INJ INTERNATIONAL NEUROUROLOGY JOURNAL

Pharmacological And Phytochemical Screening Of In-Vitro Antimicrobial Activity On Capparis Spinosa Stem

Geetika chandra[1]*,Neelam Painuly[2]*
[1]Research Scholar, School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun, India
[2]Associate Professor, School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun, India

Abstract

Capparis spinosa (Family, Capparidaceae) often referred to as "Kabra," this xerophytic plant can be found in dry areas of India and other nations. The herb has long been utilized for a number of therapeutic uses. The current study focuses on the pharmacognostical and physico-chemical characteristics of the stem of Capparis spinosa. The macroscopical and microscopical characteristics of fresh stems were examined. The stem has numerous slender, spine-adorned branches. The bark of mature branches is brownish in color and leafless. The stem has many branches, each of which has spines and is slender. Mature branches have brownish-colored bark and no leaves. The transverse portion is covered in starch grains and crystals of calcium oxalate. The anatomy of the old stem is similar, but cork has taken the role of the epidermis and hypodermis. The stem powder contains calcium oxalate crystals, starch grains, sclereids, fibers, xylem channels, epidermis, and pieces of cortex and pith. Additionally, the values of ash, water and alcohol soluble extractives, and moisture content were determined. The quality parameters developed would serve as a useful tool in standardization of C.spinosa stem.

Key Words: Capparis spinosa, Capparidaceae, Macroscopy, Microscopy, Quality parameters.

Introduction

Herbs And Herbal Medicine

India is a prosperous source of traditional medicines, many of which are of plant origin. Herbal medicine refers to the use of any plant’s seeds, berries, roots, leaves, bark, or flowers for medicinal purposes.[1-2]. Drugs come from two places: manufactured and natural. Chemical synthesis is used to make a lot of drugs that are used in health today. The most important natural sources of drugs are higher plants, bacteria, animals, and objects that live in the ocean. [3-4]. Traditional medicine is the culmination of therapeutic knowledge passed down through generations of practitioners within indigenous systems of medicine. Traditional remedies often consist of a combination of medicinal herbs, minerals, organic matter, and other similar components. Herbal pharmaceuticals encompass traditional treatments that predominantly employ medicinal plant extracts for therapeutic purposes. [5-6]. The corpus of classical Indian texts includes the Rigveda, Atharvaveda, Charak Samhita, and Sushruta Samhita. Herbal medicines, also known as traditional medicines, have been developed based on the extensive traditions of ancient civilizations and scientific knowledge passed down through generations.[7-8].

It is clear that herbal medications absolutely require "standardization" with regard to botanical, chemical, physico-chemical, and therapeutic criteria. Chemical and biological profiling is a component of the assay phase of standardization that allows for the evaluation of chemical and biological effects as well as the establishment of effective doses. This parameter also provides access to safety. Chemical investigations are still a popular technical method since they are adaptable and useful for standardization. In terms of quantitative evaluation, standardization processes are developed by analyzing the concentrations of secondary metabolites, which are thought to represent the active ingredients in herbal medications. The compounds under consideration are referred to as "marker" compounds. While some of them may not be therapeutically active, they should be present in large quantities when used. Marker analysis can help in many ways, for example, to check the strength, guess the active ingredients, and ensure the quality and consistency of botanical raw materials and produced goods. They might also help find the adulterants and keep an eye on the breakdowns, which is important for testing the security of herbs and figuring out how long a product will last. Chromatographic testing of herbal drugs is one of the most common ways to look at marker components. Thin-layer chromatography is used a lot in this testing because it is easy, cheap, and repeatable. HPTLC with a densitometry camera can also be used to make fingerprints of the chromatographic patterns and do quantitative analysis of markers. Chromatographic fingerprinting is used for plant drugs to not only identify them but also check how strong they are and how stable their chemical parts are. [9-10].

© International Neurourology Journal
DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

Plant Profile

Capparis spinosa, commonly known as the caper bush or Flinders rose, is a perennial plant characterized by its rounded, fleshy leaves and sizable white to pinkish-white flowers. Renowned for its culinary applications, the plant is primarily valued for its edible flower buds, known as capers, often used as a seasoning or garnish. Additionally, the fruit, termed caper berries, is also consumed after being salted or pickled. Other species within the Capparis genus are similarly harvested for their buds or fruits. Various parts of Capparis plants find utility in the production of medicines and cosmetics.

Native to nearly all circum-Mediterranean countries, Capparis spinosa is present in the flora of many of these regions, although its exact indigenous status remains uncertain. The Capparaceae family, to which it belongs, is speculated to have originated in the tropics and subsequently spread to the Mediterranean basin.

The taxonomic classification of Capparis spinosa is a subject of controversy and remains unsettled. The species displays significant variability, and the existence of interspecific hybrids throughout its evolutionary history adds complexity. Consequently, some scholars propose that C. spinosa comprises multiple distinct species, while others argue that it represents a singular species with various varieties or subspecies. There are also suggestions that C. spinosa may be a hybrid resulting from the crossbreeding of C. orientalis and C. sicula

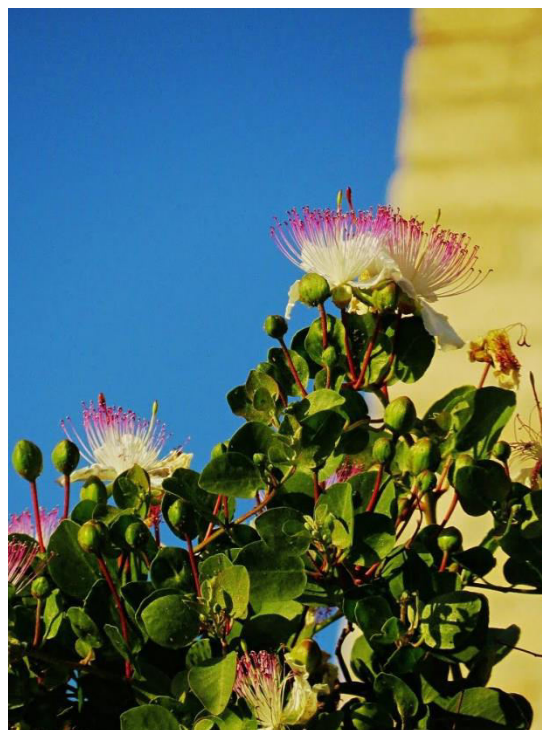


Fig 1: *Capparis spinosa* (leaves and flower buds)

Botanical Name: ***Capparis spinosa***
Family Name: Capparidaceae
Common Name: Caper bush
Part used: stem

Material And Methods

Table 1: Instruments List

INSTRUMENT	INSTRUMENT DETAIL
Round –bottom flask	Corning
Condenser	Chemglass
Heating mantle or hot plate	Cole - parmer
Electric heater	Yamato scientific
Digital PH meter	Mettler toledo
Optical microscope	Zeiss

© International Neurourology Journal

ISSN:2093-4777 | E-ISSN:2093-6931
DOI: 10.5123/inj.2023.4.in115
Vol. 27 Iss. 4 (2023)

Filtration apparatus	Milliporesigma
Rotary evaporator	Yamato scientific
Digital balance	Ohaus
Magnetic stirrer	Corning
Vernier caliper	Mitutoyo
Graduated cylinder	Corning
Volumetric pipette	Corning
Refrigerator	Thermo fisher scientific
Test tubes	Corning

Table 2: Material used

Material Name	Company Name
Capparis spinosa Methanol	Botanical garden Methane corporation
Ammonia nitrate sol	Mortar salt
Sulphuric acid	Fisher scientific
Chloroform	Fisher scientific
Dragendorff's reagent	Fisher scientific
Ethanol	Valero energy corporation
Boric acid sol	Fisher scientific
Oxalic acid sol	Fisher scientific
Folin ciocalteu sol	Fisher scientific
Lead acetate sol	Honey well
Acetic acid	Fisher scientific
Thiourea sol	Fisher scientific
Sod. Sulfide sol	Fisher scientific
Hcl acid	Honey well
Sod. Carbonate sol	Fisher scientific
Aluminum chloride	Mortar salt

Macroscopical and Microscopical Studies

The stem and its powdered components were examined to analyze their specific macroscopic and microscopic characteristics. Transverse slices of fresh, young, and elderly stems were obtained and examined. The microscopical study involved the use of a specialized CCD (charged coupled device, Lawrence and Mayo) camera, which was mounted to an Olympus magnus tilted trinocular research microscope.

Determination of Physico-chemical Parameters

Different physical and chemical properties of *C. spinosa* roots were studied.

Determination of loss on drying

10g of drugs, which were not dried beforehand and were chopped into approximately 3 mm-thick pieces, were precisely weighed and placed in a dish that had been weighed beforehand. The medication was subsequently dehydrated at a temperature of 105°C for a duration of 5 hours and then measured in terms of weight. The process of drying and weighing was repeated every hour until the difference between two consecutive weighing was less than or equal to 0.25%. The criterion for achieving constant weight was defined as the condition in which two consecutive weighing, conducted after a 30-minute drying period followed by a 30-minute cooling period in a desiccator, exhibit a difference of no more than 0.01g.

Determination of ash values

Ash values of powder of *C. spinosa* stems were determined by the following method:

(a) Determination of total ash

A precisely measured 2 g of stem powder was burned in a muffle furnace at a temperature below 450°C until all of the carbon was gone. The crucible is a silica dish that was weighed before the burn. After allowing the crucible to cool, another weight measurement was taken. If carbon-free ash could not be collected in this manner, the charred mass was exhausted with about 2 milliliters of hot water or a saturated solution of

© International Neurourology Journal
DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

ammonium nitrate. After being gathered on ash-free filter paper, the residue was dried and burned until it reached a constant weight. The proportion of ash was calculated by weighing and chilling the resultant ash and comparing it to the medicine powder that had been air-dried.

(b) Determination of acid insoluble ash

The ash obtained from the previous method was subjected to boiling for a duration of 5min with 25 milliliters of diluted hydrochloric acid (concentration: 70 grams per liter). The resulting mixture was then filtered using a filter paper that did not leave any residue in order to collecting the insoluble substances. The ash was rinsed with hot water and then heated, together with the filter paper, in a muffle furnace at a temperature of 450°C until a steady weight was achieved. The acid-insoluble ash percentage was determined relative to the air-dried powdered medication.

(c) Determination of water soluble ash

The entire ash was brought to a boil in 25ml of water for five minutes. The solids that did not dissolve were placed on ashless filter paper. After being cleaned in hot water, it was burned for 15min at a temperature not to exceed 450°C in a muffle furnace. By deducting the weight of ash from the weight of material that doesn't dissolve in water, the weight of ash that is water soluble was determined. The amount of water-soluble ash was determined using the powdered drug that had been air-dried.

Determination of extractive values

Extractive values of powder of *C. spinosa* stems were determined by the following method:

(a) Determination of water soluble extractive (Hot extraction)

4g of the powdered material were macerated in100ml of water in a closed flask for an hour while being shaken continuously. It was allowed to cool after a slow one-hour boil in a water bath, and then its weight was measured and corrected. A porcelain plate containing 25 ml of the filtrate was dried at 105°C until it reached a consistent weight by evaporation. Using the powdered medication that had been air-dried as a reference, the percentage of water-soluble extract was determined.

(b) Determination of alcohol soluble extractive (Cold extraction)

4g of air-dried powdered components were placed in a sealed flask. The components were soaked in 100ml of alcohol for a duration of 6 hrs, with the flask being shaken every hour. After being allowed to stand for 18hrs, it was promptly filtered to prevent any loss during evaporation. 25 ml of the filtrate were evaporated in a porcelain dish until all the liquid had completely evaporated, and then the remaining substance was dried at a temperature of 105°C until it reached a stable weight. The air-dried drug in powdered form was used as a benchmark to calculate the proportion of extractable substances soluble in alcohol.

Preliminary Phytochemical Screening

Test for alkaloids (Test with Dragendorff's reagent)

After 15 min of refluxing in 95% alcohol, 1 g of powder were extracted into 20 ml of liquid, which was then filtered and evaporated to dryness? 15 ml of 2N H_2SO_4 was used to dissolve the residue, and then it was filtered. The filtrate was alkalized, and then chloroform was used to extract the resulting liquid. Alkaloids were detected in the remaining residue using Dragendorff's reagent. When orange precipitates formed, we knew there were alkaloids present.

Test for flavonoid

Sinodha test: A volume of 10 ml of ethanol with a concentration of 95% by volume was used for the extraction of 1 g of powdered stem. Filtration of the finished mixture came after the extraction procedure, which lasted for 15 minutes in a water bath at boiling temperature.

Fluorescence test: A boiling water bath was used to remove 15 ml of powder from 1 g of it. The mixture was then filtered while it was still hot and evaporated until it was dry. 0.3 ml of a 3% w/v boric acid solution and 1 ml of a 10% w/v oxalic acid solution were added to the remainder. It was dried out by evaporation, and 10 ml of ether was used to remove the residue. Under U.V. light, the glow in the ethereal layer was seen. The appearance of greenish light in the ethereal layer showed that flavonoids were present.

Tests for phenolic compound

© International Neurourology Journal

DOI: 10.5123/inj.2023.4.in115

ISSN:2093-4777 | E-ISSN:2093-6931

Vol. 27 Iss. 4 (2023)

Test with fecl3: A 15-min boil in water was used to remove 10 ml of stem powder, which was then filtered. A drop of the newly made $FeCl_3$ solution was added to the filtrate. The presence of phenolics was shown by a dark green color.
Test with Folin ciocalteu reagent: A drop of Folin-ciocalteu solution was added to a drop of methanolic extract. The formation of a bluish-green color showed that phenolics were present.

Tests for tannins
The stem was powdered and then refluxed with 50 cc of water for approximately one hour on a water bath to create an aqueous extract. This extract was subsequently utilized for the subsequent experiments.

Test with lead acetate: 2 ml of a 10% w/w solution of lead acetate were added to the aqueous extract. The formation of dense, pale yellow precipitates that were only partially soluble in 1 ml of 10% acetic acid determined the presence of tannins.

Test for saponins
Forth test: In a test tube, 0.1 g of powder was agitated violently for 30 sec with five milliliters of distilled water, and the mixture was then allowed to settle for 20 min. There was a persistent foam that determined the presence of saponins.

Test for sterols and triterpenoids
Libermann burchardt's test: The drug powder, weighing 1 gramme, was mixed with 1 ml of acetic anhydride and 2 drops of H_2SO_4 on a clean floor. It was easy to mix the powder. When the powder turned red or purple, it meant that sterols and triterpenoids were present.

Salkawski's reaction: A 15 min boil in water was used to remove 10 ml of stem powder, which was then filtered. Add 2 ml of filtrate, 2 ml of chloroform, and 2 ml of pure H_2SO_4 to a shaker and mix well. Sterols and triterpenoids were present because the chloroform layer turned red and the acid layer fluoresced in a greenish yellow color.

Test for coumarins
Test with ammonia: A small quantity of ammonia was placed on a piece of filter paper. Subsequently, a small amount of methanolic extract powder was introduced. The lack of fluorescence showed the absence of coumarins.

Test with hydroxylamine hydrochloride: To the given sample, introduce a small amount of concentrated alcoholic hydroxylamine hydrochloride solution and a small amount of alcoholic potassium hydroxide. The solution underwent heating, cooling, and acidification using 0.5 N HCl. Additionally, a small amount of 1% w/v $FeCl_3$ solution was introduced. The absence of coumarins was shown by the lack of violet color development.

Tests for anthraquinone glycosides
Borntrager's test: The drug powder was removed with ether or any other organic solvent that doesn't mix with water. Ionized ether was cleaned, and ammonia was added to it. The fact that the water layer didn't turn pink or red showed that there were no anthraquinone glycosides present.

Modified Borntrager's test: The drug's aqueous extract was mixed with diluted HCl and ferric chloride. The combination underwent filtering, cooling, and heating. To shake the filtrate, ether or any other organic solvent was used. We shook the ethereal extract with diluted ammonia. The lack of anthraquinone glycosides was shown by the aqueous layer showing no development of rose pink to cherry red.

Estimation of Phytoconstituents
Estimation of total alkaloids
Preparation of extract: It took 25 ml of 2% v/v ethanol acetic acid at 100°C for 10 minutes to remove 10 g of coarse powder. The process was done twice more. The extracts were mixed together, and 2% v/v ethanol acetic acid was used to thin them out to 100 ml.

Procedure for calibration curve: The calibration curve was generated using a stock solution of bismuth nitrate pentahydrate with a concentration of 0.01% w/v. The stock solution was diluted by pipe ting off 1, 2, 3, 4, 5, 6, 7, 8, and 9 ml into individual 10 ml standard flasks and then filling the flasks to their capacity with distilled

DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

water. 5 ml of a 3% w/v thiourea solution was added to 1 ml of this solution. The solution's absorbance was measured at a wavelength of 435 nm relative to a blank sample.

Procedure for assay of alkaloids

A 5 ml sample extract was obtained, and diluted HCl was used to keep the pH between 2 and 2.5. It was then treated with 2 milliliters of 1% v/v Dragendorff's reagent (DR), and the resulting precipitates were centrifuged. By adding DR, the centrifugation was examined to ensure full precipitation. Then, alcohol was used to wash the precipitates. After discarding the filter, 2 milliliters of a 1% w/v sodium sulfide solution were applied to the residual. The resulting brownish-black precipitates were dissolved in two milliliters of strong nitric acid, heated as needed. In a standard flask, this solution was diluted to 10 ml using distilled water. 5 ml of a 3% w/v thiourea solution was added to 1 ml of the resulting solution. At 435 nm, the absorbance was measured in relation to a blank that contained thiourea and nitric acid. The standard curve, which is a constant for various concentrations, yields the factor.

Estimation of flavonoids (AlCl3 method)

Preparation of extract: 25 ml of 95% ethanol and 1 g of the powder were extracted over the course of 24 hours with 200 RPM shaking. Following filtration, 25 ml of 80% ethanol was added to the filtrate.

Calibration curve and estimation: Add 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water to the 0.5 ml of extract mentioned above. The absorbance of the reaction mixture was measured at 415 nm using a Shimadzu UV spectrophotometer after 30 minutes of incubation at room temperature. Blank was made by substituting 10% aluminum chloride with the equivalent volume of distilled water. The same process was used to plot the calibration curve of rut in (200 to 700 µg/ml), which was used to compute the percentage of total flavonoid. The results were reported as gram per hundred gram of dry weight.

Estimation of phenolics

Preparation of extract: A total of 1g of stem powder that had been dried in the air was subjected to extraction using 25 milliliters of methanol through the process of maceration for duration of 24 hours. The resulting mixture was then filtered. The filtrate was ultimately brought to a volume of 25 ml by adding methanol.

Calibration curve and estimation: 0.3 ml of the methanolic extract was combined with 1.5 ml of diluted Folin-Ciocalteu reagent (1:2) and 10 ml of distilled water. Following a 5-minute incubation period, 4 milliliters of a 20% w/v sodium carbonate solution were added, and 25 milliliters of distilled water were added. With distilled water acting as a blank, the absorbance was measured at 765 nm after 30 minutes. To find the proportion of total phenolics, the calibration curve of gallic acid (10 to 150 µg/ml) was plotted using the same technique. Gallic acid was used as a percentage to reflect the total amount of phenolics.

Estimation of saponins (Foaming index)

After precisely weighing 1 gram of the drug's fine powder, it was put into a 500ml conical flask along with 100 ml of boiling water. For half an hour, it was kept at a moderate boil. After cooling and filtering, the mixture was transferred to a 100-ml volumetric flask, where it was diluted with water until it reached 100 ml. using a series of successive amounts ranging from 1 to 10 ml, the decoction was carefully transferred into ten graduated test tubes with stoppers. The dimensions of these tubes were 16 cm in height and 16 mm in diameter. The volume of the liquid was then corrected to 10 ml in each tube by adding water. For 15 seconds at 2 Hz, the tubes were shaken vertically (lengthwise) with stoppers. After 15 minutes of standing, the tubes were lifted to measure the foam's height. The following was done to evaluate the outcomes: The foaming index is below 100 if the foam height in each tube is less than 1 cm. The volume of the plant material decoction in tube (a) is used to determine the index if any tube measures a 1 cm foam height. To get a more accurate result, create an intermediate dilution in the same way, whether this is the first or second tube in a series. A foaming index greater than 1000 indicates that the foam height in each tube exceeds 1 cm. If that's the case, you'll need to run the determination again, this time using a different sequence of decoction dilutions.

The foaming index was calculated using the following formula:

$$1000/a$$

Where a = volume (in ml) of the decoction used for preparing dilution in the tube

Where foaming to a height of 1 cm is observed

Estimation of triterpenic acids

© International Neurourology Journal

DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

INJ INTERNATIONAL NEUROUROLOGY JOURNAL

Preparation of extract: It took 50 ml of methanol and 24 hours of maceration to get 0.5 g of air-dried stem powder. The powder was then sifted. Using methanol, the end volume of the filtrate was brought down to 50 ml. After drying out 25 ml of the methanolic extract, it was mixed with chloroform that had 0.05% v/v acetic acid in it. After that, 10% w/v sodium carbonate was used to get more out of it. Formic acid, or acetic acid, was used to make the water layer more acidic. After that, chloroform was used to get more out of it, and the organic layer was drained until it was dry. Then it was mixed back together with 50 ml of alcohol and used to measure triterpenic acid.

Method: 50 ml of alcoholic solution was titrated against 0.1 N sodium hydroxide using. Phenolphthalein as an indicator.
Factor: 1 mol 0.1 N sodium hydroxide ≡ 1 mol of triterpenic acid

Preparation of Test Extracts for Pharmacological Studies

Three extracts, including the aqueous extract, alcoholic extract, and hydroalcoholic extract, were produced from the stems for the activity. 100 g of the dehydrated powdered stem were heated for two hours in a water bath with 500 ml of distilled water to obtain the aqueous extract. Following filtration, the solution was subjected to evaporation under reduced pressure, resulting in the formation of a solid extract referred to as Extract-A. An alcoholic extract was obtained by thoroughly extracting 100 g of powdered stem with 500 ml of 100% alcohol for 5 hours using a Soxhlet apparatus. Following the filtration process, the solvent was subjected to evaporation under reduced pressure, resulting in the formation of a semi-solid extract. This extract was subsequently designated as Extract-B. 100 g of powdered stem were heated for two hours in a water bath with a 50% hydroalcoholic solution to obtain a hydroalcoholic extract. Following filtration, the solution was subjected to evaporation under reduced pressure, resulting in the formation of a semi-solid extract. This extract was designated as Extract-C. The extracts A, B, and C were suspended in distilled water with acacia (1%) as a suspending agent before being administered, according to the dose expressed in mg of extract.

Screening of Antimicrobial Activity

Preparation of test extracts: Extracts A, B, and C (made according to the method in Section 5.10) was mixed with DMSO until they reached a concentration of 100 mg/ml.

Preparation of standard drugs: The standard antibiotics for bacteria and fungi and ciprofloxacin, respectively. They were dissolved in DMSO to achieve a 100 mg/mL concentration.

Microorganisms for anti-microbial screening

The stem was tested against nine bacterial strains and two fungus strains using three different extracts: aqueous, alcoholic, and hydro-alcoholic. The test organisms included gramme-positive
Factor: 1 mol 0.1 N sodium hydroxide ≡ 1 mol of triterpenic acid

Preparation of Test Extracts for Pharmacological Studies

Three extracts, including the aqueous extract, alcoholic extract, and hydro alcoholic extract, were produced from the stems for the activity. 100 g of the dehydrated powdered stem were heated for two hours in a water bath with 500 ml of distilled water to obtain the aqueous extract. Following filtration, the solution was subjected to evaporation under reduced pressure, resulting in the formation of a solid extract referred to as Extract-A. An alcoholic extract was obtained by thoroughly extracting 100 g of powdered stem with 500 ml of 100% alcohol for 5 hours using a soxhlet apparatus. Following the filtration process, the solvent were subjected to evaporation under reduced pressure, resulting in the formation of a semi-solid extract. This extract was subsequently designated as Extract-B. 100 g of powdered stem were heated for two hours in a water bath with a 50% hydro alcoholic solution to obtain a hydro alcoholic extract. Following filtration, the solution was subjected to evaporation under reduced pressure, resulting in the formation of a semi-solid extract. This extract was designated as Extract-C. The extracts A, B, and C were suspended in distilled water with acacia (1%) as a suspending agent before being administered, according to the dose expressed in mg of extract.

Screening of Antimicrobial Activity

Preparation of test extracts: Extracts A, B, and C (made according to the method in Section 5.10) was mixed with DMSO until they reached a concentration of 100 mg/ml.

Preparation of standard drugs: The standard antibiotics for bacteria and fungi and ciprofloxacin, respectively. They were dissolved in DMSO to achieve a 100 mg/mL concentration.

© International Neurourology Journal
DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

Microorganisms for anti-microbial screening
The stem was tested against nine bacterial strains and two fungus strains using three different extracts: aqueous, alcoholic, and hydro-alcoholic. The test organisms included gramme-positive.

Preparation of inoculums: At 4°C, stock cultures were maintained on the slopes of the respective medium. To produce active cultures for the tests, a loopful of stock cultures was transferred to test tubes containing Mueller Hinton Broth for bacteria and Sabouraud Dextrose Broth for fungal strains. Following that, the test tubes were incubated for 24 hours at 37°C for the bacterial strains and 72 hours at room temperature for the fungal strains without being stirred. Using the appropriate conditions, the cultures were diluted to create suspensions (100 µl) containing 108 cfu of bacteria and 104 fungal spores/ml.

Anti-microbial assay

By Agar-well Diffusion Assay: The agar-well diffusion method was used to test the antimicrobial properties of the three *C. spinosa* stem extracts on different types of bacteria.

Principle: The test organisms are placed on a plate, and the antimicrobial components of the plant extracts are allowed to soak into the medium and interact chemically with the organisms. Because there is a confluent lawn of growth, the resulting zone of inhibition has a circular shape. The diameter of the inhibitory zone is measured in millimeters.

Procedure: (a) MHA and SDA media plates were made by putting 20 ml of molten MHA and SDA media into clean petri dishes and leaving them there for 5 minutes to harden. b) The bacterial and fungal types were spread evenly on the plates with a sterile spreader to make the plates "seeded." A clean cork borer was used to make 6-mm wells in the plates after they had been dried in an aseptic way. c) A clean micropipette was used to add 50 µl of the plant extracts to each well. d) As normal antibiotics, 50 µl of ciprofloxacin and griseofulvin were used, and DMSO was used as a negative control. e) The plates were left for two hours so that the plant oils could spread out. f) The plates were then left to grow for 72 hours at room temperature for fungi and 24 hours at 37°C for bacterial strains. g) The antibiotic zone reader was used to measure the width of the clear zones of inhibition that formed around the well to test the antibacterial and antifungal activities.

Determination of Minimum Inhibitory Concentration (MIC)

By Broth Microdilution Susceptibility Test: Using micro dilution approach, the minimum inhibitory concentration (MIC) values were determined for the bacteria that showed extract sensitivity in the agar-well diffusion method.

Procedure: (a) The extract, which showed the highest inhibition in the agar-well diffusion test, was diluted in a series of steps. Then, 50 µl of the diluted extracts were introduced to test tubes containing Mueller Hinton Broth and Sabouraud's Dextrose Broth. (b) Each test tube was inoculated with 100 µl of a calibrated suspension containing 108 cfu/ml of the microorganism to be examined. (c)The tubes were then incubated at a temperature of 37°C for a duration of 18 hours (d) Ciprofloxacin was employed as the usual antibacterial agent, whereas griseofulvin was used as the antifungal agent.

After the incubation period, the tubes were visually inspected for the presence of turbidity and cloudiness. Turbidity signifies that the concentration of the extract in the medium has not effectively prevented bacterial growth. The MIC (minimum inhibitory concentration) was determined as the highest dilution that exhibited at least a 99% inhibition zone without any turbidity. For the primary screening, the extracts were tested at concentrations of 5000 µg/ml, 2500 µg/ml, 1000 mg/ml, and 500 µg/ml. Subsequently, the extracts underwent additional testing against all microbes in a second batch of dilutions, following the initial screening results.

Result and Discussion

Macroscopical Study

Root: It is spherical, wavy, deeply spread out, and tapering at the very end. Its width ranges from 1 to 6 cm, and its roots are thin and wiry. The surface is rough, with horizontal grooves and ridges and areas where it has been peeled off. The cracks are fibrous, and the colour is creamish brown on the outside and white on the inside. The smell and taste are not clear.

Stem: It is extremely branching, with slender, cylindrical, glabrous branches that can reach a height of 5 m. The width of each branch varies from 0.1 to 2.5 cm, and each branch is gently twisted at each node to give the

© International Neurourology Journal

DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

illusion of a zigzag. The internodes are 1.5 to 4 cm in length; the nodes measuring 0.5 to 2.5 cm in diameter. A pair of tiny thorns, measuring 4 to 6 mm in length and 1 to 2 mm in diameter, is present in the nodes. When a stem is young, it is usually dark **Microscopical Study**

Green, but as it ages, it turns pale green to brownish when dried, and the bark on the stems becomes whitish-gray. The stems have a distinct flavor and aroma.

Fresh samples of *C. spinosa* were cut into young and old stems, and free-hand transverse sections (T.S.) were collected. The histological characteristics of these stems were examined.

Transverse section of young stem: The transverse section (T.S.) of young stem is circular in outline and reveals the presence of following:

Epidermis: It is made up of a single layer of cells that run perpendicular to each other and is covered with a thick, papilloma covering. It shows that the stomata are sunk in at regular intervals. Trichomes are not present.
Hypodermis: The layer is beneath the epidermis and consists of 2–5 layers of elongated, densely packed cells resembling palisades, which contain chloroplasts. The arrangement of these cells is characterized by alternating intervals of elongated and lignified sclereids, which occur in groups of 5 to 20.

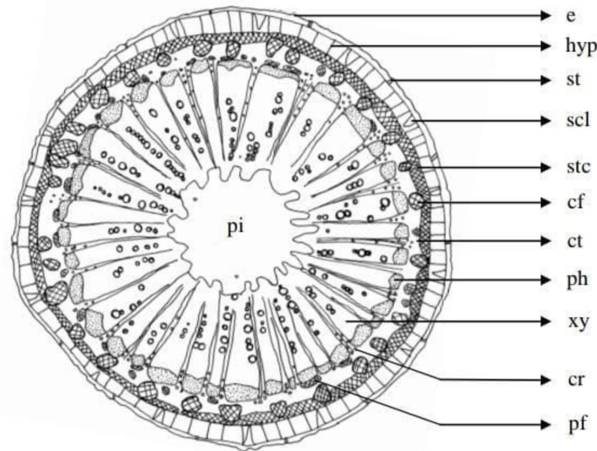
Cortex: It is composed of 5–15 layers of parenchymatous cells that are diametric. A band of thick-walled, lignified, pitted stone cells, two to three cells broad, is seen in the cortex's periphery. A collection of four to fifty tiny, lignified, thick-walled cortical fibers, as well as occasionally a few sporadic stone cells, are also seen in the cortex.

Transverse section of the old stem

The epidermis and hypodermis are made of cork instead of wood. Cork is made up of 10 to 20 layers of lignified cells that are arranged radially and laterally. There is a band of lignified stone cells below the epidermis, and then there are 5 to 8 layers of cortex. There are not many or any cortical fibers or pericyclic fibers. Secondary phloem, ceratenchyma, and secondary xylem can also be seen in the stele area. Pith is broad and well-formed.
Powder study: The stem powder is firm, pale green, and has a strong smell and taste. 2. When looking at *C. spinosa* stem powder under a microscope, the following features help identify it. 3. Pieces of skin are seen from the top, showing sunken stomata that are embedded. 4. Splits of epidermis are seen across, showing a rough cuticle and hollow stomata that are embedded. 5. Stone cells that are lignified and scarred; they can be found alone or in groups of two to twenty-five. 6. It is made up of lignified sclereids in the hypodermis. 7. Groups of lignified strands with septet end 8. Pieces of lignified cork are seen from the top. 9. Diagrammatic t.s young stem of c.spinosa. 10. Pieces of parenchymatous cells are in the brain. 11. Modularly rays are cut in a circle. 12. Simple or compound starch grains, with two or three grains in a complex starch grain.

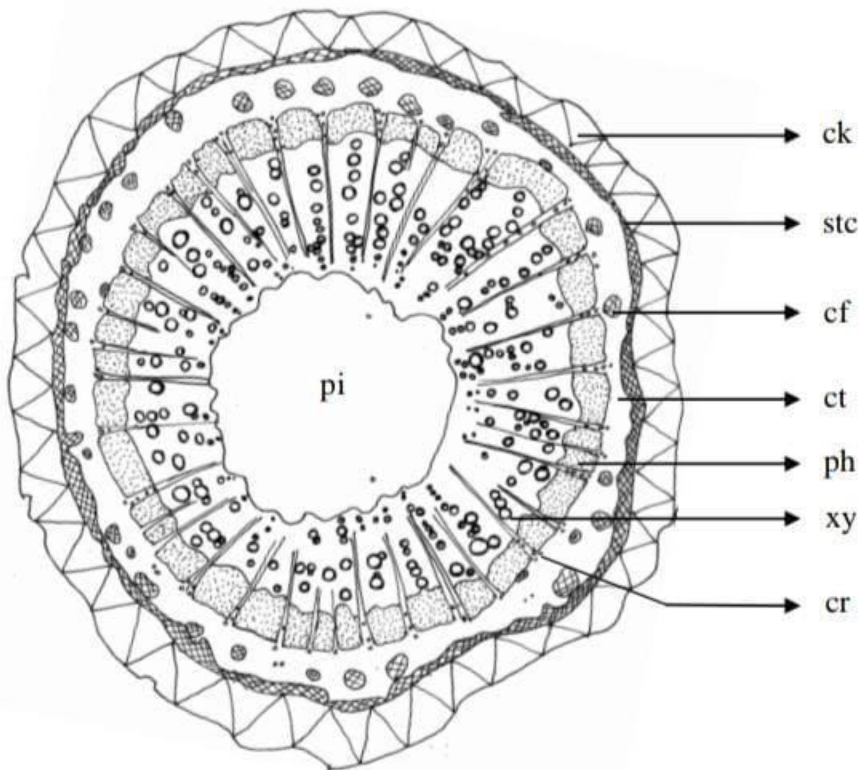
Diagrammatic T.S of young stem of c.spinosa

© International Neurourology Journal
DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)



cf, cortical fibre; cr, crystal; ct, cortex; e, epidermis; hyp, hypodermis; pf, pericyclic fibres; ph, phloem; pi, pith; scl, sclereid; st, stomata; stc, stone cells; xy, xylem.

Diagrammatic t.s.of old stems c.spinosa



cf, cortical fibre; ck, Cork; cr, crystal; ct, cortex; ph, phloem; pi, pith;stc, stone cells; xy, Xylem

Determination of Physico-chemical Parameters

The powder of *C. spinosa* stem was studied for loss on drying, ash values and Extractive values.

Table 3: Physico – chemical parameters of *C. spinosa* stem

Quality parameters	Values (% w/w +_ s.d.)
Loss on drying	44.12+_ 0.203
Ash value	
Total ash	8.13+_0.030
Acid insoluble ash	0.50+_0.019
Water soluble ash	6.03+_ 0.025
Extractive value	
Water soluble extractive	20.00+_0.135
Alcohol soluble extractive	6.67+_ 0.127

Standard deviation (+_S.D); Number of reading=3

Preliminary phytochemical screening

Alkaloids, flavonoids, phenolics, tannins, saponins, sterols, triterpenoids, and carbohydrates were found in the initial phytochemical screening.

Preliminary phytochemical screening

Table 4:

Phytoconstiuent	Test	Present (=)or absent (-)
Alkaloids	Test with dragendorff's reagent	+
Flavonoids	Shinoda test Flurecence test	+ +
Phenolic	Test with fecl3	+
Tannins	Test with gelatin Test with lead acetate	+ +
Saponins	Forth test Hemolytic zone test	+ +
Sterols and triterpenoids	Libermann burchardt's test Salkowski reaction	+ +
Carbohydrates	Molisch's test Fehling test	+ +
Cartenoids	Test with antimony trichloride	-
Coumarins	Test with ammonia Test withhydroxylamine hydrochloride	- -
Anthraquinone glycosides	Borntrager's test Modified borntrager's test	- -
Cardenolids	Kedde's test Baljet test Keller killiani's test	- - -

Estimation of phytoconstituents

Different chemical methods were used to figure out the phytoconstituents in *C. spinosa* stem powder. The drug's foaming index was also found, and the results are shown in the table.

© International Neurourology Journal
DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

Table 5: Estimation of phytoconstituents

Phytoconstituents	Amount(%w/w+_S.D.)
Alkaloids	0.041 +_ 0. 153
Flavonoids	0.13 +_ 0.236
Phenolics	0.22+_ 0.193
Triterpenic acid	0.08+_ 0.160
Carbohydrates	
Sugar	3.21+_0.026
Starch	4.57+_ 0.58

Standard deviation (S.D.) ; Number of readings (N) = 3

Table 6: Estimation of foaming index

Parameter	Result +_ S.D.
Foaming index	**200+_ 0.261**

Screening of Antimicrobial activity

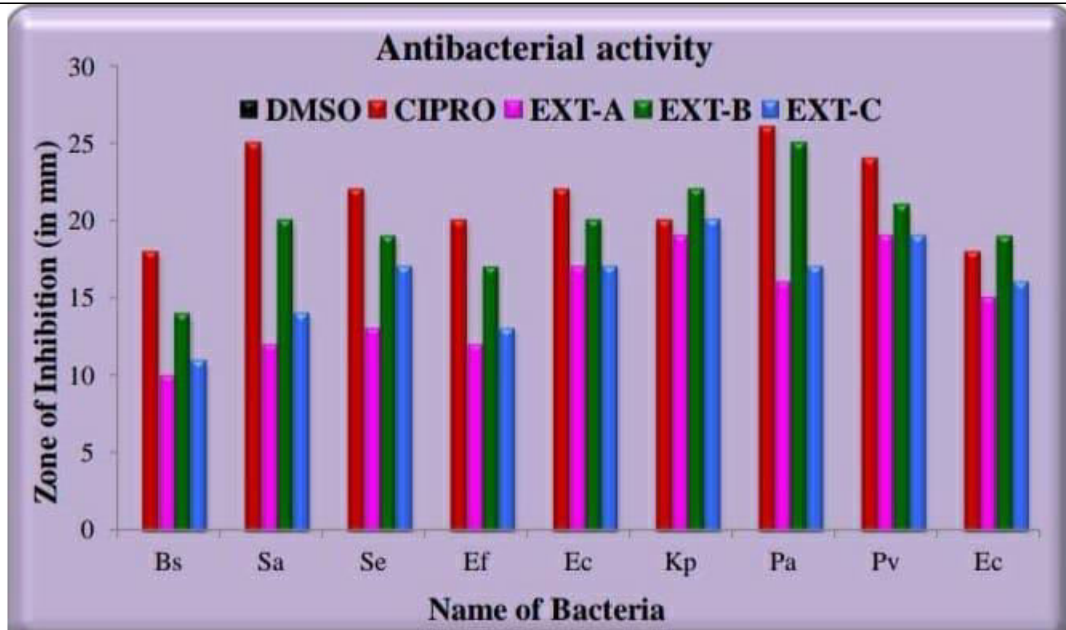
The three C. spinosa stem extracts (Extracts A, B, and C, which are aqueous, alcoholic, and 50% hydroalcoholic extracts, respectively) were tested using strains of bacteria that were Gram-positive, like Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, and Enterococcus faecalis; strains that were Gram-negative, like Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, and Erwinia Carotovora; and strains of fungus, like Candida albicans and Aspergillus niger. The table presents the findings from the stem extracts of C. spinosa antibacterial screening.

Evaluation of Antimicrobial effects of *C. spinosa* stem extracts by Agar-well Diffusion method
Table: 7

Name of Pathogens	Diameter of Zone of Inhibition (in mm)				
	DMSO	STD	EXT-A	EXT-B	EXT-C
Bacillus subtilis	-	18	10	14	11
Staphylococcus aureus	-	25	12	20	14
Staphylococcus epidermidis	-	22	13	19	17
Enterococcus faecalis	-	20	12	17	13
Escherichia coli	-	22	17	20	17
Klebsiella pneumonia	-	20	19	22	20
Pseudomonas aeruginosa	-	26	16	25	17
Proteus vulgaris	-	24	19	21	19
Erwinia carotovora	-	18	15	19	16
Candida albicans	-	25	22	25	23
Aspergillus niger	-	22			

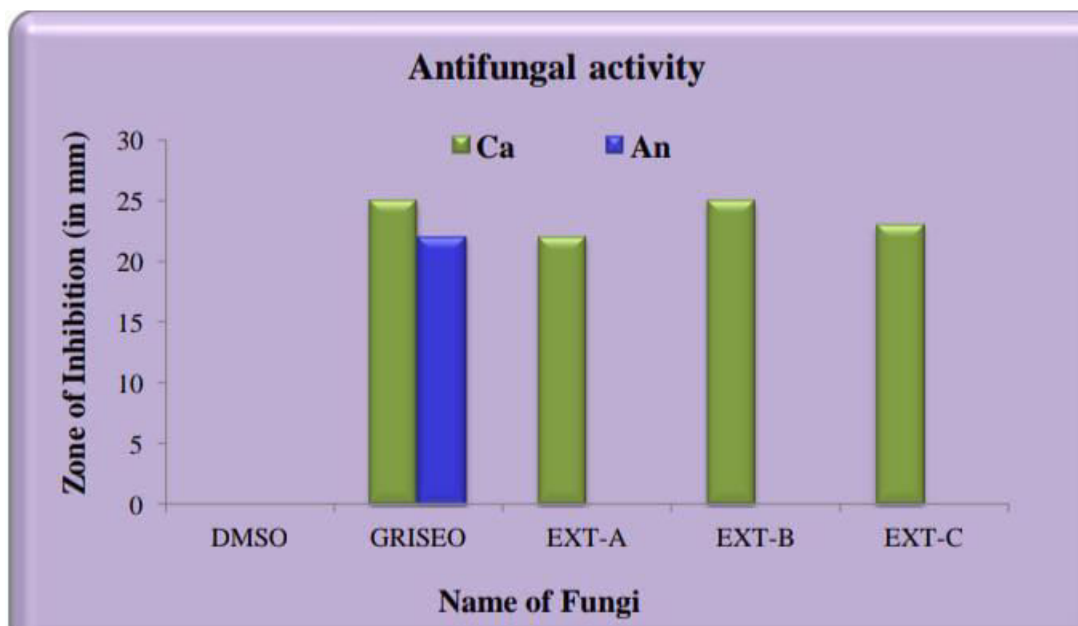
© International Neurourology Journal

DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)



Graph 1
Antibacterial activity of c.sapinosa extract

DMSO - Dimethyl sulfoxide (Negative control); Cipro - Standard drug, Ciprofloxacin; Ext-A - Extract-A; Ext-B - Extract-B; Ext-C - Extract-C; Bs - *B. subtilis*; Sa - *S. aureus*;Se - *S. epidermidis*; Ef - *E. faecalis*; Ec - *E. coli*; Kp - *K. pneumoniae*; Pa - *P. aeruginosa*;Pv - *P. vulgaris* and Ec - *E. carotovor*



Graph 2
Antifungal activity of c. spinosa stems extract

DMSO - Dimethyl sulfoxide (Negative control); Griseo - Standard drug, Griseofulvin; Ext-A - Extract-A; Ext-B - Extract-B; Ext-C - Extract-C; Ca - *Candida albicans*; An - *Aspergillus niger*

The diameter of the zone of inhibition indicates that all three of the *C. spinosa* stem extracts tested (at a concentration of 5 mg/well) demonstrated antibacterial activity, inhibiting every bacterial strain tested. However, Extract-AB (alcoholic extract) exhibited the highest activity compared to Extract-A (aqueous extract), and Extract-C (50% hydroalcoholic extract) was found to be more active against Gram negative bacteria than

© International Neurourology Journal
DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

Gram positive bacteria. Extract-B produced inhibitory zones that were quite similar to those of the normal medication for the Gram-negative bacteria pseudomonas aeruginosa, and significantly higher than that of the standard treatment for the germs Klebsiella pneumonia and Erwinia carotovora. Furthermore, considerable antifungal activity against C. albicans was identified in all of the extracts. but the extract did not inhibit Aspergillus niger. The fungal strain C. Albicans were inhibited strongly by Extract-B with the diameter of the inhibition zone similar to the standard antibiotic Griseofulvin.

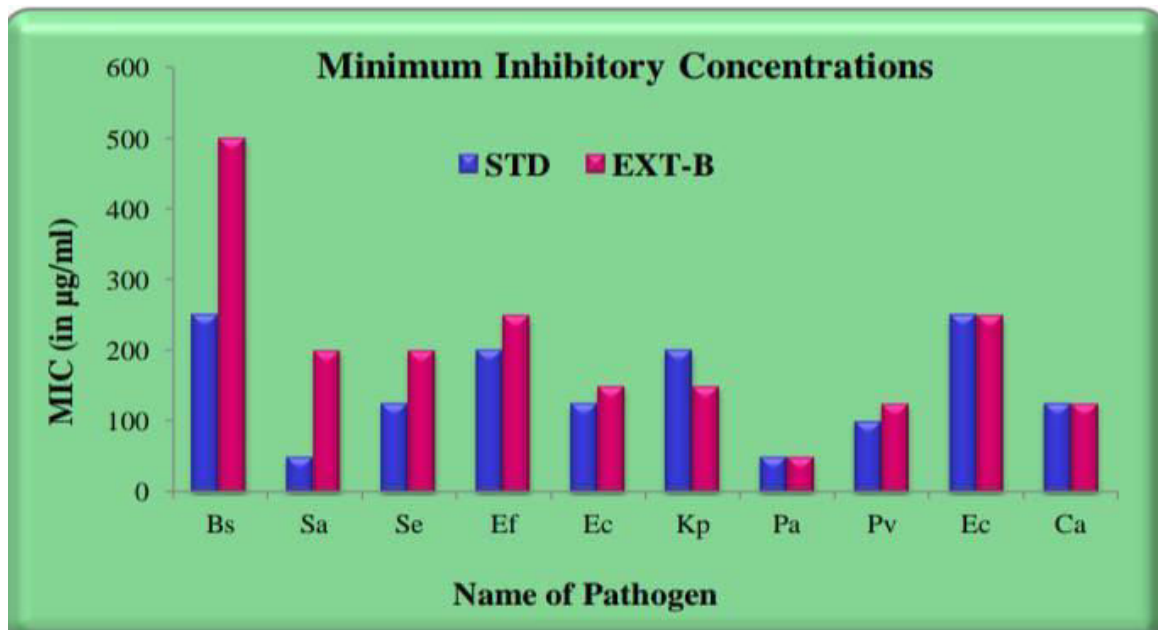
Determination of MIC

Only alcoholic extract (Extract-B) was used in the calculation of MIC values against all bacterial strains and only one fungal strain, C. albicans, based on the outcomes of the agar-well diffusion assay. The MIC values of typical antibiotics ranged from 250 to 50 ug/ml, whereas Extract-B's values were discovered to be between 500 and 50 ug/ml. In comparison to Ciprofloxacin (200 ug/ml), Extract-B's MIC value (150 ug/ml) against Klebsiella pneumonia indicates that the extract is more potent. Furthermore, it was discovered that the MIC values of Extract-B against Erwinia carotovora and pseudomonas aeruginosa were comparable to those of ciprofloxacin, and that against Candida albicans, they were comparable to those of griseofulvin, demonstrating the extract's comparable efficacy to that of standard drugs.

MIC values of Extract-B of *C. spinosa* stem for sensitive bacterial and fungal strains

Table: 8

Name of Pathogen	Minimum Inhibitory Concentration (in µg/ml)	
	Standard antibiotic (STD)	**Extract-B**
Bacillus subtilis	250	500
Staphylococcus aureus	50	200
Staphylococcus epidermidis	125	200
Enterococcus faecalis	200	250
Escherichia coli	125	150
Klebsiella pneumonia	200	150
Pseudomonas aeruginosa	50	50
Proteus vulgaris	100	125
Erwinia carotovora	250	250
Candida albicans	125	125



Graph 3

MIC values of Extract-B of *C. spinosa* stem for sensitive bacterial and fungal strains

© International Neurourology Journal
DOI: 10.5123/inj.2023.4.in115
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 27 Iss. 4 (2023)

STD - Standard antibiotic; Ext-B - Extract-B; Bs - *Bacillus subtilis*; Sa - *S. aureus*; Se - *S. epidermidis*; Ef - *E. faecalis*; Ec - *E. coli*; Kp - *K. pneumoniae*; Pa - *P. aeruginosa*; Pv - *P. vulgaris*; Ec - *E. carotovora*; Ca - *C. albicans*.

Based on the result, it is possible to conclude that the *C. spinosa* stem exhibits a broad range of antimicrobial activity to varying degrees. Particularly, alcoholic extract of stem (Extract-B) showed profound antibacterial and anti-candidal activities and could be used as anti- microbial agents in new drug therapy. The study also provides support to the traditional claims of the effectiveness of this plant in various types of infection. However, the antimicrobial study can be further extended to evaluate the potential of the plant on various other pathogenic organisms and to determine and isolate the bioactive compound responsible for the anti – microbial effect of *C. spinosa* plant.

Conclusion

Capparis spinosa Linn. (Family:Capparidaceae) is commonly known as kabar. The plant is often found in arid regions of tropical and subtropical climates. This plant has anti-inflammatory, anti-rheumatic, hepatoprotective, analgesic, diaphoretic, astringent, laxative, anthelmintic, and antibacterial qualities in Ayurvedic literature. Many ailments, including rheumatism, gout, boils and swellings, jaundice, biliousness, dropsy, diabetes, cough asthma, cardiac issues, urinary purulent discharges, piles, and ulcers are used to be treated with it in traditional medical systems. The objective of the current study was to create quality parameters for *C. spinosa* stem by methodically examining the plant material. Therefore, all pharmacognostic criteria, including physical, phytochemical, microscopy, and microscopy parameters, were assessed for the stem. The macroscopically study showed that *C. spinosa* is a small tree with practically leafless stems because its tiny, caduceus leaves are only found on immature branches. A young stem can be identified under a microscope by its layer of papillon clastic cuticle-covered epidermis embedded with an abroad cortex with lignified cortical fibers ,a narrow zone of phloem, and lignified pericyclic fibers. The lignified cork, the stone cell band beneath it, the well-developed steel area with secondary phloem, ceratenchyma, and secondary xylem are the distinguishing characteristics of the old stem.

Loss on drying of the stem was found to be 44.12% w/w, ash values, i.e. total ash, acid insoluble ash and water soluble ash were 8.13%; 0.5%; 6.03% w/w, respectively; extractive values, i.e. water soluble extractive and alcohol soluble extractive were 20% and 6.67% w/w, respectively. Heavy metals like lead, mercury and arsenic were found to be below detection limits in the stem. Study of microbiological parameters revealed total plate count, yeast and mold count within the acceptable limits, while E. coli was found to be absent.

Reference

1. Agyare C.; Aase A.; Lechtenberg M.; Niehues M.; Deters A.; Hensel A. An ethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwe-Atwima-Kwanwoma area, Ghana, J Ethnopharmacology, 2009; 125(3):393-403.
2. Crag G.; Newman D. J. Medicines for the millennia, Ann. NY Acad. Sci., 2001; 953:3-25.
3. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines –A review. Journal of Biodiversity and Conservation. 2012;4(3):101-112. DOI: 10.5897/IJBC11.163.
4. Barboza GE, Cantero JJ, Núñez C, Pacarana A, Espinar LA. Medicinal plants: A general review and a phytochemical and ethnopharmacological screening of the native Argentine Flora. Kurtz Iana. 2009; 34:7-365. ISSN:1852-5962.
5. Wachtel-Galor S, Benzie IFF. Herbal medicine. In: Benzie I, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed. Boca Raton: CRC Press/Taylor & Francis; 2011. ISBN: 13: 978-1-4398-0713-2.
6. Pal SK, Shukla Y. Herbal medicine: Current status and the future. Asian Pacific Journal of Cancer Prevention. 2003; 4:281-288.
7. Firenzuoli F, Gori L, Crupi A, Neri D. Flavonoids: risks or therapeutic opportunities? *Recent Prog Med.* 2004; 95:345–51.
8. Nutrition Business Journal (2001) US Nutrition Industry: Top 70 Supplements 1997–2000, San Diego, CA.9.
9. Agrawal OP, Raju PS. *In*: Abdin MZ, Abrion YP, editors. Global market of herbal products: Opportunities for India. Traditional system of medicine. Naroda Publishing House: New Delhi, India; 2006. p. 5-1.

10. Rama Krishnappa K. Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. New York, USA: 2003. Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Case studies from India. United Nations Publications: p. 210-5.
11. WHO Traditional Medicine Strategy 2002-2005, Document WHO/EDM/TRM/2002.1, WHO: Geneva; 2002. p.
12. Fugh-Berman A. Herb-drug interactions. Lancet 2000; 355:134-8.
13. Fugh-Berman A, Ernst E. Herb-drug interactions: Review and assessment of report reliability. Br J Clin Pharmocol2001; 52:587-95.
14. Kamboj VP (2000). Herbal Medicine. Current Science, 78, 35-9. Kew J, Morris C, Aichi A et al (1993). Arsenic and mercury intoxication due to Indian ethnic remedies. BMJ, 306, 506-7.
15. Winslow LC and Kroll DJ (1998). Herbs as medicine. Arch Interned, 158, 2192- 9.
16. Crellin, J.K., 2001. Social validation: an historian’s look at complementary/ alternative medicine. Pharm. Hist. (Lond). 31, 43-51.
17. Anisa, 2013. Consulta Publica No 34 de 6 de Agosto de 2013. Egencia Nacional de Vigilance Sanitaria. Daario Official da Unico, Brasília, me 7 de Agosto de 2013.
18. Appelhans, K., Najibullah, Ranvisa, 2013. Consulta Publica No 34 de 6 de Agosto de 2013. agencies Nacional de Vigilance Sanitaria. Daario Official da Unico, Brasília, me 7 de Agosto de 2013.
19. Appelhans, K., Najibullah, R., Frankos, V., 2013. Letter: retrospective reviews of liver-related case reports allegedly associated with Herbal life present insufficient and inaccurate data. Aliment. Pharmocol. Ther. 37, 753- 754.
20. Raynor D. K., Dickinson R., Knapp P., Long A. F., Nicolson D. J. (2011). Buyer beware? Does the information provided with herbal products available over the counter enable safe use? *BMC Med.* 9:94 10.1186/1741-7015-9-94.
21. Firenzuoli F, Gori L, Crupi A, Neri D. Flavonoids: risks or therapeutic opportunities? *Recent Prog Med.* 2004; 95:345–51.
22. Pandey MM, Rastogi S, Rawat AKS. Indian traditional Ayurvedic system of medicine and nutritional supplementation. *Evid Compl Altern Med*. 2013; 2013: 1-12.
23. Bhowmik D, Kumar KPS, Tripathi P, Chiranjib B. Traditional herbal medicines: an overview. *Arch Appl Sci Res*. 2009; 1(2): 165-77.
24. Chatterjee TK. Medicinal plants used as anti-diabetic agents. In: Herbal Options, M/s Eastern Traders, Calcutta. 1996: 1-8.
25. Shankar K1, Liao LP. Traditional systems of medicine. *Phys Med Rehabil Clin N Am*. 2004; 15(4): 725-47.
26. 26.Surh YJ. Reverse pharmacology applicable for botanical drug development - inspiration from the legacy of traditional wisdom. *J Tradit Compl Med*. 2011; 1(1): 5-7.
27. 27.Patwardhan B, Vaidya A. Natural products drug discovery: accelerating the clinical candidate development using reverse pharmacology approaches. *Indian J Exptl Biol*. 2010; 48: 220-27.
28. 28.Evans WC. Plants in medicine: the origins of Pharmacognosy. In: Trease and Evans Pharmacognosy, 15th ed., Saunders, an imprint of Elsevier. 2006: 4.
29. 29.Saumendu DR. Prospects, problems and approaches to alternative system of medicine: a review. *Internat J Ayur Herbl Med*. 2012; 2(1): 192-97.