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ORIGINAL ARTICLE

Screening of Ethanolic Extract for Antioxidant and anti-bacterial Activities of Some Selected Plants from Cholistan Desert

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Authors' Contributions

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ABSTRACT

Objective: Increase in the drug resistance and development of new strains is a source of discomfort for researchers working in developing countries. For restoration of life and health, it is essential to develop new pharmaceutical products that could replace the resistant antibiotics. Traditional medicine from Cholistan desert plays a novel role in this concern. Plant remedies and their formulations have been used extensively for the treatment of various diseases. *Tinospora cordifolia (T. cordifolia)* and *Moringa olifera (M. olifera)* possess different pharmacological activities. The aim of this study is to estimate the antioxidant and antibacterial screening of ethanolic extract of *T. cordifolia* and *M. olifera*.

Method: Antioxidant activity was done by DPPH method and anti-bacterial activity was done by agar disk diffusion and well diffusion method. Ciprofloxacin was used as the standard drug.

Results: Antioxidant activity indicates the % inhibition of 79.43345±0.16 and 89.15352±0.9 of *T. cordifolia* and *M. olifera* which is comparable with standard (Ascorbic acid) shows percent inhibition 93.74±0.14. Antibacterial activity was done with four gram negative bacterial strains and result was comparable with standard drug. In disk diffusion method ethanolic extract of *M. olifera* showed maximum zone of inhibition 30mm against *Micrococcus Luteus* and *E coli*, and *T. cordifoli, and* 20mm against *Micrococcus Luteus*. While in Well diffusion method *M. olifera* showed maximum zone of inhibition (19mm) against *Bordetella bronchiseptica and T. cordifolia* 17mm against *Micrococcus Luteus*. However, the observed zone of inhibition is less than standard Ciprofloxacin zone of inhibition.

Conclusion: It is concluded from the fact that *T. cordifolia* and *M. olifera* possess anti-bacterial potential against particular microorganism.

Keywords: *Drug Resistance, Antibacterial Activity, Cholistan, Traditional Medicine*

INTRODUCTION

Phytotherapy is the science of consuming plants to treat diseases [1]. The term medicinal plants refer to a diversity of herbs that have therapeutic characteristics. These floras are an ironic cause of chemicals that can be recycled to improve medicine integration [2]. Various parts of medicinal herbs will be recycled by altered kinds of seeds, roots, leaves, fruit, skin, flowers, or the whole plant. The chemicals that work in many slices of curative herbs have uninterrupted or unplanned therapeutic effects and are used as medicinal properties [3]. Herbal medicines are made into powders, infusions or poultices and are administered in a variety of ways [4]. In Pakistan, nearly two thousand herbal forms have been accepted to have biological possessions [5]. Pakistan is blessed with a special kind of biodiversity. For instance, Cholistan has a suitable atmosphere and high quality of soil, so the country is blessed with unique type of medicinal plants [6]. Cholistan dessert is located in south of Bahawalpur district and it contains rare flora of its own type. These medicinal plants are used by the local desert people to treat the various ailments. These natural floras are empirically utilized traditionally without knowing their chemical composition and scientific literature [7]. There is plenty of information on the usage of remedial foliage, however if this outdated understanding is not quickly investigated and documented, there are clues that it will be eliminated for future generations [8]. So, this traditionally treasure must be protected for the future development in field of medicine.

T. cordifolia (Guduchi), is the member of family Menispermaceae. *T. cordifolia* traditionally used in cough, fever, emaciation in children, bites of poisonous insects, eye disorder, diarrhea, dysentery, and cancer etc. It contains steroids, alkaloids, glycosides, steroids, phenolic compounds. Its leaves are rich in protein (11.2%) and calcium and phosphorus [9].

M. olifera (Sohanjana) belongs to family of Moringacea*. M. olifera* containing a wide range of essential micronutrients. Traditionally it is used for various ailments like anemia, anxiety, blood purifier, bronchitis, chest congestion, cough, cholera, eye and ear diseases, joints pain, tuberculosis [10].

After COVID-19, microorganisms have developed more resistance against commercially used antibiotics so the development of new antibiotic and antioxidants are necessary to combat this virulent situation in the community. The aim of this study is to evaluate the antioxidant and antibacterial activity of the *T. corifolia* and *M. olifera*as a potential traditional medicinal plant using Disk Diffusion & Well Diffusion Methods.

Collection and Identification of Plants

M. olifera (Sohanjana) and *T. cordifolia* (Guduchi) both plants were collected from the Cholistan desert near Bahawalpur, South Punjab, Pakistan. The plants were authenticated and identified by Dr.Ghulam Sarwar's Assistant Professor Department of Botany, The Islamia University of Bahawalpur and voucher numbers were obtained for *T. Cordifolia* 155/ Botany and *M. olifera* 156/ Botany respectively.

Preparation of Crude Extract

Fresh leaves of *M. olifera* and the stem of *T. cordifolia* were washed to remove dust and then carefully screened to prevent any adulteration. Then leaves of *M. olifera* were shade dried over a period of 5 days and stem of *T. cordifolia*take was dried for one and half months. The dried plants were roughly crushed into fine particles via a mortar and pestle and then grounded with a grinder available in an industrial laboratory at Khawaja Fareed Campus, IUB. The powdered material of plants was liquefied with Ethanol for 15 days with constant stirring. After 15 days the dry material of the plant was first filtered with muslin fabric and then strained over the Whatman strainer paper no.1. After that, the filtered material was again filtered, and the extract was prepared by using a rotary evaporator. This extract was preserved in the glass jar for antioxidant and anti-bacterial activities [11].

Antioxidant Activity

DPPH Free Radical Scavenging Assay

Stock samples of (1.0 mg / mL) were diluted to a final concentration of 5 mg/mL, in ethanol. 1 mL of ethanol solution of 0.3 Mm, DPPH was added to 2.5 mL of sample solutions for different concentrations and was allowed to react at room temperature. After 30 min the absorbance was taken at 518 nm and is converted to a percentage antioxidant activity (AA) using the following:

$$AA\% = 100 - [(Abs_{sample} - Abs_{blank}) * 100] / Abs_{control}$$

Ethanol (1.0 mL) and plant solution (2.5 mL) were used without the solution [12]. DPPH solution (1.0 mL; 0.3 mM) and ethanol (2.5 mL) were used as negative controls. Good controls are those that use

standard solutions. IC50 values are calculated by reversing the line of episodes in which the abscissa represents the focus of the extraction of tested plants and the lubrication of the normal percentage of antioxidant activity from three different trials [13].

Anti-Bacterial Activity

Bacterial Strains

Bacterial strains used for the antibacterial assay are given below in table **1**.

E.coli and *Microaures letus* were given by Pakistan's First Fungal Culture Bank (FCBP), Institute of Agricultural Sciences (IAGS), Punjab University, Lahore, in the form of stock culture agar. *Pseudomonas aeruginosa* and *Bordetella bronchiseptica* were isolated from micro-biological Lab of Khawaja Fareed Campus.The Islamaia University of Bahawalpur.

Nutrient Media

For conducting this study following media were used.

- Nutrient Broth Media
- Nutrient Agar Media

Preparation of Inoculum for Anti-Bacterial Activity

Inoculums were ready by taking a few colonies of suitable bacteria from 24 h old cultures and mixed with a 10 ml sterile medium nutrition. The turbidity was used to 0.5 McFarland, a variable rate equal to the cell size of 108 CFU / ml [15].

Well Method Used for Anti-bacterial Activity

The protocol described in a previous research was followed [16] after doing minor modifications. Petri dishes and nutritious agar media were sterilized in an autoclave. Nutrient agar was poured into Petri containers and allowed to remain in the laminar flow area for solidification. On agar surface, bacterial cultures had rows followed by the formation of three holes for 6mm the width of each Petri container. 60 µL of ciprofloxacin (1 mg/ml) and both extract solutions (1.25, 2.5, and 5 mg/ml) are added to the sources using a micropipette. All these Petri plates were placed in an incubator at 37 ° C for 18-24 hours. After incubation, the protected areas were measured to measure antibacterial activity. The results were taken by taking an average of three tests.

Disk Diffusion Method used for Anti-bacterial Activity

All the glassware and other material that were used for antibacterial activity were autoclaved at 121 ˚C for 15 minutes. Then dry all glassware in a hot air oven at 180 ˚C used for 30 minutes. Then all the Petri plates were put in a laminar stream lid for aseptic settings. Sterilized nutrient agar media was cooled at 35 ˚C. Then, 20 ml of sterilized nutrient agar was poured into all Petri plates and allowed to solidify at room temperature. 30 µl of 1.25, 2.5, and5 mg/ml of concentrate was applied to a 6mm filter paper disc and allowed to dry and then mounted on the inoculated Petri dishes. After the filtration of the filter paper bacterial culture, Petri plates were placed in the refrigerator spread and moved to the incubator for incubation at 37◦C for 24 hours. Normal ciprofloxacin was used as a positive control of 30 µl per filter paper disc and dimethyl sulphoxide as the negative control to compare antibacterial impact. The inhibition zone was then measured at millimeters (mm). The procedure is made in threes.

Table 1. Bacterial Strains.

S. No	Bacterial strains	Types	Voucher #
1	*Microaureus lutes*	Gram –ve	072
2	*Escherichia coli(E. coli)*	Gram –ve	088
3	*Pseudomonas aeruginosa*	Gram –ve	147
4	*Bordetella bronchiseptica*	Gram –ve	100

RESULTS

Antioxidant Assays

Both plants show strong antioxidant potential. The experiment was done three times.

Results of antioxidant activity are given below in Table **2**.

Antibacterial activity

Table **3** shows that *M. olifera* shows better result against *Micrococcus Luteus, E. coli*than *Pseudomonas Aeruginosa* and *Brodetella bromchisseptica*. Table **4** shows that T. cordifolia also showed better result against *Micrococcus Luteus*than *E. coli, Pseudomonas aeruginosa* and *Brodetella bromchisseptica*. According to Table **5** *M. olifera* shows better result against *Bordetella bronchiseptica.* Lastly, Table **6** showed T. cordifolia have better zone of inhibition against ***Micrococcus Luteus*** then against other tested bacterial strains. All these results was less than control group Ciprofloxacin

Table 2. % Incubation and IC^{50} of *T. cordifolia* and *M. olifera.*

Tested Drugs	Conc. (mg/ml)	Inhibitory %	IC^{50}n (mg/ml)
T. cordifolia	5	79.43345±0.16	2.1
M. olifera	5	89.15352±0.9	0.4
Ascorbic Acid	0.5 (mmol/ml)	93.74±0.14	0.0039±0.5

Table 3. Anti-bacterial Activity of ethanolic extract of *M. olifera* by Disk Diffusion Method.

Ethanoloc Extract *M. olifera* Dose (mg/ml)	*E. coli* Zone of inhibition (mm)	*Micrococcus Luteus* Zone of inhibition (mm)	*Bordetella Bronchiseptica* Zone of inhibition (mm)	*Pseudomonas aeruginosa* Zone ofinhibition (mm)
1.25	08±00	09±00	05±00	05±00
2.5	20±00	20±00	18±00	20±00
5	30±00	30±00	25±00	29±00
Ciprofloxacin				
5	35±00	35±00	35±00	40±00
Negative control				
5	00±00	00±00	00±00	00±00

Table 4. Anti-bacterial Activity of ethanolic extract *T. cordifolia* by Disk Diffusion Method.

Ethanoloc Extract *Tinospora Cordifolia* Dose (mg/ml)	*E. coli* Zone of inhibition (mm)	*Micrococcus Luteus* Zone of inhibition (mm)	*Bordetella Bronchiseptica* Zone of inhibition (mm)	*Pseudomonas aeruginosa* Zone ofinhibition (mm)
1.25	03±00	04±00	03±00	05±00
2.5	10±00	15±00	09±00	10±00
5	19±00	22±00	19±00	20±00
Ciprofloxacin				
5	35±00	35±00	35±00	40±00
Negative control				
5	00±00	00±00	00±00	00±00

Table 5. Anti-bacterial Activity of ethanolic extract of *M. olifera* by well Diffusion Method.

Ethanoloc Extract *M. olifera* Dose(mg/ml)	*E. coli* Zone of inhibition (mm)	*Micrococcus Luteus* Zone of inhibition (mm)	*Bordetella Bronchiseptica* Zone of inhibition (mm)	*Pseudomonas aeruginosa* Zone ofinhibition (mm)
1.25	04±00	04±00	06±00	05±00
2.5	09±00	11±00	09±00	10±00
5	16±00	18±00	19±00	17±00
Ciprofloxacin				
5	35±00	40±00	35±00	40±00
Negative control				
5	00±00	00±00	00±00	00±00

Table 6. Anti-bacterial Activity of ethanolic extract of *T. cordifolia* by Well Diffusion Method.

Ethanoloc Extract *M. olifera* Dose (mg/ml)	*E. coli* Zone of inhibition (mm)	*Micrococcus Luteus* Zone of inhibition (mm)	*Bordetella Bronchiseptica* Zone of inhibition (mm)	*Pseudomonas aeruginosa* Zone ofinhibition (mm)
1.25	04±00	03±00	03±00	02±00
2.5	09±00	09±00	05±00	08±00
5	16±00	17±00	12±00	15±00
Ciprofloxacin				
5	35±00	40±00	35±00	40±00
Negative control				
5	00±00	00±00	00±00	00±00

DISCUSSION

Plants play a vital role in the development of a new patent drug [17]. Antioxidant and anti-bacterial activities of a compound are primarily investigated during new drug development [18]. Microbial resistance has been increasing day by day. So, for the safety of mankind new drugs have been launched in market to reduce this burden [19]. Phytomedicine are not very costly and proving their tremendous effect in their areas. Now it's time to use the cheap and effective source to control the burden of increasing resistance and prevention of infectious diseases [20]. Efficacy of the plant remedy is reported in research articles [21].The main objective of this study was to evaluate the antioxidant and antibacterial effect of ethanolic extracts of *T. cordifolia* and *M. olifera* by using both disk diffusion and well diffusion methods against gram-negative bacteria. In Table **2** antioxidant activity indicates the %inhibition of 79.43345±0.16 of *T. cordifolia*and 89.15352±0.9of *M. olifera*. In Table **3** and **4**, the antibacterial activity of ethanolic extract of *M. olifera* showed zone of inhibition of 30mm against *Micrococcus Luteus* and *E coli*, and *T. cordifolia* showed 20mm against *Micrococcus Luteus* by disc diffusion method respectively. While Table **5** and **6** showed the results of well diffusion method on *M. olifera that* showed maximum Zone of inhibition (19mm) against *Bordetella bronchiseptica and T. cordifolia, and 17mm* against *Micrococcus Luteus* respectively. The well diffusion method showed good results but less effective than Ciprofloxacin as a control drug. Presence of active secondary metabolites hinders the rate of microbial growth and metabolism. Tannin and flavonoids are the main active constituents of *M. olifera* and*T. cordifolia,* which are responsible for the growth inhibition of microorganism [23] and multidrug resistance [24].

Furthermore, it is essential to isolate and purify the compounds present in *M. olifera* and *T. cordifolia.* The

study findings will allow the researcher to recommend them as an alternative synthetic antibiotic.

CONCLUSION

Current studies have reported ethanolic extracts of*M. olifera* and *T. cordifolia* showed good susceptibility against all the pathogens at different concentrations when comparison was done against the standard ciprofloxacin. The anti-oxidative potential of *M. olifera* and *T. cordifolia* by DPPH method gives IC^{50} value 0.4mg/ml and 2.1mg/ml respectively that showed good antioxidant effects of these tested plants.

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