucher specimen (GC.Bot.Herb.954) was prepared and deposited in the herbarium of the Government College University, Lahore, Pakistan. The fruits were washed with tap water and dried under shade (20-25°C) for 10 to 15 days followed by drying and further pulverized into fine powder. About 60g fruit powder was dissolved in different solvents separately one by one to make different solutions. Different solvents selected were petroleum ether (DPF), chloroform (DCF), methanol (DMF) and water (DWF) (200 mL ×2, of each solvent) and further each solvent-extract solution was shifted on continuous shaking for three days. The extracts were evaporated until dryness in vacuum based incubator and storage of each extract was done at cold temperature for future use (Malik *et al.*, 2015). The yield of extraction was calculated as:

$$\text{Extraction yield (\%)} = \frac{\text{Wt. of the dried extract (g)}}{\text{Wt. of the plant powder (g)}} \times 100$$

**Preliminary phytochemical assay:** Extracts in different solvents were freshly prepared and stored in large quantity for performing different types of assays. The extracts were further modified by following previously developed methods of phytochemical analyses (Harborne, 1998; Kokate, 2000) for the purpose of phytochemical detection, *viz.* flavonoids, terpenoids, alkaloids, phenolics and glycosides.

**Antibacterial screening:** *In-vitro* antimicrobial potential of the fruit extracts was performed against four pathogens including *Echerichia coli* (ATCC-2590), *Pseudomonas aeruginosa (*ATCC-2591*), Staphylococcus saprophyticus (*ATCC-2592*)* and *Staphylococcus aureus* (ATCC-2593) using agar well diffusion method (Oretaga *et al*., 1996). Nutrient agar was prepared by following method of Cruickshank *et al*., 1975. Bacterial slants were prepared, bacteria were inoculated, and slant culture was incubated at 37ºC for 24 hours to be used as bacterial inoculum (Jett *et al*., 1997). The agar plate was inoculated by spread plate technique (Wise, 2010). Antimicrobial activity was checked for each fruit extract which was made in different solvents independently. Stopper borer No. 4 was used to burrow a uniform gap (approximately 8.0 mm) in the medium. Crude extracts (0.5 mL) were poured in wells. On account of the extension of the same experiment to check antibacterial activity of positive control was applied in the same way as plant extracts. (Amikacin, 30 µg).

**Antifungal screening:** The fruit extracts were screened for antifungal potential against *A. parasiticus* and *R. oryzae* by agar well diffusion method on potato dextrose agar (PDA) as described by Malik *et al*., (2010). Antibacterial standards were Carbenicillin (Py 100), Ampicillin (AM 10), Trimethoprim (SXT 25) Ampicillin (AM 10), Kanamycin sulphate (5 mg/ml), and antifungal standards were Ketoconazole (5mg/ml), Fluconazole (5mg/ml)

After the incubation period of 24 hours for bacteria and 72 hr for fungi in incubator, the zone of inhibition produced by organic fruit extracts against microorganisms was estimated by comparing both zones produced by standard drugs and the fruit extract. The zones of the inhibition for bacterial strain were also calculated by using pure solvents as control. The zone of inhibition was calculated by following the method of (Koch, 1994).

$$\text{Zone of inhibition} = \text{Zone of inhibition}_{(\text{plant extract})} - \text{Zone of inhibition}_{(\text{control})}$$

1,1-Diphenyl-2-picrylhydrazyl **radical scavenging assay**

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical test was used to measure free radical scavenging potential of extracts as described by Erasto *et al*. (2004) with minor changes. Equal volumes (2.0 mL) each of crude extracts (0.5 mg/mL) and DPPH were taken in test tubes and wrapped with aluminum foil for providing dark environment, incubated at 30°C in an incubator with control. Control solution consisted of equal volume of DPPH and solvents were considered as blank ($A_{blank}$). The change in color was measured at 517 nm. Butylated hydroxytoluene (BHT) and α-tocopherol were considered as positive controls. The radical scavenging assay (RSA) was measured using following formula:

$$\text{Inhibition of DPPH} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

**Total antioxidant assay:** The method of Prieto *et al*. (1999) was utilized to estimate antioxidant capability of the fruit extracts. Two standards (BHT and α-tocopherol) were used for this purpose. A 0.1 ml of fruit extracts with a concentration of 0.5 mg/ml and 1.9 ml of reagents (4 mM of ammonium molybdate, 28 mM sodium phosphate and 0.6 M of sulphuric acid) were mixed in a test tube, covered and placed into at a temperature of 95ºC for 90 minutes. The samples were drawn out of thermal block, cooled to room temperature (25ºC) absorbance of fruit extract samples and control was measured at 695 nm against blank.

**Total phenolic contents:** Makkar *et al*. (2006) protocol was used to measure total phenolic contents of the fruit extracts. A volume of 0.1 ml fruit extracts with a concentration of 0.5 mg/mL, 0.1 mL of 2N folin Ciocalteu and 2.8 mL of 10% sodium carbonate were mixed in a test tube. After 40 minutes wait at room temperature, the absorbance was measured at 725 nm.

Total phenolic content = mg of Gallic acid equivalent/gm of fruit extracts

**Mean ± S.E. (standard error):** All the *in vitro* experimental results were presented as mean ± S.E. (standard error) of three replicates and statistical analyses were performed using BIOSTAT statistical software.

## RESULTS

**Preliminary phytochemical investigation:** Physical appearance, color, yield and phytochemical screening of different extracts of *D. malabarica* fruit are described in Table 1, The highest yield of extracted phytochemicals were obtained from methanolic extract i.e 1.94%, while chloroform (1.49%), petroleum ether (1.49%), and water (1.36%) extracts also produced significant amount of yield. The yield demonstrated the presence of higher quantity of flavonoids, terpenoids and phenolics in the fruit extracts. Alkaloids, glycosides and saponins were also noted in few extracts.

**Antimicrobial activity:** Results showed that among all antibacterial standard discs Carbenicillin (Py 100), Ampicillin (AM 10), Trimethoprim (SXT 25) Ampicillin (AM 10), Kanamycin sulphate (5 mg/ml), and antifungal standards Ketoconazole (5mg/ml), Fluconazole (5mg/ml), Ketoconazole had maximum inhibition zone against *A. parasiticus*. The maximum inhibition zone was observed in Amikacin against all bacterial strain, which demonstrated its distinctive antibacterial potential against various bacterial strains (Figures 1-3).

The findings of the antimicrobial screening of extracts against two fungal and four bacterial strains are given in Fig-4. The concentrated extracts of *D. malabarica* fruit indicated diverse inhibitory impacts against every microorganism tested,

**1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity:** Free radical is scavenging action of different fruit extracts are presented in Fig-5. Results showed that DPF yielded the highest free radical scavenging activity i.e. 83.46% against the standard control i.e. BHT 77.3%.

**Total antioxidant assay:** Antioxidant potential of *D. malabarica* fruit extract is presented in Fig-6. The investigation of the result showed that methanolic and petroleum ether extract had the highest antioxidant activity among all antioxidants.

**Total phenolic contents:** The total phenolic content of *D. malabarica* fruit extracts are presented in Fig-7 and 8. The DWF and DPF showed the highest concentrations of phenolic substances followed by the DMF and DCF.

**Table 1: Phytochemical, yield, color and consistency of extracts of Diospyros malabrica fruit.**

| Part used | Solvents | Extraction yield (%) | Color and consistency | Terpenoids | Alkaloids | Glycosides | Phenolics | Saponins |
|---|---|---|---|---|---|---|---|---|
| **Fruit** | Petroleum Ether | 1.49 | Yellowish gum | +++ | + | ++ | ++ | ++ |
| | Chloroform | 1.49 | Greenish gum | ++ | + | + | + | + |
| | Methanol | 1.94 | Reddish gum | +++ | ++ | + | ++ | ++ |
| | Aqueous | 1.36 | Reddish gum | ++ | + | + | + | + |

Key of Table; +++ defines maximum yield, ++ denotes moderate level of concentration while + denotes scarce amount.

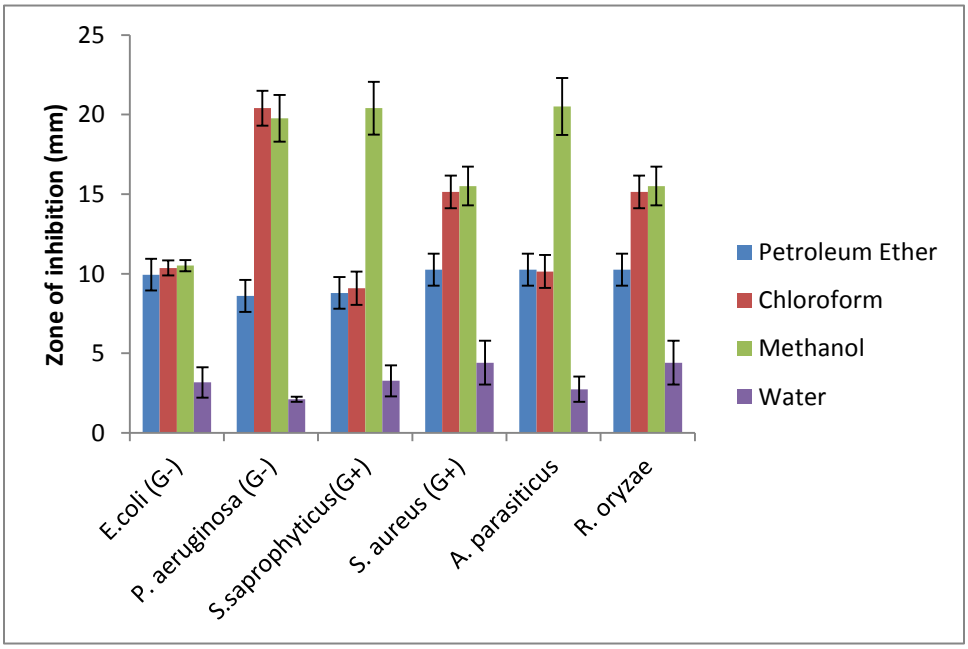


**Figure-1: Zone of inhibition (mm) by pure solvents against microorganisms (Blanks)**

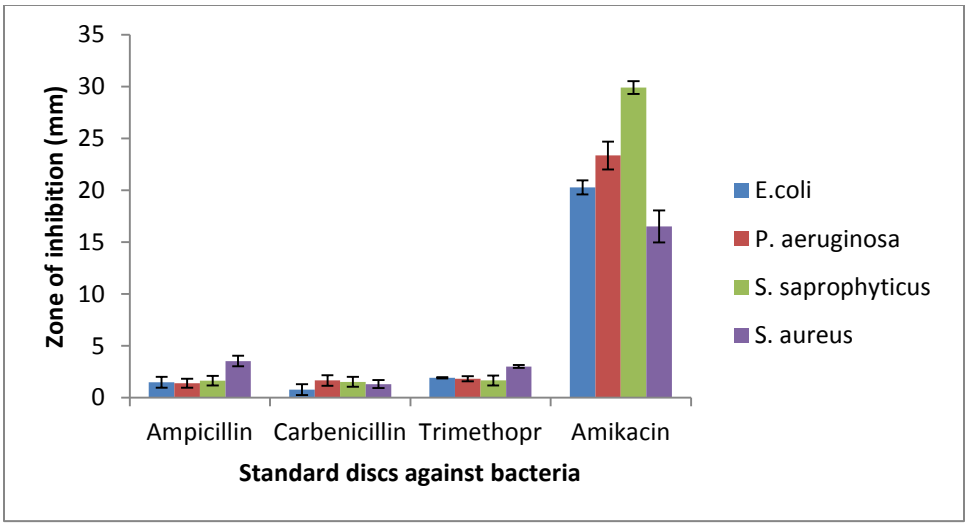


**Figure 2: Zone of inhibition (mm) by standard drugs against bacterial strains positive control for antibacterial activity.**

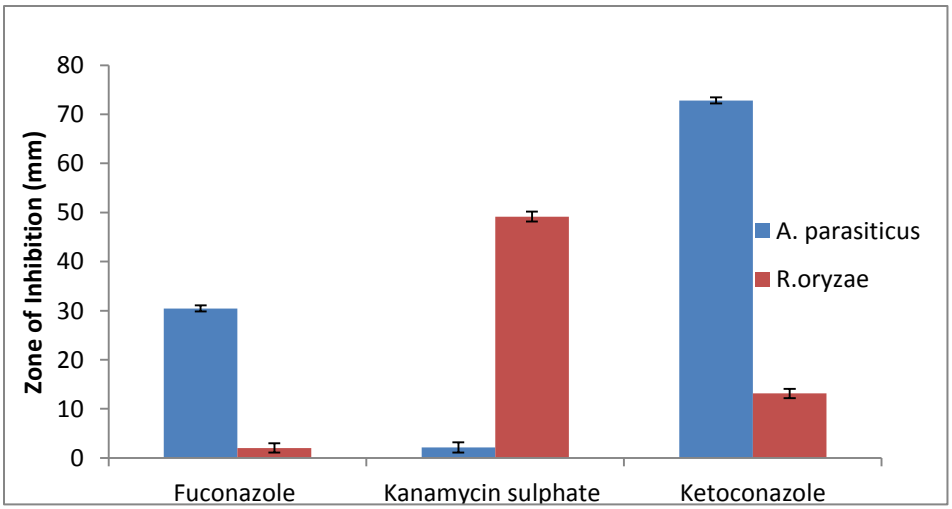


**Figure 3: Zone of Inhibition by fungal medicines against fungal strains (Positive control for antifungal activity)**

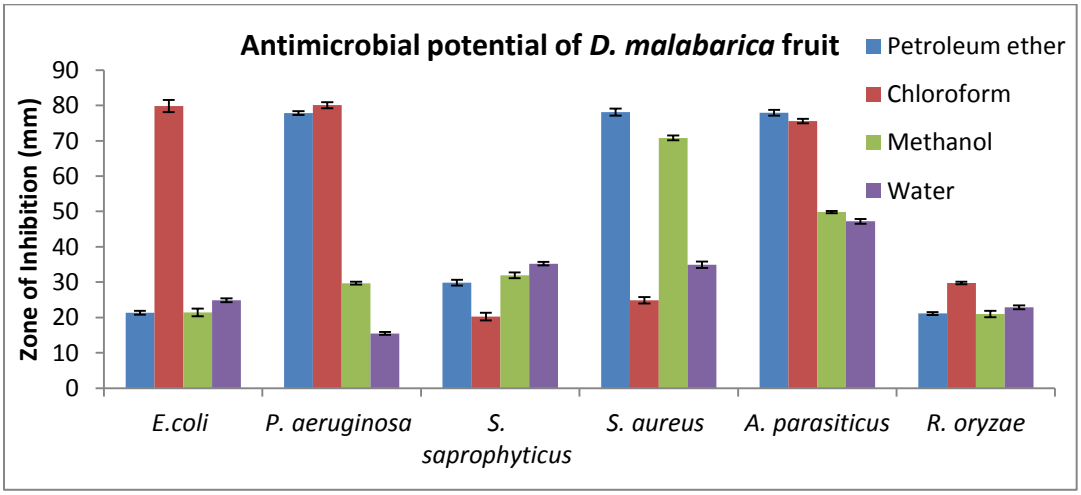


**Figure 4: Zone of Inhibition by fruit extracts against all microorganisms (Anti bacterial and antifungl activity of samples or plant fruit extracts)**

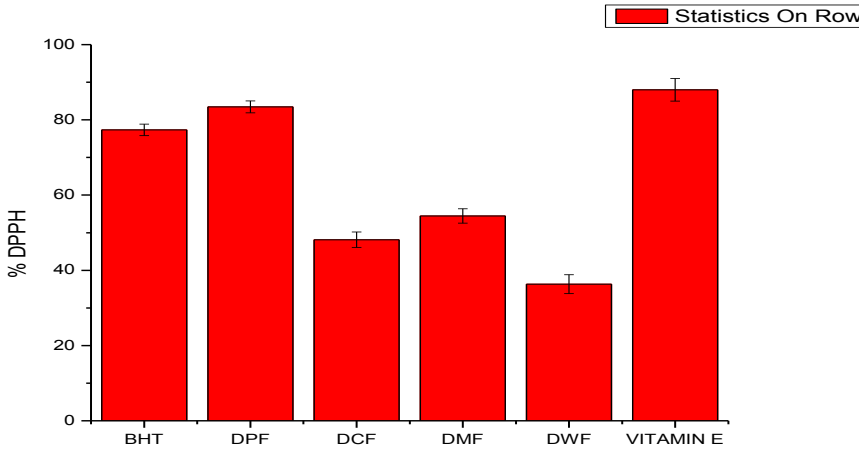


**Figure 5: % DPPH Free Radical Scavenging Activity of *D.malabarica* fruit extracts (DPF, DCF, DMF and DWF) against standards where as BHT and Vitamin E are serving as standards positive controls.**

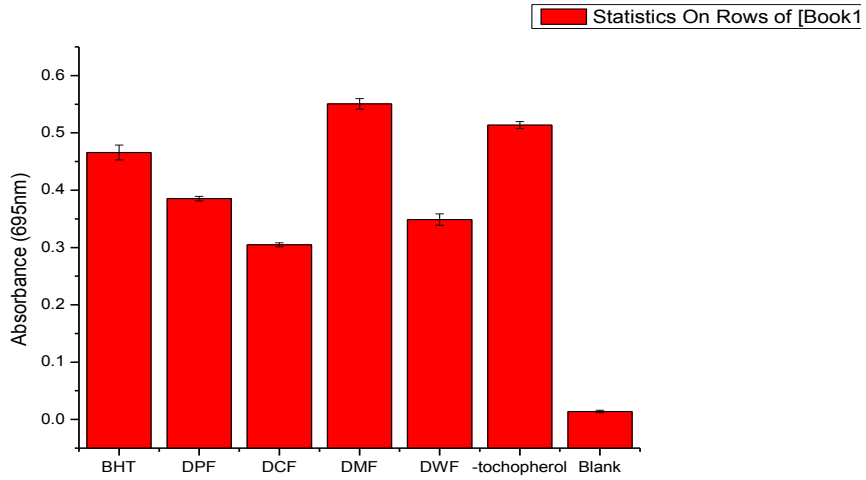


**Figure 6: Total antioxidant potential of *D. malabarica* fruit extracts (DPF, DCF, DMF and DWF) against standards or positive controls (while tocopherol and BHT are positive controls)**

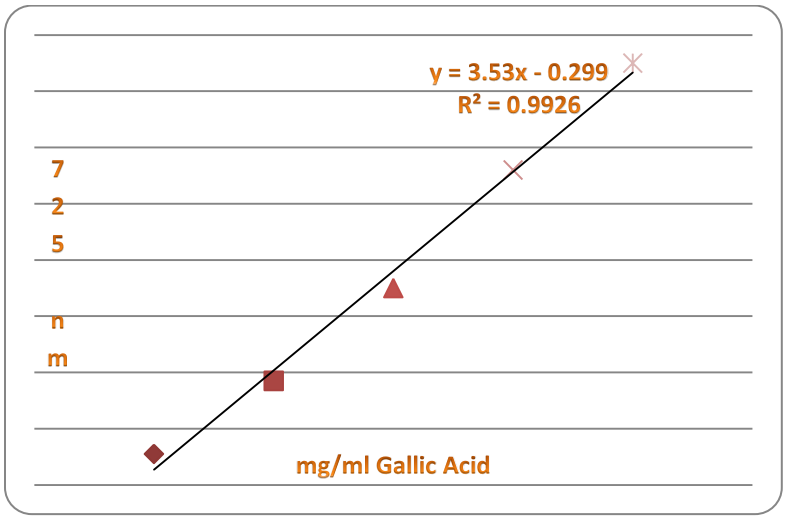


**Figure 7: Gallic acid standard curve: A galic acid standard curve is made to determine phenolic acid quantity.** Firstly a stock of galic acid is prepared in phenol and than diluted to different dilutions. Then the absorbance of these dilutions are taken at 760nm. The absorbtion values are plotted against concentrations to get standard curve.

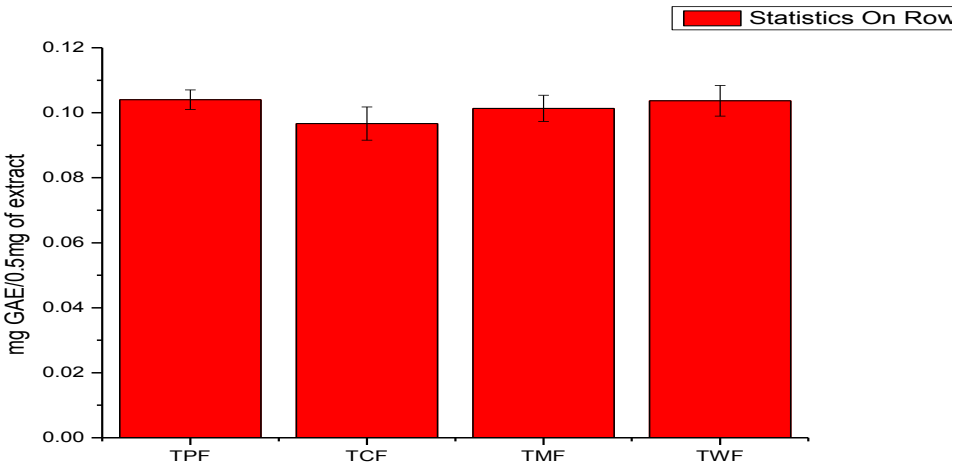


**Figure 8: Total phenolic contents in *D. malabarica* fruit extracts**

## DISCUSSION

At present, there is a requirement for new antimicrobial medicines and with the least adverse effects. The fruit extracts of *D. malabarica* showed to contain sufficient amounts of bioactive agents namely alkaloids, terpenoids, and flavonoids. Literature data show that plants containing various bioactive agents and their byproducts have powerful antibacterial and antifungal activities (Lwu *et al*., 1999). For example, terpenoids and flavonoids have strong antimicrobial activity because of their property to form complex with extracellular as well assoluble proteins and also with bacterial cell walls (Achmad *et al*., 2019). Flavonoids are the promising antioxidant as they disturb microbial metabolic activity. These compounds may also be viable against fungal microbes (Souza-Moreira *et al*., 2019) and viruses (Cowan, 1999). Alkaloids are likewise successful against viral and protozoan contaminations and ailments as they intercalate with DNA of microorganisms (Cowan, 1999). Saponins are bacteriostatic, antiviral and have expectorant properties (Cseke *et al*., 2006). Essentially, terpenoids are notable for antimicrobial and cancer prevention properties. A few tannins were also reported as cytotoxic and anticancer (Aguinaldo *et al*., 2005). Due to the presence of these bioactive phytochemicals in the fruit of *D. malabarica*, it is used as curative in local remote areas of Pakistan.

In our research, the fruit extracts of *D. malabarica* plant revealed critical antimicrobial activity against *S. saprophyticus*, *S. aureus*, *E. coli*, *P. aeruginosa*, *A. parasiticus* and *R. oryza* even more than many standard antibacterial and antifungal drugs. The presence of terpenoids, alkaloids, and flavonoids and their unique combinations in these extracts might also be the explanation behind great antimicrobial action. It was noted that fruit extracts have various inhibitory zones against these microbial strains indicating their dependency on solubility and polarity. Further in *vitro* assays on a broad range of microbes or pure solvents may explore this fruit's broader antimicrobial potential.

Typically, antioxidant are substances that scavenge free radicals before their binding with cellular compounds and alter their ability to damage DNA leading to mutation and cancer formation. They can significantly decrease the damage brought about by free radicals by protecting lipids, proteins, enzymes, carbohydrates and cellular membranes (Halliwell and Gutteridge, 1995). In fact, any imbalance of cell reactive oxidative species and antioxidants leads to the oxidative stress and damage to the cell (Ighodaro *et al*., 2018; Vasdev and Singal, 2006). Oxidative stress is the fundamental stimulation of aging, neurodegenerative ailments like Alzheimer's and harmful diseases like carcinomas (Morry *et al*., 2017; Uttara *et al*., 2009). This lead us to the need of new powerful cancer preventive agents that can battle these diseases with least or no adverse effects. In our research, the fruit extracts of *D. malabarica* exhibited antioxidant potential with about 60-80 % DPPH inhibition. Additionally, these extracts have high number of total antioxidants and good choice for an immediate antioxidant source. Our research coincides with the finding of Akond *et al*., (2010) who suggested that antioxidant activity of the fruit extracts may be because of the higher concentration of phenolic compounds that react individually with DPPH or may be because of their combined or synergistic effect. Sannigrahi *et al*. (2010) also indicated that higher antioxidants potential may be due to the occurrence of reactive oxygen species, produced as a result of plant metabolic reactions. However, the occurrence of major conjugated antioxidants i.e. phenolics, hydroxycinnamic acids alkaloids, flavonoids, and anthocyaninscan affect total antioxidant values.

**Conclusion:** This investigation revealed that the aqueous and organic extracts of *D. malabarica* fruit displayed strong antimicrobial and antioxidant potentials. It is

therefore proposed that the organic extracts of *D.malabarica* fruit should be propagated as modern and least harmful antimicrobial drugs and antioxidants to combat the conditions arising from infectious agents or cell damage. Further research is needed to develop methods for efficient extraction and antimicrobial and antioxidant potential determination of these plants.

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