



Antibacterial Activity of *Datura metel* Linn. (TALONG-PUNAY) Fruit Extract

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Authors' contributions

This work was carried out in collaboration among all authors. Author MCGV is the principal investigator who designed the study and performed the antibacterial activity of the fruit extract. Authors KMCL and FMSSD performed the phytochemical analysis of the fruit extract. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i2130758

Editor(s):

(1) Dr. Paola Angelini, University of Perugia, Italy.

Reviewers:

(1) Thamer Mutlag Jasim, Laboratory Clinical Science, Iraq.

(2) Hayet Edziri, University of Monastir, Tunisia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/60665>

Original Research Articles

Received 20 June 2020
Accepted 26 August 2020
Published 05 September 2020

ABSTRACT

Aim: The objective of this study is to determine the phytochemical constituent and antibacterial effect of fruit extract of *Datura metel* against *Staphylococcus aureus* and *Escherichia coli*.

Methods: The extract of the sample underwent a phytochemical screening to identify the secondary metabolites present. Also, an antibacterial test was carried out to test its effectiveness, it was tested by impregnating the respective disc on Mueller-Hilton agar streaked with *Staphylococcus aureus* and *Escherichia coli*.

Results: *Datura metel* fruit extract was observed to have a good antibacterial activity on the two bacteria and this was due to the presence of the phytochemical compounds terpenoids, saponin, alkaloids, steroid, flavonoids, and tannins.

Conclusion: The sample *Datura metel* fruit extract could be used in the field of pharmaceutical because of its antimicrobial activity and present secondary metabolites.

Keywords: Antibacterial activity; *Datura metel* Linn; phytochemical screening.

1. INTRODUCTION

Medicinal plants are used for the treatment of several microbial and non-microbial diseases due to their valuable effects in healthcare. The affordability, reliability, and low toxicity of medicinal plants in therapeutic use has made them popular and acceptable by all religions for implementation in medical health care all over the world. Medicinal plants are indeed the first material used as an alternative medicine against many diseases. Several plants have therapeutic and pharmaceutical effects, such as antimicrobials, antioxidant, anti-infection and antitumor activities. Furthermore, there has been a lot of interest in the chemical composition of plants as sources of new antibacterial agents [1]. Different extracts from traditional medicinal plants have been tested. Many reports have shown the effectiveness of traditional herbs against microorganisms.

Datura metel is a highly potential functional and valuable medicinal plants. The whole plant contains *scopolamine* and *atropine* which increase gradually with the progress of developmental growth, and are most pronounced when the plant is at the end of its reproductive stage. Furthermore, plants contain the alkaloids *hyocyanine*, *hyoscine* and *atropine*. The total alkaloid content of the leaves is 0.426%, which is mainly *atropine*. The seeds contain 0.426% alkaloids, which is mainly *hyoscyanine*. The roots contain 0.35% *hyoscyanine* [2].

A colorless crystalline constituent, daturilin has been obtained from the acid-insoluble fractions of the alcoholic extracts of *D. metel* leaves. These compounds were recognized as withametelin C, D and E [3]. Previous studies showed that the cultured callus of *D. metel* contained cholesterol and 5 α -pregnane-3 β ,20 β diol. It also demonstrated the presence of C28 sterol 3 β , 24 β -dihydroxy-ergosta-5-25-dienolide and the withanolide 12-deoxywithastramonolide in "in vitro" propagated shoots of *D. metel* [4].

Also known as angel's trumpet or devil's trumpet, *Datura metel* is a medicinal plant whose use dates back as far as 3000 years. It contains tropane alkaloids and is used as a sedative, antispasmodic, antitussive, bronchodilator, anodyne, and has hypnotic and mydriatic effects. It has a wide range of

application in India, including in the treatment of epilepsy, hysteria, insanity, heart diseases, and for fever with catarrh, diarrhea and skin diseases. A poultice of the crushed leaves is used to relieve pain. In China, the plant is used in the treatment of asthma [2].

For this reason, *Datura metel* fruit extract was evaluated for its phytochemical property and antibacterial activity since this will not only counteract the large number of bacterial diseases in our country but will also be a new source of antibacterial medicine to replace expensive and chemically made antibacterial medicines which may be harmful to humans.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

The matured fruit of *D. metel* was collected from Lope de Vega, Northern Samar. The matured fruits were washed with distilled water and were sun-dried. One thousand (1000) grams of segregated matured fruit of *D. metel* was pounded using mortar and pestle in order to extract the juice, which was then filtered using cheesecloth to separate the solid parts from the liquid extract. After filtration, the extract was placed in a sterilized bottle and was labeled for easy identification.

2.2 Phytochemical Analysis

Phytochemical tests were done to find out the presence of secondary metabolites, such as alkaloids, carbohydrates, flavonoids, tannins, steroids, terpenoids and saponin.

2.2.1 Test for alkaloids (Meyer's Test)

The extract of *D. metel* was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent [5]. The samples were then observed for the presence of turbidity or yellow precipitate [6].

2.2.2 Test for saponin

The sample extract with 2 mL volume was added with 20 mL of distilled water and was agitated in a graduated cylinder for 15 minutes. The

formation of 1cm foam indicates the presence of saponin [7].

2.2.3 Test for steroids

A volume of 0.5 mL of acetic anhydride and 0.5 mL of chloroform was treated with 4 mL of extract. Then, a concentrated solution of sulphuric acid was added slowly and when a green bluish color appear, it is an indication of steroids [5].

2.2.4 Test for terpenoids

A volume of 0.5 mL of acetic anhydride and 0.5 mL of chloroform was treated with 4 mL of extract. Then, a concentrated solution of sulphuric acid was added slowly and when a red violet color appear, it is an indication of terpenoid [5].

2.2.5 Test for carbohydrates

Treat the test solution with few drops of alcoholic alpha-naphthol. Add 0.2 mL of concentrated solution of Sulphuric acid slowly through the sides of the test tubes, a purple to violet color ring appears at the junction [8].

2.2.6 Test for tannins

A volume of 0.5 mL of extract solution was added with 1.0 mL of water and 1-2 drops of ferric chloride solution. Blue color was observed for garlic tannins and green black for catecholic tannins [9].

2.2.7 Test for flavonoids

Shinoda's test was followed for the presence of flavonoids. Few drops of concentrated hydrochloric acid and magnesium filings were added in 1.0 mL of ethanolic extract. Appearance of pink or magenta red color indicates the presence of flavonoids [7].

2.3 Antibacterial Activity

2.3.1 Preparation of culture media

Measure about 9 grms of Mueller-Hinton Agar and suspend it in a 250 mL distilled water. Let the mixture boil to completely dissolve the medium. Sterilize it by using oven at 100°C for 15 minutes. Then allow it to cool down about 50°C and aseptically pour it to individual petri dishes. Allow it to solidify.

2.3.2 Source of test organisms

The cultured *Staphylococcus aureus* and *Escherichia coli* was purchased in Philippine Collection of Microorganisms, National Institute of Molecular Biology (BIOTECH) of the University of the Philippines Los Banos, Laguna.

2.3.3 Preparation of the positive control

Chloramphenicol was used as positive control for *Staphylococcus aureus* (Gram positive bacteria) and Tetracycline was used as positive control *Escherichia coli* (Gram negative bacteria). Pound a commercial tablet of an antibiotic without removing it from its shelf. After turning it into a powder form, open the sachet and place the powdered tablet in a sterile petri dish. Add 1mL distilled water to totally dissolve the powdered tablet.

2.3.4 Preparation of sensitivity disc

Cut the Whattman filter paper No. 1 into round disc shapes using a puncher. The number of filter paper discs is dependent on the number of trials in the study. Place each labeled disc in petri dishes and sterilize at 121°C for 15 minutes in an oven. After sterilizing those filter discs, soak them in the *D. metel* fruit extract and commercial antibiotic for 1 hr.

2.3.5 Antibacterial screening

The base layer plates were prepared by prepouring 30 mL Mueller-Hinton agar medium into a sterile petri dish to be used for assaying bacteria. A suspension of the test microorganisms was prepared by mixing 1 mL of the organisms to 99 mL of Mueller-Hinton agar. A filter paper disc from a Whattman assay disc was prepared. A metal puncher was used in cutting the paper discs. They were wrapped in sets of 10 in an aluminum foil and sterilized in the oven at 121°C for 15 minutes.

With sterile forceps, the individual disc was picked and was immersed in the plant extract and then placed in a sterile petri dish with bacterial inocula for bacteria. Dissolved chloramphenicol was used as standard antibiotic for gram positive bacteria and dissolved tetracycline for gram negative bacteria. All discs were soaked separately for 15 minutes. Each disc was kept in the center of each plate for possible antibacterial activity which was demonstrated by producing a clear zone around the discs. Each plate was incubated at 37°C for

24 hours to allow maximum growth of the microorganisms. The antibacterial activity of the test samples was determined by measuring diameter of the inhibition zone expressed in millimeter [10].

3. RESULTS AND DISCUSSION

The phytochemical screening of *D. metel* revealed the presence of important pharmacological active substances as well as medicinal and nutritional potentials in the fruits. As shown in Table 1, the secondary metabolites present in the fruit extract of *D. metel* were alkaloids, steroids, tannins and terpenoids. In the test for the presence of alkaloids, the formation of white precipitate using Meyer’s reagent was observed which is a positive result for alkaloids. In the test for the presence of steroids, a color change from violet to green was observed which is a positive result for steroids and red violet color for terpenoids. In the presence of tannin, a formation of jelly-like precipitate was observed.

The microbiological efficacy of *Datura metel* fruit extract as manifested by its being able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* is presented on Table 2.

As shown in the table, the effectiveness of *Datura metel* fruit extract in inhibiting the growth

of *Staphylococcus aureus* and *Escherichia coli* showed its antibacterial activity. However, results showed that the fruit extract is more effective in inhibiting the growth of *Escherichia coli* with 9.0 mm mean zone of inhibition than *Staphylococcus aureus* with 7.3 mm mean zone of inhibition.

This implies that *Datura metel* fruit extract showed the promise of being a good source of antibacterial activity because it can inhibit the growth of both gram-positive and gram-negative bacteria. Thus, it can be a novel source of antimicrobial drugs.

Results of investigation somehow showed that the methanolic crude extract and its derived fractions from both dry and fresh leaves of *D. metel* showed small and moderate antibacterial potential, respectively against *S. aureus* and *E. coli* [11]. Likewise, it also showed that the ethanol and ethyl acetate extracts of the mature leaves of *D. metel* indeed exhibit antibacterial potential against *S. aureus* and *E. coli* [8]. Meanwhile, utilizing crude aqueous and ethanolic extracts of the different plant parts, manifested that among the bacterial isolates tested, *S. aureus* was most inhibited by the ethanol extract [12].

Comparison of *Datura metel* fruit extract and the positive control chloramphenicol against *Staphylococcus aureus* are shown in Table 3.

Table 1. Phytochemical screening of *Datura metel* fruit extract

Secondary Metabolites	Result of <i>Datura metel</i> fruit extract
Alkaloids	Positive
Saponin	Negative
Tannin	Positive
Terpenoids	Positive
Steroids	Positive
Carbohydrates	Negative
Flavonoids	Negative

Table 2. Effectiveness of *Datura metel* fruit extract in inhibiting microbial growth

Organisms	Mean zone of inhibition (mm)
<i>Staphylococcus aureus</i>	7.3
<i>Escherichia coli</i>	9.0

Table 3. Comparison between the effectiveness of *Datura metel* fruit extract versus chloramphenicol

Bacteria	<i>Datura metel</i>	Chloramphenicol	Level of Significance		Interpretation
	Mean (mm)	Mean (mm)	Computed t-value	Tabular t-value	
<i>S. aureus</i>	7.3	13.3	-3.95	-2.78	Significant

Table 4. Comparison between the effectiveness of *Datura metel* fruit extract versus tetracycline

Bacteria	<i>Datura metel</i>	Tetracycline	Level of significance		Interpretation
	Mean (mm)	Mean (mm)	Computed t-value	Tabular t-value	
<i>E. coli</i>	9.0	11.3	-1.1	-2.78	No significant

As shown in the table, the mean zone of inhibition of *Datura metel* on *Staphylococcus aureus* was 7.3 mm while the mean zone of inhibition of chloramphenicol was 13 mm. Looking at it on the surface, chloramphenicol has greater effect on *Staphylococcus aureus* compared to *Datura metel* fruit extract, as shown by its difference in the mean zone of inhibition of both antimicrobial agents.

Statistically analyzing the result utilizing the t-test, the computed t-value of -3.95 was found greater than the tabular t-value of -2.78, with 4 degrees of freedom. Thus, the null hypothesis is accepted, which means that there is a significant difference between *Datura metel* and chloramphenicol in inhibiting the growth of *Staphylococcus aureus*.

Comparison of *Datura metel* fruit extract and the positive control chloramphenicol against *Staphylococcus aureus* are shown in Table 4.

Result show that the mean zone of inhibition of *Datura metel* fruit extract was 9.0 mm, while the mean zone of inhibition of Tetracycline was 11.3 mm. It shows that the chloramphenicol has greater mean zone of inhibition against *E. coli* compared with *Datura metel* fruit extract.

Statistical test however, showed that the computed t-value of -1.1 is below the critical value of the tabular t-value of -2.78 with 4 degrees of freedom, hence the null hypothesis is rejected, which means that the *Datura metel* fruit extract is not significantly different from the commercial antibacterial drug chloramphenicol in terms of its antibacterial activity against the bacterial isolate tested. This means that *Datura metel* fruit extract is comparable to the positive control in inhibiting the growth of *E. coli*.

Thus, it implies that the *Datura metel* fruit extract can be used as an alternative for chloramphenicol in preventing the growth of *E. coli*, and it can also be a source of an ingredient to inhibit bacterial growth, particularly gram-negative bacteria.

4. CONCLUSION

Extract of *Datura metel* is not comparable to chloramphenicol in inhibiting the growth of *Staphylococcus aureus*. Chloramphenicol is still more effective than the *Datura metel* fruit extract. Meanwhile, the *Datura metel* fruit extract was comparable to Tetracycline in inhibiting the growth of *E. coli*. Phytochemical analysis showed that the antibacterial activity of *Datura metel* was due to the presence of phytochemical compounds such as tannin, alkaloid, terpenoid and steroid. Thus, research is needed towards identification of active components present in the extracts and which could be used in the field of pharmaceuticals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the University Research and Development Services of the University of Eastern Philippines for their continued support for the conduct of this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

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