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EVALUATION OF *IN VITRO* PHARMACOLOGICAL ACTIVITIES OF *DIOSPYROS MALABARICA KOSTEL* FRUIT

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ABSTRACT

Objective: To explore the *in vitro* pharmacological activities of fruit of *Diospyros malabarica*. **Method:** 70% ethanolic extracts of *Diospyros malabarica* was screened against ten pathogenic bacteria, five strains of gram +ve, five strains of gram –ve bacteria and fungi by using disc diffusion method. Moreover, to study the toxicity of this fruit, we conducted a saltwater shrimp lethality bioassay based on the ability to kill laboratory cultured brine shrimps *(Artemiasalina)*. **Results:** Fruits extracts were found to exhibit antimicrobial activity against bacterial pathogens with the zone of inhibition ranges between 13mm to 19mm. Fruit Extracts was also tested for its antifungal activity against fungal strains exhibiting zone of inhibition ranges from 14 mm to 19 mm. The fruit extract of *Diospyros malabarica* shows significant lethality towards brine shrimps at LD50 value of 93.55 ppm. **Conclusion:** Plant extracts is potentially effective in suppressing microbial and fungal growth. As fruit extracts has significant LD50 value thus it can be further used for cancer cell line studies.

Keywords: *Diospyros malabarica*, Fruits extracts, brine shrimps, pathogenic bacteria

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INTRODUCTION

Medicinal plants are great source for obtaining antimicrobial agents. Many potent drugs are obtained from plants and are used medicinally in various countries. A vast variety of plant extracts have been utilized as raw drugs which contains various medicinal properties. Although a lot of plants have been qualified for antibacterial and antifungal activities and majority of them have not been fully evaluated. *Diospyros* species exhibited antiviral activity. The researchers revealed the inhibitory activity of *D. kaki* may be due to the aggregation of protein extracted from *Diospyros kaki* could reduce viral infection of influenza virus adenoviruses, coxsackie viruses, mouse norovirus, rotavirus and feline calicivirus. Similarly, ethyl acetate extracts of *D. glans bark, containing ursane and* and lupanetipo, exhibited a potent activity against the multiplication of the dengue virus, estimated by NS5 and NS5 RNA-dependent RNA polymerase [**1**]. Keeping in view the wide potentiality of plants as sources for antibacterial agents as *in vitro* investigation was undertaken to check the *Diospyros malabarica* fruits for antifungal and antimicrobial activities.

MATERIALS AND METHODS

All the chemicals were purchased from Sigma–Aldrich, USA and Merck Germany. *Diospyros malabarica* Fruits were collected from University of Agriculture Faisalabad. The plant was identified by Dr. Mansoor Hameed, Associate Professor Department of Botany, University of Agriculture, Faisalabad, Voucher # 73-1-2019.

Preparation of the Extract

Thinly sliced fresh fruits were shade dried, crushed using a mechanical grinder, passed through sieve i.e. 40 mesh, and preserved in an airtight container for further use. The powdered fruits were triple macerated in 70% ethanol for total seven days with occasional shaking. Solvent was evaporated using rotary evaporator under reduced pressure, maintaining temperature of 40 ºC. Temperature of

chiller was maintained at 5 ºC. Syrupy extracts were obtained, poured in beaker and dried at room temperature. Refrigerated at 4 ºC for further use.

Antibacterial Activity of Plant Extract

Bacterial Strains

The antibacterial activity of *D. malabarica* fruit extract was evaluated against Gram positive bacteria [*Staphylococcus aureus* and *Bacillus cereus, Staphylococcus epidermidis, Staphylococcus Haemolyticus, methicillin resistant staphylococcus aureus* (MRSA)] and Gram negative bacteria i.e; *Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa, Pseudomonas fluorescens and Klebsiella pneumonae*. The bacterial strains were obtained from the culture collection of Botany and Microbiology Department.

Inoculums Preparation

Mueller-Hilton media was used to culture bacterial strains at 35 ºC overnight using agar slants. The bacterial growth was harvested using 5 ml of sterile saline water, its absorbance was adjusted at 580 µm and diluted to attain viable cell count of 107 CFU/ml using spectrophotometer, absorbance was recorded at 580 nm.

Antibacterial Activity

Antimicrobial activity was carried out using disc diffusion method. Sterile filter paper discs were used to obtain final concentration of 10 mg/disc. Mueller-Hilton agar medium was used, 10 ml sterilized media was poured into pre-sterilized Petri dishes followed by the addition of 15 ml of seeded medium which was previously inoculated with bacterial suspension (100 ml of medium/1 ml of 107 CFU) to manage 105 CFU/ml of medium. Sterile filter paper discs impregnated with fruit extracts at concentration of (10 mg/ml) were placed over the petri dishes containing Mueller-Hilton agar. Gentamycin impregnated discs (30 µg) were used as positive control. Plates were kept in the refrigerator at 5°C for 2 h, incubated at 35 ºC for 24 h. Zones of inhibitions of both control and fruit extracts were measured by Vernier caliper, and considered as indication for antibacterial activity [**2**].

Antifungal Activity

Disc diffusion method was used to test antifungal activity. The sabouraud deaxtrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 µg ml 1 concentrations of the extracts was placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Disc impregnated with solvent methanol followed by drying off was used as negative control and Clotrimazole (30 µg disc 1) used as positive control. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm [**3**].

Brine Shrimp Lethality Assay

Brine shrimps eggs (*Artemia salina*) were hatched in artificial sea water in a conical shaped vessel (1L), seawater solution was prepared using sea salt 38 g/L under constant aeration for 48 h. After hatching, active larvae were collected from brighter portion of the hatching chamber.10 larvae were added into a glass test tube having 4-5ml of artificial sea water solution. Experiments were conducted along with control (vehicle treated). Different concentrations (10-100 µg/mL) of the test substances in a set of three tubes per dose. 10 ppm fruit extracts were added in 1st set of three test tubes, 50 ppm in 2nd and 100 ppm in 3rd set of test tubes. Temperature was maintained between 28-30 ºC. After 24 hrs number of survived larvae were counted [**4**]. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LD50 values were obtained from the best-fit line plotted concentration verses percentage lethality.

RESULTS

Antibacterial Activity

Antibacterial activity against five strains of Gram positive bacteria (*S. aureus* and *B. cereus, S. epidermis, S. haemolyticus and MRSA)* and five strains of Gram negative bacteria (*E. coli, S. typhi and P. aeruginosa*, *P. fluorescens and K. pneumonae*) using disc diffusion method was carried out. Results revealed that *D. malabarica* fruit extracts exhibit potentially effect in suppressing growth of all the testing microbes. Chloramphenecol was used as standard, when tested by disc diffusion method. Results suggested that *Staph. haemolyticus* was the most resistant strain to fruit extract with 13 mm zone of inhibition (ZOI) followed by *Pseudomonas aeruginosa, Pseudomonas fluorescens* gave ZOI 14 mm while *Klebsiella pneumonae* and *E. Coli* were the most susceptible strains with ZOI of 19 mm.

Antifungal Assay

Fruit extract of *Diospyros malabarica* was found to exert antifungal activity against fungal strains (**Table 3**). Fruit extracts shows 19mm ZOI against *Fusarium albican,* 19 mm ZOI against *fusarium albicans* and *Mucor racemosu,* followed by 18 mm, 17 mm,16 mm,14 mm against *Mucor mucedo, Aspergillus flavus, Candida albicans, Aspergillus nigar* and *Rhizopus stolonifer* respectively.

Brine Shrimp Lethality Assay

The degree of lethality was found to be directly proportional to the concentration of the extract of *Diospyros malabarica's* fruit as shown in the **Table 3.** The LD_{50} values of the fruit extract was

obtained by a plot of percentage of the shrimp larvae killed against the concentrations of the extract and the best-fit line was obtained from the data by means of regression analysis. This significant lethality of fruit extracts to brine shrimp is an indicative of the presence of potent cytotoxic components **Figure 1. Table 3** shows that 6, 12 and 15 brine shrimp larvae are killed by Fruit extract at the dose level of 10 ppm, 50 ppm and 100 ppm respectively. LD_{50} value is calculated for the cytotoxicity of fruit extract by using probit analysis which is 93.55. Results showed that concentration of the fruit extracts is directly proportional to the number of killed shrimps. 100 µg/ml was found to be maximum lethal dose and 10 µg/ml was minimum lethal dose.

Table 1: Antibacterial activity of hydro alcoholic fruit extract of *Diospyros malabarica*.

Bacteria strains	Inhibition zone diameter (mm)	
	Sample (hrdroalcholic fruit extract)	**Standard (**Gentamycin)
Staphylococcus aureus	16mm	25mm
Klebsiella pneumonae	19mm	23mm
Pseudomonas aeruginosa	14mm	20mm
Salmonella typhi	18mm	25mm
Escherichia coli	19mm	22mm
Bacillus subtilis	17mm	25mm
Pseudomonas fluorescens	14mm	25mm
Staphylococcus epidermidis	16mm	24mm
Staph. Haemolyticus	13mm	20mm
(MRSA)	15mm	22mm

Table 2: Antifungal activity of fruit of *Diospyros malabarica.*

Fungi strains	Inhibition zone diameter (mm)	
	Hydroalcholic fruit extracts	Chloramphenicol
Candida albicans	16mm	21mm
Fusarium oxysporum	19mm	22mm
Aspergillus nigar	14mm	20mm
Mucor mucedo	18mm	22mm
Mucor racemosus	19mm	22mm
Aspergillus flavus	17mm	21mm
Rhizopus stolonifer	14mm	20mm

Table 3: Brine shrimp lethality data of *D. malabarica's* fruit extract.

S/No	Sample	No of deaths /30 larvae			LD_{50}
		10ppm	50ppm	100ppm	
1	Hydro alcoholic Ext. of *Diospyros malabarica*	06	12	15	93.55 ppm

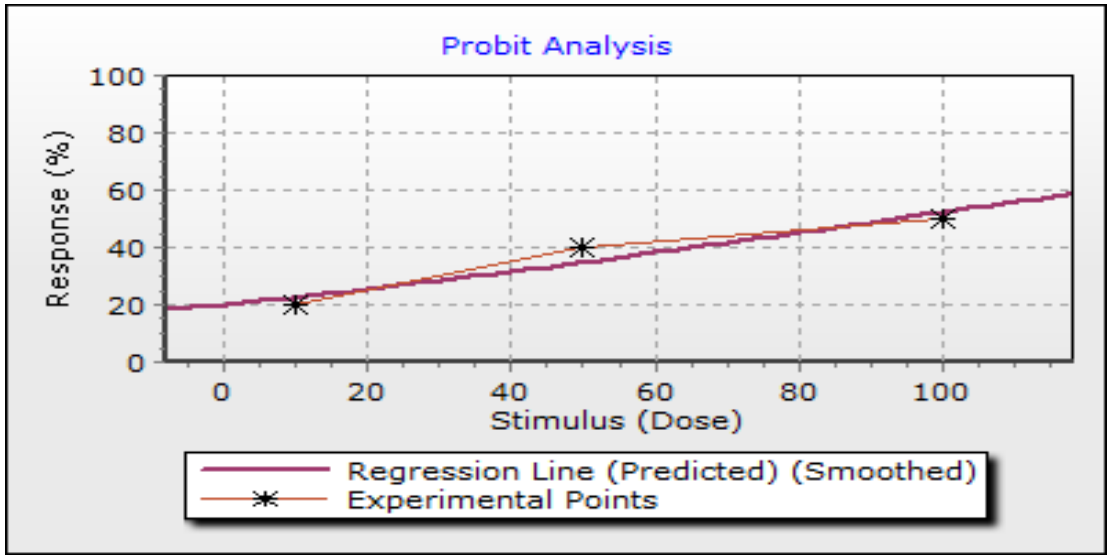

Figure 1: Graphical representation of brine shrimp lethality assay.

DISCUSSION

Antibacterial and Antifungal Activity

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. Ethanolic fruit extracts of *D. malabarica*, showed activity against *P. aeruginosa, Klebsiella pneumonae, Salmonella typhi, Pseudomonas fluorescens, Staphylococcus epidermidis, Staph. Haemolyticus, Staphylococcus aureus, MRSA.*

The highest ZOI was observed for Klebsiella pneumonae and E. coli 19 mm followed by 18 mm for Salmonella typhi and 17 mm for Bacillus subtilis. According to Rasamison and coworkers, ethanol extracts of *D. gacilips* shows reasonable antimicrobial activity towards *Klesbsiella pneumonia* and *Staphylococcus aureau* [**5**]. Ethyl acetate extracts of *D. malabarica* shows the highest activity (24 mm) against *P. aeruginosa* while ethyl acetate, methanol and aqueous extracts shows second highest activity against *staphylococcus aureus and P. aeruginosa* with 13 mm zone of inhibition. Moreover, methanol extract of *D. malabarica* leaves (12 mm) shows activity against *ESBL Klebsiella* [**6**]. With comparing our study, it was evaluated that hydro alcholic extracts of *D. malabarica* fruit shows least activity against *Staphylococcus haemolyticus* (13mm) and (14mm) against *P. aeruginosa* and strongest activity against *Kleibsiella pneumonia* (19mm). Various antimicrobial compounds are responsible for the antibacterial effect of *D. malabarica* fruits. Fruits of *D. malabarica* have been effectively proven for their utilization as source for antimicrobial compounds.

Similarly Fruit extract of *Diospyros malabarica* was found to exert antifungal activity against fungal strains. Fruit extract shows 19 mm zone of inhibition against *fusarium albicans* and *Mucor racemosu*, followed by 18 mm,17 mm,16 mm,14 mm for *Mucor mucedo, Aspergillus flavus, Candida albicans, Aspergillus nigar and Rhizopus stolonifer* respectively. When we compare graphs of both anti bacterial and anti-fungal activities of *D. malabarica* fruits extracts it can be clearly seen that Fungal strains are more sensitive to the fruit extract as compare to bacterial culture. According to Borges-Argáez and colleagues plumbagin have efficacy against the *fungi Candida albicans, Aspergillus niger*, and *Colletotrichum gloeosporioides*, derived from hexane fraction of *D. anisandra* [**7**]. Hence it is concluded that *Diospyros* specie exhibit significant activity against fungal strains.

Brine Shrimp Lethality Bioassay

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. In the present study the brine shrimp lethality of extract of fruits of *Diospyros malabarica* was determined using the procedure of [**8**]. The LD_{50} value of the brine shrimp obtained for fruit extract. It is evident that 6, 12 and 15 brine shrimp larvae are killed by Fruit extract at the dose level of 10 ppm, 50 ppm and 100 ppm respectively. LD_{50} value for fruit extract is 93.55 ppm. The degree of lethality was found to be directly proportional to

the concentration of the extract. The LD_{50} values of the fruit extract was obtained by a plot of percentage of the shrimp larvae killed against the concentrations of the extracts and the best-fit line was obtained from the data by means of regression analysis. This significant lethality of fruit extract to brine shrimp is an indicative of the presence of potent cytotoxic components which warrants further investigation. Concentration of the fruit extracts was directly proportional to degree of lethality. Percentage of killed shrimps was plotted against fruit extracts concentrations to obtained LD_{50} value of fruit extracts.

CONCLUSION

The study scientifically proves the importance of plant products in development of a potent antibacterial agent.

REFERENCES

1. Hazra S, Ghosh S, Sarma MD, Sharma S, Das M, Saudagar P, Prajapati VK, Dubey VK, Sundar S and Hazra B. Evaluation of a diospyrin derivative as antileishmanial agent and potential modulator of ornithine decarboxylase of Leishmania donovani. Experimental parasitology, 135(2), 407-413, 2013.
2. Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN and Bakri MM. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J Bio Sci, 25(2), 361-366, 2018
3. Mahesh B and Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World j agri sci, 4(5), 839-843, 2008.
4. Krishnaraju AV, Rao TV, Sundararaju D, Vanisree M, Tsay HS and Subbaraju GV. Assessment of bioactivity of Indian medicinal plants using brine shrimp (Artemia salina) lethality assay. Int J Appl Sci Eng, 3(2), 125-34, 2005.
5. Rasamison V E, Rakotondraibe H L, Razafintsalama V, Rakotonandrasana S, Ratsimbason MA and Rafidinarivo E. Chemical constituents from stems and leaves of Diospyros gracilipes Hiern and the antimicrobial and cytotoxic principles. J Pharmacog and Phytochem 5(2),109, 2016.
6. Gayathri R, Balaraju K, Jasmine DJ, Vijayakumar A, Amutha R and Agastian P. Antimicrobial Activity of Diospyros malabarica (Desr.) Kostel., Using Different Solvent Extracts: An In Vitro Study. IUP J Life Sci, 3(3), 2009.
7. Borges-Argáez R, Canche-Chay C I, Peña-Rodríguez LM, Said-Fernández S. and Molina-Salinas, GM. Antimicrobial activity of Diospyros anisandra. Fitoterapia. 78(5), 370-372, 2007
8. Sarah QS, Anny FC and Misbahuddin M. Brine shrimp lethality assay. Bangladesh J Pharma, 12(2), 2017.