Journal of Analytical & Bioanalytical Techniques

ISSN: 2155-9872

Aynalem HG, Workie HG, J Anal Bioanal Tech 2021, 12:7

Research Article Open Access

Investigation of Antibacterial Activities of the Leaf Extracts of *Capparis Tomentosa* (Gumero)

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Received date: September 02, 2021; **Accepted date:** September 16, 2021; **Published date:** September 23, 2021

Abstract

Capparis tomentosa plant is one of the commonly used traditional medicinal plants. It is one of the varieties of *Capparis* species that is used for different traditional medicinal purposes. In herbal and traditional medicine, *C. tomentosa* used to treat rheumatism, madness, snakebite, chest pain, jaundice, malaria, headache, coughs, pneumonia, constipation, infertility and to prevent abortions. The present study was conducted to investigate antibacterial activity of different solvent extracts of the leaf of *Capparis tomentosa*. Phytochemical studies of the extracts of *Capparis tomentosa* has been carried out and their results revealed the presence of tannins, saponins, alkaloids, glycosides, flavonoids, phenols, steroids and Terpenes. Each of the extract(methanolic, ethyl acetate and pet ether) exhibited antibacterial activity methanolic extract showed the highest antibacterial activity(13.7 mm) while petroleum ether extract had the lowest antibacterial activity (6.2 mm) on the four bacteria strain tested (*Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aurous* and *Streptococcus pyogenes*).

Keywords: *Capparis tomentosa*; Antibacterial activity; Solvent extract; Bacteria strain

Introduction

Background of medicinal plant

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines which have made large contributions to human health. Although many drugs that come from trees generally have been replaced by more potent synthetic ones, trees remain a source for some drug ingredients. Medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anemia for a long time now [1].

Infectious disease caused by bacteria, fungi, viruses and parasites are still a major threat to human and animal health. The impact is particularly large in developing countries due to lack of availabilities of modern medicines and the emergence of widespread drug resistance. The development of drug resistance as well as lack of availabilities of certain antibiotics has led to the search of new antimicrobial agent mainly among plant extracts with the goal to discover new chemical structures, which overcome the above problems. Current research on natural molecules and products primarily focuses on natural plants since they are locally available, less costly and can be selected based on their ethno-medicinal uses.

The therapeutic value of the plants is due to the presence of some chemical substances within the plant tissues. Which produce a definite physiological action on the human body include: - Alkaloid, flavonoid, glycosides and others. A wide range of medicinal plant parts used for extract as raw drugs. This awareness is tremendously increased finding of new antimicrobial bioactive compound/s.

Capparis tomentosa belongs to the family Capparaceae commonly referred to as Woody Caper Bush in English [9]. It is an indigenous South African plant that grows naturally in the savanna Forest of Western, Eastern and Southern Africa. It is a scrambling shrub, sometimes maturing into a tree that can grow as high as 10 meters tall and is covered with scattered spines. In herbal and traditional medicine, *C. tomentosa* used to treat rheumatism, madness, snakebite, chest pain, jaundice, malaria, headache, coughs, pneumonia, constipation, infertility and to prevent abortions. It is used to treat leprosy, tuberculosis and gonorrhea. The roots are boiled in water and half a cup of this infusion is drunk three times per day to manage cough and chest pain.

Drug resistance has become a major clinical and public health problem in the world today is serious in developing countries like Ethiopia, where rates of resistance are higher than in developed nations. It is necessary to investigate the presence of phytochemicals from plants in order to validate their therapeutic use and to identify the active constituents which may act as lead compounds in drug discovery, innovation and development of safe, more effective, affordable and readily available antimalarial, anti-bacterial and anti-fungal agents.

Capparis tomentosa is one of the best known woody species with magico-medicinal properties, and it is commonly used in ritual ceremonies. This plant is traditionally used to treat various diseases such as madness, snakebite, headache, impotence, sterility and etc. In spite of having a wide application in traditional medicine, little is known about the Phytochemistry and pharmacological activities of *Capparis tomentosa*. Preliminary screening showed pronounced antimicrobial activity, which warrants more detailed studies. The reports on the toxicity of various plant parts are contradictory, and more research is needed before methods for safe usage as medicine.

The main Objective of this study was to carry out phytochemical investigations and evaluation of biological activities of leaf extracts of *Capparis tomentosa*.

The specific objectives of this study were:

- To extract phytochemicals from the leaf using different solvent.
- To screen the major phytochemicals in each extract.
- To assess the antibacterial activities of each extract.
- To compare the antibacterial activity of the extracts with the standards.

The use of natural products as medicines has been described throughout history in the form of traditional medicines, remedies, potions and oils with many of these bioactive natural products still being unidentified. This test suggests that there is possibility to isolate potential antimicrobial drugs from this medicinal plant. The finding of this study will also hint that potential lead molecule can be isolated from this medicinal plant that can be a base for synthesis of effective antimicrobial drugs [2].

Materials and Methods

Study design and study area

Bahir Dar city is situated on the southern shore of Lake Tana which is located north western part of Ethiopia approximately 565 km from Addis Ababa, is the capital of Amhara National Regional State (ANRS).The Experiment was conducted at Bahir Dar University department of chemistry post graduate research laboratory, which is located on latitude 11°59' N and longitude 37° 39' E, at an altitude of 1840 m above sea level.

Materials

Plant materials: The fresh leaf of *Capparis tomentosa* was collected from Bahir Dar city around Lack Tana in December 2018 and was ready for investigation**.**

Instrument and apparatus: Beaker, volumetric flask, plastic containers, analytical balance (RADWAG; PS 360/C/1, China), sieve 1mm, heating mantle, rotary evaporator (RE-2S-VD, German), pH meter (HI 99161, China), UV spectrophotometer Cary 60 Agilent technologies (China).

Chemicals and reagents: The analytical grade chemicals and reagents used for this study were distilled water, ethyl acetate, 99.9% methanol, 99.9% petroleum ether, 10% ferric chloride (FeCl3) (British drug house Ltd. England), Wagner's reagent (Iodine in potassium iodide), aluminum chloride (AlCl3), sodium nitrite(NaNO2) (Blulux Laboratories (P) Ltd.-121001),37%Hydrochloric acid (Bluluxlaboratories(p)Ltd., India), 99%Sulphuricacid (Bluluxlaboratories (p) Ltd., India), Sodium hydroxide pellets AR 98% (Breckland Scientific Supplies), nitric acid (HNO3), Sodium carbonate(Blulux Laboratories (P) Ltd.-121001), NaH2PO4, Na2HPO4, , trichloroacetic acid (Blulux Laboratories (P) Ltd.-121001), Potassium hexacyanoferrate(II), Iron chloride, 30% ammonia solution. 99.9 % petroleum ether (Blulux Laboratories (P) Ltd.-121001), 98.8% acetone, 99.8 %Chloroform. Standard antibiotic disc (Gentamycin10µg/disc) and Mueller Hinton agar (Blulux Laboratories (P) Ltd.-121001) were used for antimicrobial test [3].

Instrumentation: The necessary apparatus and instruments used for this study were electronic beam balance for mass measurement, Rotary evaporator for concentrating the filtrate to dryness, electrical shaker to mix the mixture well, volumetric flask, beaker, conical flask with different, Whiteman No.1 filtrate paper, aluminum foil, micropipette, Incubated agar, cuvettes, and others were used for different purposes. Separatory funnel different size Beakers (50 mL, 100 mL, 150 mL, 1500 mL and 2000 mL flasks for maceration. and others were used for different purposes.

Methods

Sample preparation: The selected samples were thoroughly washed with tap water to remove all the dust particles. Then the cleaned samples were air dried in the room temperature (25°C) with aluminium foil until to remove the moisture present. The dried samples were ground and homogenized by using mortar and pestle and sieved. The powdered samples were kept in an airtight container.

Extraction of Capparis tomentosa leaf: 200 g powdered plant sample was successively extracted with petroleum ether, ethyl acetate and methanol using maceration technique for 48 hr in each solvent. The extract was filtered and residual solvent from each extract was removed using Rotary evaporator. The resulting semidried mass of each fraction was stored in shade area until used for experiments.

Qualitative phytochemical screening test

Test for alkaloids (Wagner's reagent test): Extracts were dissolved individually in dilute hydrochloric acid and filtered. Then the filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/ reddish precipitate indicates the presence of alkaloids.

Test for terpenoids (Salkowski's Test): 2 mL of chloroform was added to plant extract (0.5 g) in a test tube. Then concentrated sulfuric acid was added to this mixture that would result in reddish brown interface confirming the presence of terpenoids.

Test for steroids (Salkowski's test): 1 mL of concentrated H_2SO_4 was added carefully along the sides of the test tubes to 2 mL of each extract. A red color was produced in the chloroform layer and confirms the presence of steroids.

Test for flavonoids: 2mL of the extract was treated with 2 mL of dilute NH3 solution and a few drops of concentrated H2SO4. A formation of yellow color indicates the presence of flavonoids.

Test for Saponins: To a little amount of each of the sample in a test tube, 2 mL of distilled water was added and vigorously shaken for 15 minutes. Formation of 1 cm foam confirms a positive result.

Test for phenols (ferric chloride test): 10 mL of each extract solution and 2 mL of distilled water followed by drops of 10% aqueous FeCl3 solution was added. Formation of blue or green indicates the presence of phenols.

Test for tannins (ferric chloride test): 2mL of the aqueous extract was added to 2 mL of water, a 1 to 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins.

Test for cardiac glycosides: A small amount of each extract was dissolved in 1 mL of water and the aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides [4].

Antibacterial activity

Agar diffusion method: The 5 mm diameter sterile discs (Whatman No 3 paper) were placed on the surface of the inoculated

Agar in Petri dishes, and 20 µL each test solutions were applied onto the discs. After addition of test solutions on the discs, the extract was allowed to diffuse for 5 minutes and the plates were then be kept in an incubator at 37°C for 24 hrs. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with ruler and results was expressed as Mean ± Std. of replicate tests. Standard discs of the antibiotic disc (gentamycin, 10 µg/disc) were serving as the positive antibacterial control. For negative control the same volume (20 µL) DMSO poured on paper disks was used. Antibacterial activity was recorded if the zone of inhibition was greater than 6 mm. The disk diffusion assay was used as a preliminary test to select the most efficient extracts.

Method of data analysis: The data for antibacterial activities were expressed as the average of three measurements, and all the remaining data were expressed as mean ± standard deviations of triplicates using MS Excel 2010, IBM SPSS statistics 21 (one way Anova) to show the significant difference of antibacterial activities of different extracts.

Results and Discussion

The finely divided powder of the leaf of *Capparis tomentosa* (200 g) was subjected to successive extraction by petroleum ether, ethyl acetate and methanol respectively. The extracts were analyzed for phytochemical screening. A number of assays were conducted to test antibacterial activities of each extracts since antibacterial activities of different solvent extracts involved in different mechanisms of action.

Yield of the extracts of *Capparis tomentosa:*

Successive extraction 200 g leaf powder of Capparis tomentosa gave the highest yield with methanol (10.5 g) followed by ethyl acetate (5.82 g) and petroleum ether (4.709 g).

Phytochemical analysis

Qualitative preliminary phytochemical analysis: To promote the proper use of phyto-medicine and to determine their potential as sources for new drugs, it is essential to study phytochemical constituents present in the plant species. In order to prop up species from traditional location to world medicinal plants category qualitative preliminary phytochemical analysis was performed initially with different chemicals & reagents to detect the nature of phytochemical constituents and their presence in the leaf extracts of *Capparis tomentosa*. The presence of secondary metabolites like phenols, steroids, terpenoids, flavonoids, tannins, alkaloids, saponins and glycosides. Phytochemical studies of the extracts of *Capparis tomentosa* has been carried out and their results revealed the presence of tannins, saponins, alkaloids, glycosides, flavonoids, phenols, steroids and Terpenes.

Phytochemical constitute of *Capparis tomentosa* leaf extract varied with the different solvent (methanol. ethyl acetate and petroleum ether) extracts. Most of phytochemicals tested were found in methanolic extract while in petroleum ether and ethyl acetate extracts half of them were absent.

Antibacterial activities of *Capparis tomentosa* leaf extracts: Different extracts from the leaf part of Capparis tomentosa plant demonstrated antibacterial activities against gram-positive and gram-negative bacteria strains. As shown in Tables 12 and 13, most of the diluted extract showed antibacterial activity compared to DMSO (negative control) which had inhibition zone of 5 mm (size of formed well). The methanol extract exhibited maximum zone of inhibition against the four bacteria strain as compared to the other extracts inhibition activity of *C. tomentosa leaf. Escherichia coli* were the most susceptible bacteria among the four bacteria strain tested in this study. Among the gram positive bacteria, *Staphylococcus aurous* was the more susceptible than *Streptococcus pyogenes* in methanolic extract of *Capparis tomentosa* as showed in the figure 30, but in ethyl acetate *Streptococcus pyogenes* was more susceptible than *Staphylococcus aurous.*

As showed in the figure 4 the lowest inhibition activity was recorded in petroleum ether extract of *C. tomentosa.* The antibacterial activity of most dilutions of each extract was statistically significant ($P \leq 0.05$) compared to the negative control (DMSO) and displayed similar potency with that of gentamycin, a standard drug used as positive control in this study. Most of the extract showed antibacterial activity but methanolic extract has a pronounced effect against the four bacteria strain(*Klebsiella pneumonia, Escherichia coli, Staphylococcus aurous and Streptococcus pyogenes*) and ethyl acetate extract also had a high antibacterial activity next to methanol extract of *Capparis tomentosa.* Petroleum ether extract had antibacterial activity but much lower than methanol and ethyl acetate extract.

Antibacterial activity decreases as the concentration of the plant extract decrease. This stud was confirmed by the previous report which was stated as followed, extract of *Capparis tomentosa* showed activity of 4 mg/ml against Staphylococcus aurous and 1 mg/ml against Streptococcus pyogenes, whereas the methanol extract was active against *Streptococcus pyogenes* at 4 mg/ml. The highest Zone of inhibition was observed on the methanolic extract which have a diameter of 13.7 mm, this is the highest of all the measured value of each extract but lower than the standard antibiotic drug(gentamycin)which had a diameter 30 mm inhibition zone on Escherichia coli bacteria(negative bacteria) at a concentration of 300 µg/mL. At this concentration ethyl acetate extract and petroleum ether extract showed inhibition zone of 11mm and 9.6 mm on the same bacteria respectively, whereas the standard gentamycin had the highest antibacterial activity (30 mm) and hence supported the results of the present study.

The results showed that for all microorganisms the activity decreased by decreasing the concentration of the extracts. The antibacterial activity of different solvent extracts of Capparis tomentosa leaf extract against Staphylococcus aurous was found to display an inhibition zones within the range between 6.2 and 13.7 mm. The highest activity was observed in the methanol extract (13.7 mm), while there is lower activity on the petroleum ether and ethyl acetate extracts against Staphylococcus aurous as shown in the figure 4 and 32 mm. Where the reference gentamycin (10 µg /disk) gave zone inhibition of 29 and 30 mm. Different extracts of *Capparis tomentosa* showed antibacterial activity against E. coli, which was highly susceptible than the other bacteria strain tested. Table 10 &11 Showed inhibition zone ranged from 6.2 to 13.7 mm in which the lowest activity (6.2 mm) was obtained from the petroleum ether extract of *Capparis tomentosa* and the highest activity (13.7 mm) was recorded from methanolic extract. The leaf extract of *Capparis tomentosa* were showed antibacterial activity against Klebsiella pneumonia, the inhibition zone ranged from 6.2 to 12 mm. The highest activity showed in methanol (12 mm) while the lowest activity showed in petroleum ether extract (6.2 mm). The highest activity of C. tomentosa plant extract was obtained from methanolic extract.

It was observed that most of C. tomentosa extracts showed antibacterial activity. There was a significant difference in antibacterial activity between the different concentrations of the Capparis tomentosa leaf extracts. In the first case (methanol extract) had the most antibacterial activity and there was a significant difference between the different concentration (300,150, 75 and 37.5 ppm) since the p-value (p=0.000) which was less than 0.05 at 95% confidence level. Most of extract had significance difference in inhibition activity at different concentrations of C. tomentosa leaf extract as showed in the table above. Except ethyl acetate extract on Escherichia coli which had p-value (p=0.068) in all the other extracts of C. tomentosa had p-value ($p \leq 0.05$) there was a significant difference between the different concentrations each of the C. tomentosa leaf extracts antibacterial activity [5].

The most susceptible bacteria on methanolic extract of Capparis tomentosa leaf extract was Escherichia coli(gram negative bacteria) which had a maximum mean zone of in inhibition and the next was Staphylococcus aurous (gram positive bacteria). Streptococcus pyogenes (gram positive bacteria) was the lest inhibited (susceptible) bacteria on Methanolic extract of the Capparis tomentosa leaf which was shown on the mean plot of methanolic extract inhibition zone vs. the four bacteria strain tested in this study. Therefore methanolic extract of Capparis tomentosa leaf was most effective in inhibiting Escherichia coli bacteria, but it was less effective in the case of Streptococcus pyogenes which was most resistant to this extract.

Conclusion and Recommendation

Conclusion

The powdered leaf of *Capparis tomentosa* was subjected to successive extraction with petroleum ether, ethyl acetate and methanol respectively and the solvent were removed by Rotary evaporators 06-04(C). Phytochemical works were then conducted on the three extract sample of the leaf of *Capparis tomentosa*. The results of phytochemical work on the extract revealed the presence of alkaloids, phenols, flavonoids, tannins, terpenes, saponins, and glycosides on the extract sample. Antibacterial effects of leaf extract of *Capparis tomentosa* showed different degrees of inhibition against both Gram positive and Gram negative bacteria. The methanol and ethyl acetate extracts showed higher antibacterial activity than petroleum ether extracts.

Recommendation

- Isolation and characterization of bioactive compounds on the crude extracts of the stems, flowers, root, leaf and fruits of Capparis tomentosa plant using different solvent system should be done.
- Anti-microbial studies by using other kinds of bacteria and fungi on the crude extracts and pure compounds should be done.
- Determination of the cytotoxicity level of the extracts from all parts of the plant should also be done.

Acknowledgement

All special thanks and praised to Almighty God who helped me from starting up to this level without any obstacles.

I would like to forward my deepest gratitude to my advisor Yeshitila Asteraye (PhD); His guidance helped me in all the time of research and writing of this thesis. His communicative enthusiasm, patience, motivation, and his encouragement has been great help.

I am greatly thankful to college of Natural Science of Bahir Dar University for providing scholarship for MSc. study. I would like to thank to Dr. AlemuTesfaye (department head of chemistry) and staff members of chemistry department for supplying and facilitating instruments and chemical for this study.

Finally, my special thanks belong to my families for giving me their perpetual love, support, and encouragement throughout my life and I am also grateful to my friends. Thanks to GOD to reach for this day.

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