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ANTIBACTERIAL ACTIVITY OF ROOTS OF CAPPARIS SPINOSA

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ABSTRACT

This present was an attempt to study the antibacterial activity of roots of the plant *Capparis spinosa* the extracts of the roots were obtained by successive extraction with solvents according to increasing polarity. These extracts were subjected to photochemical screening and tests for antibacterial activity. Disc diffusion method was chosen to elucidate the antibacterial activity of the extracts, using penicillin as a reference standard. The activity was tested **on *Bacillus cereus, Staphylococcus aureus, E. coli and Pseudomonas aeruginosa* variety of bacteria.** All the extracts except the aqueous extract were found to have antibacterial activity against ***Staphylococcus aureus* and *Pseudomonas aeruginosa*** cultures, among which the **petroleum ether** extract was seen to have maximum activity.

KEYWORDS: Capparis spinose, Bacillus cereus, Staphylococcus aureus, E. coli and Pseudomonas aeruginosa.

INTRODUCTION

Bacteria are microorganisms that have circular double-stranded DNA and (except for mycoplasmas) cell walls. Most bacteria live extracellularly. Some bacteria (eg, Salmonella typhi; Neisseria gonorrhoeae; Legionella, Mycobacterium, Chlamydia, and Chlamydophila spp) preferentially reside and replicate intracellularly. Some bacteria such as chlamydiae and rickettsiae are obligate intracellular pathogens (ie, able to grow, reproduce, and cause disease only within the cells of the host); others (eg, Salmonella typhi, Brucella sp, Francisella tularensis, N. gonorrhoeae, N. meningitidis, Legionella and Listeria spp, Mycobacterium tuberculosis) are facultative intracellular pathogens.

Many bacteria are present in humans as normal flora, often in large numbers and in many areas (eg, in the GI tract). Only a few bacterial species are human pathogens.

Bacteria are classified by the following criteria.

a) Morphology: Bacteria may be cylindric (bacilli), spherical (cocci), or spiral (spirochetes). A few coccal, many bacillary and most spirochetal species are motile.

b) Staining: The most common stain for general bacterial identification is Gram stain. Gram-positive bacteria retain crystal violet dye (appearing dark blue) after iodine fixation and alcohol decolorization; gram-negative bacteria do not. Gram-negative bacteria have an additional outer membrane containing lipopolysaccharide (endotoxin), increasing the virulence of these bacteria. (For other factors that enhance bacterial pathogenicity. Ziehl-Neelsen stain (acid-fast stain) is used to identify mainly mycobacteria, particularly M. tuberculosis. It also can identify Nocardia sp. Carbolfuchsin is applied with heat, followed by decolorization with hydrochloric acid and ethanol and counterstaining with methylene blue.

c) Encapsulation: Some bacteria are enclosed in capsules; for some encapsulated bacteria (eg, Streptococcus pneumoniae, Haemophilus influenzae), the capsule helps protect them from ingestion by phagocytes. Encapsulation increases bacterial virulence.

d) Oxygen requirements: Aerobic bacteria (obligate aerobes) require O_2 to produce energy and to grow in culture. They produce energy using aerobic cellular respiration.

Anaerobic bacteria (obligate anaerobes) do not require O_2 and do not grow in culture if air is present. They produce energy using fermentation or anaerobic respiration. Anaerobic bacteria are common in the GI tract, vagina, dental crevices, and wounds when blood supply is impaired.

Facultative bacteria can grow with or without O_2. They produce energy by fermentation or anaerobic respiration when O_2 is absent and by aerobic cellular respiration when O_2 is present. Microaerophilic bacteria prefer a reduced O_2 tension (eg, 2 to 10%). Chlamydiae are obligate intracellular parasites that acquire energy from the host cell and do not produce it themselves.

Table-1.

Classification of Common Pathogenic Bacteria	
Type	**Bacteria**
Obligate aerobic	
Gram-negative cocci	Moraxella catarrhalis, Neisseria gonorrhoeae, N. meningitidis
Gram-positive bacilli	C. jeikeium
Acid-fast bacilli	Mycobacterium avium complex, M. kansasii, M. leprae, M. tuberculosis, Nocardia sp
Nonfermentative, non-Enterobacteriaceae	Acinetobacter calcoaceticus, Flavobacterium meningosepticum, Pseudomonas aeruginosa, P. alcaligenes, other Pseudomonas sp, Stenotrophomonas maltophilia
Fastidious gram-negative coccobacilli and bacilli	Brucella, Bordetella, Francisella, and Legionella spp
Treponemataceae (spiral bacteria)	Leptospira sp
Obligate anaerobic	
Gram-negative bacilli	Bacteroides fragilis, other Bacteroides sp, Fusobacterium sp, Prevotella sp
Gram-negative cocci	Veillonella sp
Gram-positive cocci	Peptococcus niger, Peptostreptococcus sp
Non–spore-forming gram-positive bacilli	Actinomyces, Bifidobacterium, Eubacterium, and Propionibacterium spp
Endospore-forming gram-positive bacilli	Clostridium botulinum, C. perfringens, C. tetani, other Clostridium sp
Facultative anaerobic	
Gram-positive cocci, catalase-positive	Staphylococcus aureus (coagulase-positive), S. epidermidis (coagulase-negative), other coagulase-negative staphylococci
Gram-positive cocci, catalase-negative	Enterococcus faecalis, E. faecium, Streptococcus agalactiae (group B streptococcus), S. bovis, S. pneumoniae, S. pyogenes (group A streptococcus), viridans group streptococci (S. mutans, S. mitis, S. salivarius, S. sanguis), S. anginosus group (S. anginosus, S. milleri, S. constellatus), Gemella morbillorum
Gram-positive bacilli	Bacillus anthracis, Erysipelothrix rhusiopathiae, Gardnerella vaginalis (gram-variable)
Gram-negative bacilli	Enterobacteriaceae (*Citrobacter* sp, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella* sp, *Morganella morganii*, *Proteus* sp, *Providencia rettgeri*, *Salmonella typhi*, other *Salmonella* sp, *Serratia marcescens*, *Shigella* sp, *Yersinia enterocolitica*, *Y. pestis*)
Fermentative, non-Enterobacteriaceae	Aeromonas hydrophila, Chromobacterium violaceum, Pasturella multocida, Plesiomonas shigelloides
Fastidious gram-negative coccobacilli and bacilli	Actinobacillus actinomycetemcomitans, Bartonella bacilliformis, B. henselae, B. quintana, Eikenella corrodens, Haemophilus influenzae, other Haemophilus sp
Mycoplasma	Mycoplasma pneumoniae
Treponemataceae (spiral bacteria)	Borrelia burgdorferi, Treponema pallidum
Microaerophilic	
Curved bacilli	Campylobacter jejuni, Helicobacter pylori, Vibrio cholerae, V. vulnificus

Obligate intracellular parasitic	
Chlamydiaceae	Chlamydia trachomatis, Chlamydophila pneumoniae, C. psittaci
Coxiellaceae	Coxiella burnetii
Rickettsiales	Rickettsia prowazekii, R. rickettsii, R. typhi, R. tsutsugamushi, Ehrlichia chaffeensis, Anaplasma phagocytophilum

The present study is aimed at Phytochemical screening and Anti-bacterial activity on roots of Capparis spinosa belonging to the family capparidaceae.

METHODOLOGY

Roots of *Capparis spinosa* were collected. They were dried, powdered and stored in an air tight container for further use.

EXTRACTION OF PLANTS

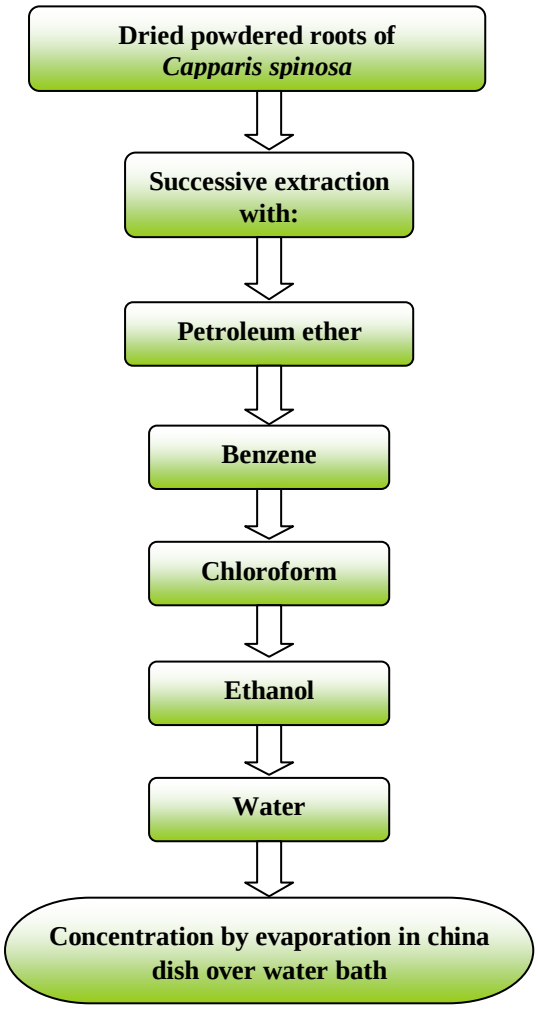
Successive solvent extraction

The method is based on the extraction of active constituents present in the crude drug using solvents ranging from non-polar to polar. The solvents used were Petroleum ether, benzene, chloroform, ethanol and water. The extraction was carried out in a soxhlet apparatus. The apparatus included a soxhlet tube in which the drug was placed, a round bottomed flask for collection of extract, a condenser for reflux and a heating mantle to supply heat. The extract collected in the round bottomed flask was evaporated in china dish.

PROCEDURE

Plant extracts were prepared by successive Soxhlet extraction method. The plant materials (leaves) were powdered and extracted successively with different solvents (non-polar to polar). The solvents used were Petroleum ether, Benzene, Chloroform, Ethanol and Water. The plant extracts were filtered through Whatman No. 1 filter paper into beaker. The filtrates were dried until a constant dry weight of each extracts was obtained. The residues were stored at 4°C for further use.

Flow chat of extraction procedure



PHYTOCHEMICAL SCREENING

Detection of Tannins

The extracts are dissolved in the respective solvents and subjected to the following tests.

a) **Test with 5% $FeCl_3$ solution:** formation of a blue-black or greenish yellow colour indicates the presence of tannins.
b) **With Lead Acetate:** formation of a white precipitate indicates the presence of tannins.
c) **With Acetic Acid:** formation of red colour indicates the presence of tannins.
d) **With dilute Iodine:** formation of a transient red colour indicates the presence if tannins.

Detection of Alkaloids

Small portions of extracts were dissolved in the respective solvents and subjected to the following tests:

a) **Dragendorff's test** (Potassium bismuth iodide solution)**:** Extracts were treated with dragendorff's reagent. Formation of reddish-brown precipitate indicates the presence of alkaloids.
b) **Mayer's test (**Potassium mercuric iodide solution)**:** Extracts were treated with Mayer's reagent. Formation of cream coloured precipitate indicates presence of alkaloids.
c) **Hager's test** (saturated solution of picric acid)**:** Extracts were treated with Hager's reagent. Formation of yellow precipitate indicates presence of alkaloids.
d) **Wagner's test (**Iodine and Potassium iodide)**:** Extracts were treated with Wagner's reagent. Formation of a reddish brown precipitate indicates the presence of alkaloids.

Detection of Glycosides

Extracts were dissolved in the respective solvents and subjected to the following tests.

Cardiac glycosides

a) **Baljet's test:** The extract is treated with sodium picrate solution. Formation of a yellow colour indicates the presence of cardiac glycosides.
b) **Legal's test** (for cardinolides)**:** The extracts were treated with pyridine and 1ml of sodium nitroprusside. A pink to red colour indicates the presence of cardinolides.
c) **Kellar-killiani test:** The extract is treated with glacial acetic acid and 1drop of FeCl3 and concentrated sulfuric acid. Formation of a reddish brown precipitate indicates the presence of cardiac glycosides.
d) **Liebermann's test (**for bufadienolides): Extracts were treated with 3ml acetic anhydride and heated. To it a few drops of concentrated sulfuric acid was added. Formation of a blue colour indicates the presence of bufadienolides.

Detection of Steriods

a) **Salkwoski test:** Extract is treated 2ml chloroform and 2ml concentrated sulfuric acid. If the chloroform layer appears red and acid layer shows greenish yellow fluorescence, it indicates the presence of steroids.
b) **Liebermann-Burchard test:** The extract is treated with chloroform and 1-2ml acetic anhydride followed by 2 drops of concentrated sulfuric acid. The appearance of an initial red colour which changes to blue then green indicates the presence of steroidal glycosides.
c) **Liebermann test:** the extract is treated with 3ml acetic anhydride and heated. After cooling, a few drops of concentrated sulfuric acid are added. A blue colour indicates the presence of steroids.

Detection of Saponin glycosides

a) **Foam test:** water is added to the extract and shaken vigorously. Formation of foam indicates the presence of saponin glycosides.

Detection of Flavonoid glycosides

a) **Shinoda test:** The extract is treated with 5ml of 95%ethanol and a few drops of concentrated hydrochloric acid and 0.5gms of magnesium turnings. A pink colour indicates the presence of flavonoids.
b) **Lead acetate test:** The extract is treated with lead acetate. Formation of a yellow colour precipitate indicates the presence of flavonoids.
c) **Sodium hydroxide test:** the extract is treated with sodium hydroxide. A yellow colour which is decolourized by acids indicates the presence of flavonoids.

Anthraquinone glycosides

a) **Borntrager's test:** The extract is heated with dilute sulfuric acid and filtered. To the filtrate an equal volume of chloroform is added and shaken well. The organic layer is separated and to it ammonia is added. A pink to red colour in the ammonical layer indicates the presence of anthraquinone glycoside.
b) **Modified Borntrager's test:** Dilute $FeCl_3$ is added and heated and then the extract is heated with dilute sulfuric acid and filtered. To the filtrate an equal volume of chloroform is added and shaken well. The organic layer is separated and to it ammonia is added. A pink to red colour in the ammonical layer indicates the presence of anthraquinone glycoside.

Detection of Carbohydrates

Extracts were dissolved individually and filtered. The filtrates were used to test for the presence of carbohydrates.

a) **Molisch test:** Filtrate is treated with 2drops of molisch reagent (alcoholic α- naphthol solution) in a test tube and 2ml of concentrated sulfuric acid was carefully added along the sides of the test tube. Formation of a violet ring at the junction of two layers indicates the presence of carbohydrates.

b) **Benedict's test:** Filtrate was treated with Benedict's reagent and heated on a water bath. Formation of red precipitate indicates the presence of reducing sugars.
c) **Fehling's test:** Filtrates were heated with equal quantities of Fehling's A and B solutions. Formation of a brick red precipitate indicates the presence of reducing sugars.

Detection of Proteins
The extracts were dissolved in the respective solvents and subjected to the following tests.

a) **Biuret's test:** The extract is treated with 4% sodium hydroxide and a few drops of 1% Copper sulfate solution. Formation of a violet to pink colour indicates the presence of proteins.
b) **Million's test:** The extract is treated with 5ml million's reagent. Formation of a white precipitate which turns red on warming indicates the presence of proteins.

ANTIBACTERIAL ACTIVITY OF EXTRACTS
The extracts obtained by successive extraction were subjected to tests for antibacterial activity.

Nutrient Media
Beef extract : 10.0gms
Peptone : 10.0gms
Sodium chloride : 5.0gms
Agar : 12.0gms
Distilled water : 1000ml

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria. The inhibition can be measured by either dilution method or by diffusion method. The serial dilution method is more useful in determining the antibacterial activity quantitatively. This method is not of much use in detection tests and also for evaluation of a large number of samples.

The method adopted for this investigation was the disc-diffusion method as it has the advantage of showing both inhibition and control growth (outside the zone of inhibition) of fastidious organisms on the same plate.

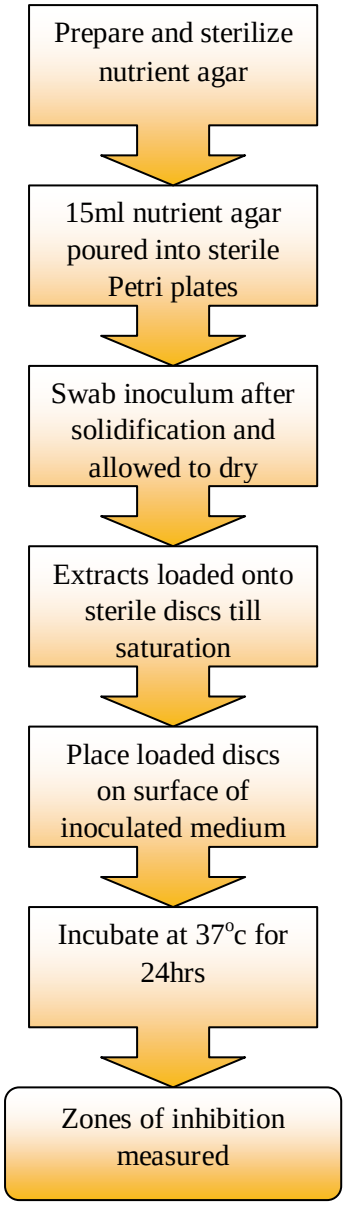
Preliminary Antibacterial Screening
The activity of all five extracts were evaluated by disc diffusion method. This method is based on diffusion of antibacterial component from disc to the surrounding inoculated nutrient agar medium, so that the growth of microorganisms is inhibited as circular zone around the disc. Three gram negative organisms and one gram positive organisms were Selected viz. *viz* **Pseudomonas aeruginosa, Escherichia coli, klebsiella pneumonia, Bacillus subtilis** respectively for the present study**.**

Organism used
Standard cultures viz **Pseudomonas aeruginosa, Escherichia coli, klebsiella pneumonia, Bacillus subtilis** were obtained from Microbiology department of Deccan school of pharmacy, Hyderabad**.** Staining techniques and biochemical reactions identified the microorganisms. The organisms were maintained by sub-culturing at regular intervals in nutrient agar medium.

Preparation of inoculum
Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Nutrient agar broth for bacteria and incubated without agitation for 24 hrs at 37°C. The cultures were diluted with fresh Nutrient agar.

Antibacterial susceptibility test
The disc diffusion method was used to screen the antibacterial activity. In vitro antibacterial activity was screened by using nutrient agar media obtained from Himedia (Mumbai). The nutrient agar plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 5 minutes and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The different extracts and isolated compounds were loaded on 3mm sterile disc till saturation. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones are formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate by using standard drugs (10 units/disc Penicillin; for bacteria).

Flow chart for antibacterial susceptibility test



Anti-bacterial

Anything that destroys bacteria or suppresses their growth or their ability to reproduce. Heat, chemicals such as chlorine, and antibiotic drugs all have antibacterial properties. Many antibacterial products for cleaning and handwashing are sold today. Such products do not reduce the risk for symptoms of viral infectious diseases in otherwise healthy persons. This does not preclude the potential contribution of antibacterial products to reducing symptoms of bacterial diseases in the home.

Antibacterial Drugs

Antibacterial drugs are derived from bacteria or molds or are synthesized de novo. Technically, "antibiotic" refers only to antimicrobials derived from bacteria or molds but is often (including in The Manual) used synonymously with "antibacterial drug."

Antibiotics have many mechanisms of action, including inhibiting cell wall synthesis, activating enzymes that destroy the cell wall, increasing cell membrane permeability, and interfering with protein synthesis and nucleic acid metabolism.

Antibiotics sometimes interact with other drugs, raising or lowering serum levels of other drugs by increasing or decreasing their metabolism or by various other mechanisms. The most clinically important interactions involve drugs with a low therapeutic ratio (ie, toxic levels are close to therapeutic levels). Also, other drugs can increase or decrease levels of antibiotics.

Many antibiotics are chemically related and are thus grouped into classes. Although drugs within each class share structural and functional similarities, they often have different pharmacology and spectra of activity.

RESULTS

COLLECTION OF PLANT MATERIAL

Fresh roots of the plant *Capparis spinosa* were collected and dried.

PERCENTAGE YIELD OF EXTRACTS

EXTRACT	COLOUR OF EXTRACT	% YIELD (W/W)
Petroleum ether	Bottle green	5.3%
Benzene	Brown	5.2%
Chloroform	Dark Green	4.9%
Ethanol	Green	4.6%
Aqueous	Brown	6.6%

PHYTOCHEMICAL SCREENING

Chemical constituent	Test	P. E. Ext.	Ben.Ext	Chl. Ext	EtOH.Ext.	Aqu. Ext
Tannins	Ferric chloride test	+	+	-	+	-
	Lead acetate test	+	+	-	+	-
	Acetic acid sol.	-	-	-	+	-
	Dil. Iodine sol.	+	-	-	+	-
Alkaloids	Mayer's test	+	-	+	-	-
	Dragendroff's test	+	-	+	-	-
	Hager's test	+	-	+	-	-
	Wagner's test	+	-	+	-	-
Glycoside						
A. Cardiac glycosides	Baljet's test	+	-	-	-	-
	Legal's test	+	-	+	-	-
	Keller-killiani test	+	-	-	-	-
	Liebermann's test	+	-	+	-	-
B. Steroids	Salkowski test	+	-	+	-	-
	Liebermann-burchard test	+	-	+	-	-
	Liebermann's test	+	-	+	-	-
C.Saponins	Foam test	+	+	+	+	+
D. Flavonoids	Schinoda test	-	-	-	-	+
	Lead acetate test	-	-	-	-	+
	NaOH test	-	-	-	-	+
E. Anthraquinones	Borntrager's test	-	-	-	-	-
	Modified-borntrager's test	+	-	-	-	-
Carbohydrates	Molisch test	+	+	+	+	+
	Fehling's test	-	-	+	+	+
	Benedict's test	-	-	+	+	+
Proteins	Biuret's test	-	-	-	-	-
	Millon's test	-	-	-	-	-

ANTI BACTERIAL ACTIVITY

The Extracts were subjected to antibacterial activity using different micro organisms.

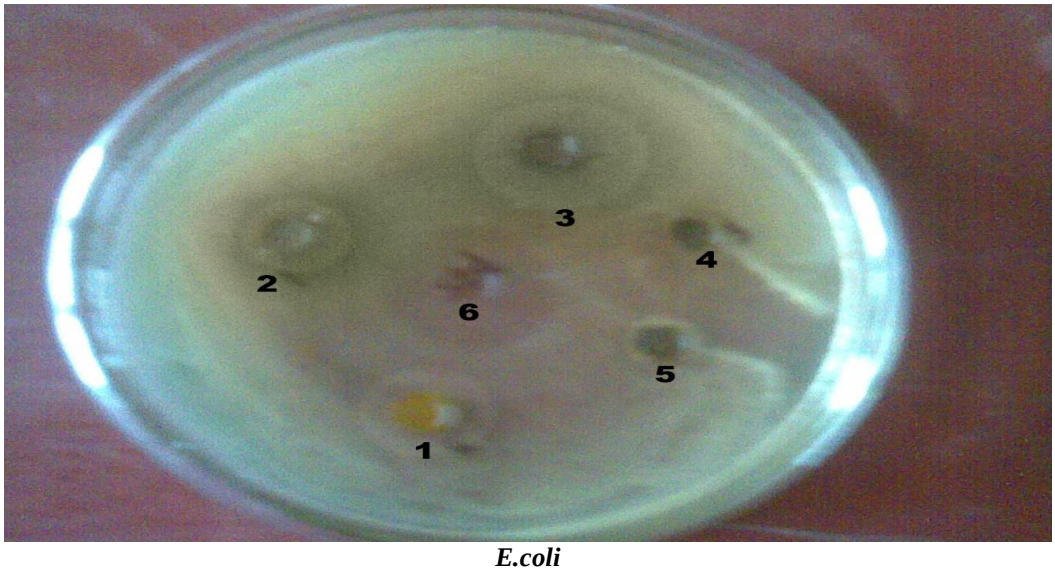
Zone of inhibition of different extracts

Organisms	Zone of inhibition of extracts					STD
	P.E	B.E	C.E	EtOH.E	Aq.E	
1. *P.aeruginosa*	NI	NI	NI	6mm	5mm	20 mm
2. *E.coli*	4mm	3.8mm	4.3mm	3mm	2.8mm	20 mm
3. *B.subtilis*	6mm	6.2mm	1mm	4mm	3mm	20 mm
4. *K.pneumoniae*	1.5mm	1.7mm	1.7mm	4mm	2mm	20 mm

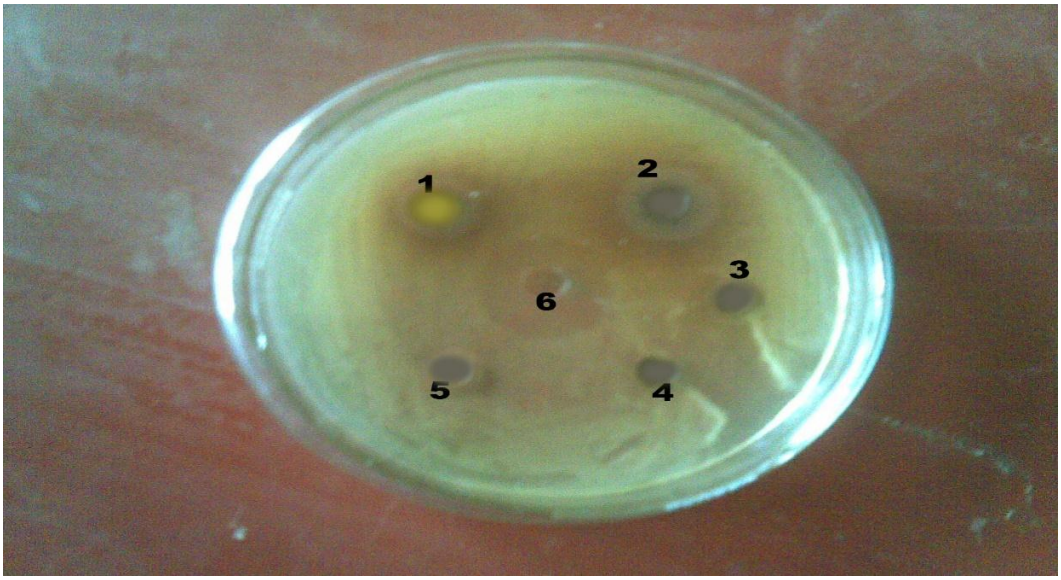
NI = No Inhibition
P.E = Petroleum ether extract
B.E = Benzene extract
C.E = Chloroform extract
EtOH. E = Ethanolic extract
Aq.E = Aqueous extract
STD =Standard (Penicillin 10 units/disc)

Antibacterial activity of different extracts

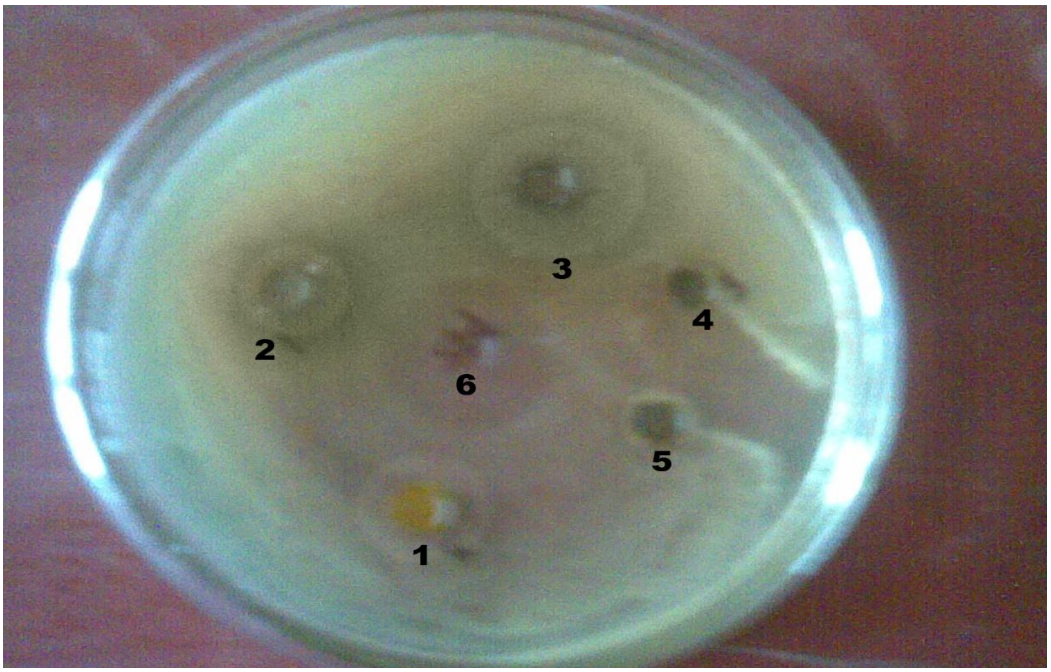
P.aeruginosa



E.coli



B.subtilis



K.pneumoniae

P.E – Petroleum ether extract **B.E**-Benzene extract
C.E-Chloroform extract **EtOH**-Ethanolic extract
Aq.E – Aqueous extract **Std**-Standard Pencillin.

CONCLUSION

The present project is an attempt to study the extraction, phytochemical screening and antibacterial properties of roots of the plant *Capparis spinosa.*

The dried roots were powdered and extracted successively with different solvents based on polarity (petroleum ether, Benzene, chloroform, ethanol and water).

The concentrated extracts were subjected to phytochemical screening. All the extracts were tested for presence of antibacterial activity.

The petroleum ether, benzene extact chloroform, and ethanolic extracts exhibited antibacterial activity **against *Staphylococcus aureus* and *Pseudomonas aeruginosa* cultures.**

The maximum activity was shown by **petroleum ether extract.**

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