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Phytochemical Screening Of Different Fractions And Anti-Bacterial Activity Of Caralluma Tuberculata

Sayyida Aiza Bukhari[1], Behramand[2], Ashfaq Ahmad Shah Bukhari[3], Syed Sikandar Shah[4], Umm-e-Aiman[5], Muhammad Haidar Zaman[6*]

[1](a) Department of Agricultural Science and Engineering, Hohai University, Nanjing, China. (B) Department of Chemistry, Islamia College Peshawar, Pakistan
[2]Associate Professor, Department of Chemistry, Islamia College Peshawar, Pakistan
[3]Assistant Professor, Department of Physiology, RAK College of Medical Science, Ras Al Khaimah Medical and Health Sciences University, UAE
[4]Assistant Professor, Department of Clinical Pharmacy and Pharmacology, RAK College of Pharmacy, Ras Al Khaimah Medical and Health Sciences University, UAE
[5]Department of Chemistry, Islamia College Peshawar, Pakistan
[6](a) Assistant Professor, Department of Health and Biological Sciences, Abasyn University, Peshawar, Pakistan (b) IBD, Nan Shi Fu Zhang, Nanjing Normal University, Nanjing, China*

***Corresponding author:** Dr. Muhammad Haidar Zaman*
**(a) Assistant Professor, Department of Health and Biological Sciences, Abasyn University, Peshawar, Pakistan. (b) IBD, Nan Shi Fu Zhang, Nanjing Normal University, Nanjing, China.*
Email: dr.mhaidarzaman@gmail.com

Abstract

Caralluma Tuberculata is an important medicinal plant and belongs to *Apocynaceae* family. The succulent stem of the plant is widely used to treat several diseases including diabetes, rheumatism, leprosy, peptic ulcer, inflammation, jaundice, dysentery, constipation, stomach pain, hepatitis B and C. This dissertation reports fractional extraction, phytochemical screening and their anti-bacterial activities. Fractions of different polarities were obtained through fractional extraction with n-hexane, dichloromethane, ethyl acetate, methanol and distilled water respectively. The phytochemical analysis revealed the presence of phytosterols, carbohydrates, terpenoids, and steroids in all four fractions. Alkaloids were present in all fractions except the methanol fraction. Flavonoids were present in all except n-hexane fraction. Tannins were only present in dichloromethane fraction. Saponins were present in all fractions except dichloromethane fraction. Amino acids were only present in methanol fraction. The antibacterial activities of all the fractions were evaluated against two bacterial strains *Escherichia coli* and *Citrobacter freundii*. Streptomycin was used as standard. Plant extracts of methanol fraction showed a maximum zone of inhibition against *Escherichia coli* and *Citrobacter freundii*. The highest % inhibition (96%) at 100µg/ml concentration relative to standard (100%) was showed by methanol fraction against *Escherichia coli*. In case of *Citrobacter freundii* methanol fraction showed 92% inhibition at 100µg/ml relative to standard (100%). While comparing different fractions of *Caralluma tuberculata*, methanol and

CC License CC-BY-NC-SA 4.0	ethyl acetate fractions appear to have highest zone of inhibition as compared to the dichloromethane and n-hexane fractions against both *Escherichia coli* and *Citrobacter freundii.*

INTRODUCTION

Natural Products

Natural products are chemical substances or compounds made by living things that can be found in nature. Any substance created by life is considered a natural product in the broadest sense [1]. The use of natural products as medicines has been documented throughout history in the form of customary drugs, cures, potions, and oils, many of which are bioactive natural substances that are yet unknown today [3]. Since the 1850s, organic chemists have been deeply interested in these novel phytochemicals and have intensively studied their chemical characteristics [2]. The natural products that plants make are enormously diverse in structure [4]. Natural products typically have more complicated molecular structures, making it more challenging to extract, purify, or synthesize enough of an NCE of interest for discovery and development operations [1].

These natural products play crucial roles in how plants interact with their biotic and abiotic environments. For instance, they can behave as substances that protect plants from herbivores and pathogens, as floral pigments that draw pollinators, or as hormones or signal molecules [4]. They can be formed via the primary or secondary metabolic pathways [14]. Natural products are often divided into two major classes:

- Primary Metabolites
- Secondary Metabolites

1.1.1 Primary Metabolite

A primary metabolite directly affects healthy growth, development, and reproduction [14]. Primary metabolites are essential to the survival of the species because they are actively involved in photosynthesis and respiration [5].

Examples of primary metabolites are carbohydrates, proteins, fats and oils etc [14].

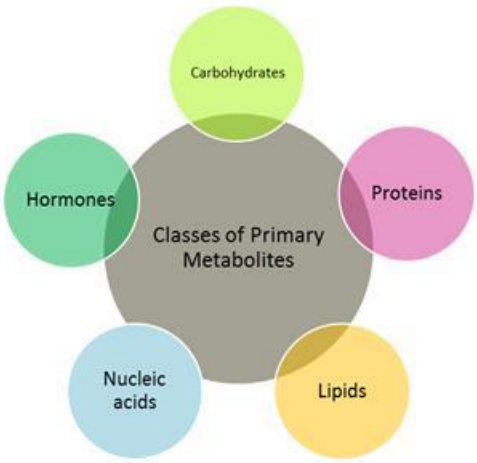

Figure 1. Classes of Primary metabolites

Secondary Metabolites

Secondary metabolites are organic compounds that are not necessary for an organism's regular growth and development [5]. Although secondary metabolites do not directly contribute to an organism's growth, development, or reproduction, they do have an ecological purpose [14]. Absence of secondary metabolites does not result in instant death, but rather a long-term reduction in the organism's ability to survive. These compounds are a remarkably diversified collection of natural products produced by a wide range of organisms, including plants, fungus, bacteria, algae, and mammals [5].

Depending on the kind of secondary metabolite generated, a plant's secondary metabolite can be found in the plant's leaves, stem, root, or bark. Example of the most bioactive secondary metabolites are the Alkaloids, Tannins, Flavonoids and Phenolic compounds.

Figure 2. Some major classes of secondary metabolites

Medicinal Plants

Since ancient times, numerous diseases have been treated using plants, such as fruits, vegetables, spices, medicinal herbs etc. [6]. The plant kingdom is a treasure trove of potential medications, and in recent years, there has been an increased understanding of the significance of medicinal plants [15]. The development of natural, home-made remedies for numerous diseases has been significantly aided by the high biodiversity of medicinal plants. Plants are a rich source of secondary metabolites with a wide range of structural characteristics that can be used to create novel, therapeutically significant compounds [6]. Drugs made from plants are widely available, inexpensive, safe, and effective, and they seldom ever cause side effects [15]. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological actions on the human body [16]. In medicinal plants, leaves, vegetables, and roots, phytochemicals are naturally occurring compounds with defense mechanisms that help them fend off a variety of diseases [17]. Medicinal plants have a wide range of therapeutic effects, including analgesic, antiviral, anticancer, and antimalarial properties [18]. The World Health Organization (WHO) believes that medicinal plants are the finest source for a wide range of medications. Approximately 80% of people in developed nations take traditional medicines, which contain ingredients derived from medicinal plants [15]. Herbal medicine is the main source of primary healthcare for people in underdeveloped nations, according to the World Health Organization [19].

Importance of Medicinal Plants

Without plants, there would be no life. Plants are the fundamental building blocks of medicine. The majority of medicines come from medicinal plants [8]. The health of individuals and societies depends greatly on medicinal plants [22]. The medicinal plants are crucial sources of materials that help maintain the body's good health and conditions and are used to cure both human and plant ailments. About two-thirds of the world's population relies on medicinal plants for primary healthcare. In the medical field, medicinal plants are highly valued due to their improved adaptability and compatibility with the human body, which results in fewer side effects and higher cultural acceptance [9]. The medical efficacy of plants and herbs should be evaluated in both current and future studies because it is projected that they will play a crucial role in the medical profession, particularly in the treatment of serious diseases like cancer [10].

Medicinal plants are thought to be a reservoir of a wide variety of bioactive substances with a range of medicinal qualities. Medicinal plants have a wide range of therapeutic effects, including analgesic, antiviral, anticancer, and antimalarial properties. One of the main challenges to human health in the world is cancer. Recently, there has been a rise in interest among scientists in the therapeutic potential of plants used as medicine as a source of possible anticancer drugs. Vinca alkaloids, vinblastine, vincristine, and cytotoxic podophyllotoxins are among of the very first plant-based anticancer medications [18]. Around the world, between 35,000 and 70,000 plant species are reportedly employed in folk medicine [13].

Phytochemistry and Phytochemicals

The study of plant compounds, particularly secondary metabolites produced as a kind of defense against infections, herbivores, ultraviolet radiation, and environmental dangers, is known as Phytochemistry. The structural makeup of these metabolites, the biosynthetic pathways, the roles, the mechanisms of action in biological systems, as well as their uses in industry, commerce, and medicine are all taken into account by

Phytochemistry [30]. Phytochemicals, which are naturally occurring chemical substances found in plants and derived from the Greek term phyto, which means "plant,"[31]. Primary and secondary constituents are the two categories under which phytochemicals fall.
Primary ingredients include proteins, common carbohydrates, and chlorophyll, whereas secondary chemicals contain Terpenoids, Inorganic phenols and alkaloids [17]. The phytoconstituents in the plant extract had an impact on cell growth and proliferation [33]. The human body does not depend on phytochemicals as a necessary food or for life support, but they do have substantial health benefits, including the ability to prevent or treat some diseases [31].

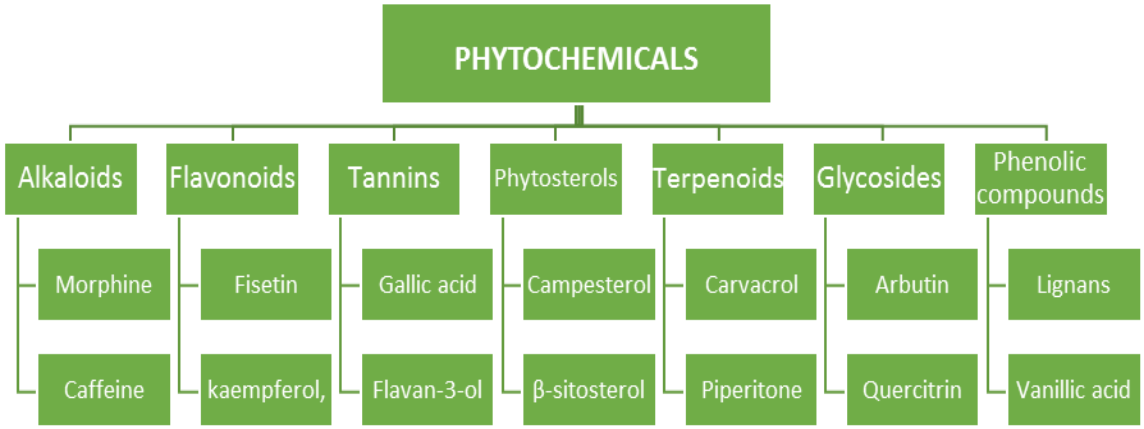

Figure 3. Classification of phytochemicals

Caralluma Tuberulata
Caralluma tuberculata belongs to family *Asclepiadaceae* and the genus *Caralluma. Caralluma tuberculata* is a perennial herb, succulent found mostly in mountainous regions of Pakistan, India, the United Arab Emirates, Saudi Arabia, and the regions to the southeast of Egypt, Iran, and Nigeria. The common names of *Caralluma tuberculata* are Pamanke or Pamaney, Chunga and Bitter cress in Pushto, Urdu, and English respectively. *Caralluma* is a member of the *Asclepiadaceae* family, it is medicinally an important genus. *Caralluma* is classified into 200 genera and 2500 species. About 200 species of the *Caralluma* genus can be found throughout Africa and Asia. *Caralluma* species are xerophytes and can endure dry circumstances for a considerable amount of time. The majority of these species are indigenous to the Arabian Peninsula and the Indian subcontinent. *Caralluma tuberculata* is erect fleshy, leafless, succulent herb. Stem is angular, succulent and 15cm in height. Angular stem is devoid of leaves and has small flowers. Branches are tetragonal in shape and 8 to 13 mm broad. The margins of branches are dentate which contain delicate spines. Flowers are in terminal cymes and pedicellate. The plant can be utilized as a cooked vegetable, added to tea, used as a dry powder, or chewed raw. It has significant therapeutic values [24-26]

Kingdom	***Plantae***
Clade	*Angiosperm*
Family	*Apocynaceae*
Sub Family	*Asclepiadaceae*
Genus	*Caralluma*
Specie	*C. tuberculata*
Botanical name	*Caralluma tuberculata*
Common name	*Pamanke*

Table 1. Botanical description of *Caralluma tuberculata*

Figure 4. photographic view of *caralluma tuberculata*

Medicinal Uses of *Caralluma Tuberculata*

For hundreds of years, numerous diseases have been treated with *Caralluma* species as part of traditional medicine**.** The plant's succulent stem is frequently used to cure a variety of conditions, including hepatitis B and C, diabetes, rheumatism, leprosy, peptic ulcer, inflammation, jaundice, and dysentery. According to scientific evidence, the plant has positive benefits that include antihyperglycemic, antibacterial, antifungal, and antinociceptive [25]. Additionally, it has been used to treat constipation, diarrhea, and stomach pain. Dried form is consumed with water to alleviate jaundice. Similarly, its paste in fresh form is applied to skin blemishes and acne. In many countries *Caralluma tuberculata* is used in fresh form, chewed as it acts as a blood purifier. The movement of joints can also be improved by *Caralluma* extract. It improves the amount of synovial fluids produced, which is what gives joints their flexibility and effectiveness. It is used as a tonic to strengthen joints so they can support large loads. *Caralluma tuberculata* is an effective antipyretic agent [24].

Phytochemistry of *Caralluma Tuberculata*

Various phytochemicals are isolated from *Caralluma tuberculata* including terpenes, glycosides, sterols, pregnane etc. According to a chemical investigation of its extract, it contains terpenes such as amyrin, α amyrin cinnamate, α amyrin acetate, lupeol (α and β).It also contains pregnanes, which include various type of caratubersides. The extract also contains a variety of sterols, primarily taraxasterol, β-sitosterol, and its various glucosides [25]. H.C. Dutt et al., 2012 carried out a study which demonstrated the presence of Flavone glycosides, bourcerin, dihydrobourcerin, caratubersides and pregnene glycosides in *Caralluma tuberculata*.These compounds show antimalarial, antitrypanosomal, antiplasmodial, hypoglycemic, and protection of gastric mucosa against the injuries caused by 80% ethanol, hypertonic saline and indomethacin. Russelioside B is a pregnane glycoside isolated from *Caralluma tuberculata* by Abdel-Sattar et al. 2017. Russelioside B has anti-inflammatory, anti-obesity, antidiabetic, antibacterial, anti-gastric ulcer, and antibiofilm effects. It may also be effective in the treatment of rheumatoid arthritis . Several pregnane glycosides isolated from organic extracts of *Caralluma tuberculata* showed cytotoxicity against the MRC5 human diploid. A. waheed et al., 2011carried a study and isolated pregnane and andostrane gylcosides from *Caralluma tuberculata*. These compounds show inhibition on three cancer line cells: MCF-7 estrogen-dependent and MDA-MB-468 estrogen-independent breast cancer cells, Caco-2 human colonic cells. S. N. Alqahtani et al., 2014 isolated three flavonoid compounds. The first compound has the molecular formula a C27H30O15 and is identified as luteolin-4'-O-β-D-glucopyranosyl-(2-1)-α-L-rhamnopyranoside. The second identified flavonoid is kaempferol-7-O-β-D-glucopyranosyl-(2-1)-α-L-rhamnopyranoside (molecular formula C27H30O15). The third compound is kaempferol-3-O-β-D-glucopyranosyl- (6-1)-α-L-rhamnopyranoside having molecular formula C27H30O15. Ahmad et al isolated two pregnane glycosides named caratubenides A and B from *Caralluma tuberculata* shown in figure below. Penicilloside E was isolated by Abdel-Sattar et al. 2008 which was very effective against antitrypanosomal activity. Other compounds such as anthocyanin, saponins, cumarine, betacyanin, tannins and alkaloids are also found in *Caralluma tuberculata.* [65-8]

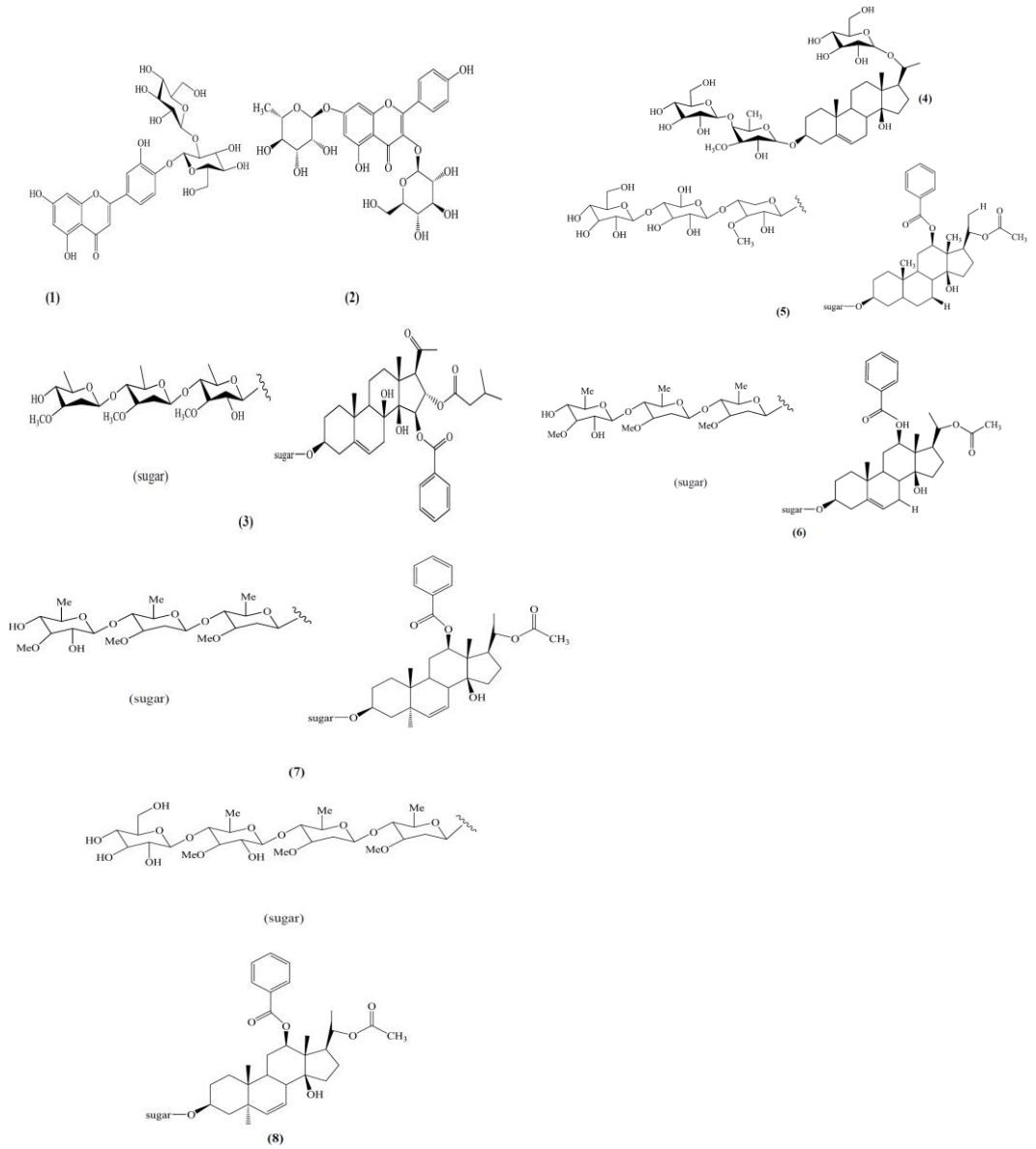

Figure 5. Different Glycosides compounds and a flavonoid compound isolated from *Caralluma tuberculata*. (1) Flavone glycoside, (2) kaempferol 3-O-β-D-glucopyranoside (Flavonoid), (3) penicilloside E, (4) Russelioside B, (5) Pragnane Glycoside, (6) Caratuberside C, (7) Caratuberside E (8) Caratuberside F

1.5 Aims and Objectives

Major objectives of our work include:

1. Fractional extraction of *Caralluma tuberculata* in different solvents.
2. Phytochemical screening of different fractions for the detection of various classes of natural products
3. Analysis of the plant fractions for their anti-bacterial properties against *Citrobacter freundii* and *Escherichia coli.*

MATERIALS

Caralluma tuberculata (dried powdered sample), required glasswares and instruments (Soxhlet apparatus), Different chemicals.

Chemicals Used

n-hexane, Dichloromethane, ethyl acetate, methanol, hydrochloric acid HCl, sulphuric acid(H2SO4), Acetic acid, NaOH, ammonium chloride, Hager's reagent, Wagers reagent, lead acetate, ferric chloride, Biuret reagent, Benedict's reagent, Fehling's solution, Molisch reagent.

METHODOLOGY

Collection of Plant

The selected plant *Caralluma tuberculata* was collected in the month of December, 2021 from District Bunir, Khyber Pakhtunkhwa, Pakistan. The collected plant was well washed and chopped into small pieces. The plant was dried in shade for four weeks and powdered by grinder. The plant material was stored in air tight container.

Figure 6. Chopped plant (Caralluma Tuberculata)

Extraction and Fractionation

30g of dried finely powdered plant material was put within a porous bag (thimble) made of filter paper and the bag is sealed tightly. Thimble was placed in the extraction chamber to stop the siphon tube from being clogged when powdered plant material is added. The round bottom flask was filled with the extraction solvents before the thimble was placed within the extraction chamber. During fractionation, the chosen solvents were introduced in the sequence of increasing polarity: n-hexane, dichloromethane, ethyl acetate, methanol, and water, respectively. After that, the solvent was heated by heating mantel, and maintain temperature according to the boiling point of the solvent. The solvent evaporates, travels through the condenser, condenses, and flows downward to the extraction chamber, where it interacts with the plant material to extract it. When the solvent level in the extraction chamber reaches the top of the siphon, the solvent and the extracted plant material flow back to the flask. The process is continued until all of the plant material has been extracted and a point reach when a solvent flowing from extraction chamber does not leave any residue behind. The same procedure was repeated for other solvents and we obtained fractions of n-hexane, dichloromethane, ethyl acetate, methanol and water.

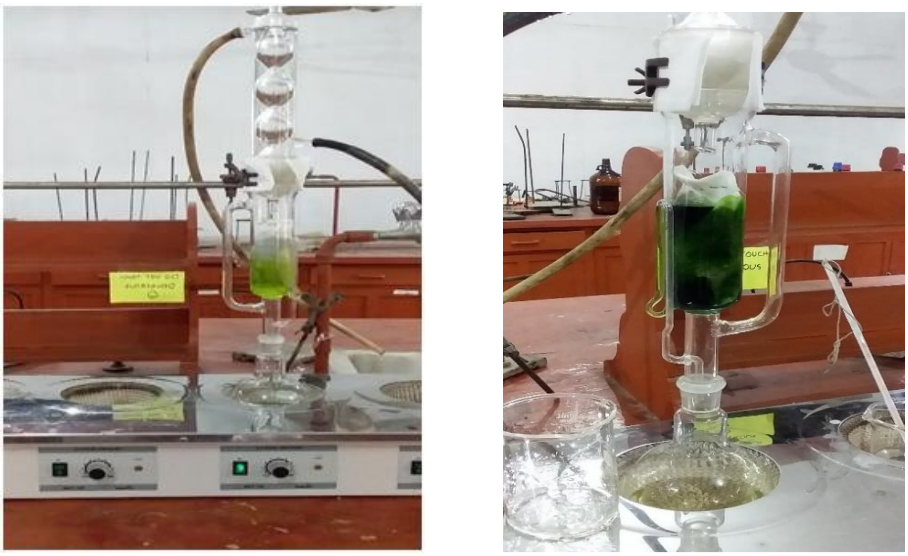

Figure 7. Soxhlet extraction of *Caralluma tuberculata* with (a) n-Hexane (b) Dichloromethane

Qualitative Phytochemical Screening
Various screening tests were conducted for different classes of natural products. These tests were carried out for all the fractions.

Detection of Alkaloids
The alkaloids were detected using Hager's test and Wagner's test. In Hager's test, 2ml of n-hexane extract was taken in test tube and few drops of Hager's reagent was added. The formation of yellow precipitates indicates the presences of alkaloids. During Wagner's test, 2ml of plant extract was taken in test tube and few drops of Wagner's reagent was added to it sidewise. A reddish-brown precipitate indicates the presence of alkaloids.

Detection of Flavonides
2ml of n-hexane extract was taken in test tube and 1ml of 2% NaOH was added to it, followed by few drops of HCl. The solution turns colorless indicates the presence of flavonoids.

Detection of Tannins
Tannins were detected using Ferric chloride and Lead acetate test. In Ferric chloride test, 2ml extract was taken in a test tube and 1ml of ferric chloride was added to it. A dark green color indicates the presence of tannins. During Lead acetate test, 1ml of lead acetate solution was added to 2ml of each extract. Formation of white precipitates indicates presence of tannins.

Detection of Terpenoids
2ml of each extract was mixed with 1ml chloroform and wait for few seconds. Then add 2ml of concentrated H2SO4. A reddish brown colour indicates the presence of terpenoids.

Detection of Saponins
2ml of extract and 5ml of water was taken in test tube and vigorously shaken. The appearance of frothing or foam indicates the presence of saponins.

Detection of Steroids
2ml of extract was taken in test tube and 2ml of acetic acid was added to it, followed by conc. H2SO4.The appearance of blue or green mixture indicates the presence of steroids.

Detection of Carbohydrates
Carbohydrates were detected using Molish's and Benedict's test. In Molish's test, 2ml of extract was taken in test tube and 1ml Molish reagent was added to it, followed by the addition of few drops of concentrated H2SO4 along the sides of the test tube. Formation of violet ring indicates the presence of carbohydrates. During Benedict's test, 2ml of extract was taken in test tube along with 1ml of Benedict's reagent. The mixture was heated on water bath for 2 minutes. The presence of carbohydrates is indicated by distinctively colored precipitates. Depending on the type and quantity of sugar, the color can range from green to dark red (brick) or rusty-brown.

Detection of Reducing Sugars
Reducing sugars were detected by Fehling's test. In Fehling's test, 2ml of extract was taken in test tube and 1ml of Fehling A and Fehling B solution was added to it. The test tube was kept in water bath until the mixture started boiling. The orange red precipitates formation indicate the presence of reducing sugars.

Detection of Phytosterols
2ml of extract was taken in a test tube and few drops of conc H2SO4 was added to it. Wait for few minutes. Formation of brown ring indicates presence of phytosterols.

Detection of Phenolic Compounds
Phenolic compounds were detected by Ferric chloride test. In Ferric chloride test, 2ml of extract was taken in test tube and 2-3 drops of ferric chloride was added to it. Formation of dark green or bluish black color indicates the presence of phenolic compounds.

Detection of Proteins and Amino Acids

Proteins and amino acids were detected by Biuret and Ninhydrin test. In Biuret test, 2ml of extract was taken in test tube and 1ml of biuret reagent was added to it. The mixture was well shaken and was allowed to stand for 5 minutes. The purple colour does not form which indicates the absence of proteins and amino acids. During Ninhydrin test, first ninhydrin solution was prepared. Then take 2ml of extract in a test tube and 1ml ninhydrin solution was added to it. Boil it for few minutes. Formation of blue color indicates the presence of amino acids.

Detection of Glycosides

2ml of extract was diluted with 3ml of water in a test tube. 2ml of glacial acetic acid was added to it followed by few drops of H2SO4. The formation of brown ring, violet ring or greenish ring indicates the presence of glycosides [27,28].

Figure 8. Different tests performed

Anti-Bacterial Activities

The anti-bacterial efficacy of *Cralluma Tuberculata* extract against *Citrobacter freundi* and *Escherichia coli* was investigated using extracts obtained in various solvents. The successive method was followed. The bacterial strains were cultivated on broth and left to incubate for 24 hours. Then, a stock solution was made by taking 20 g of agar with 1000 ml of distilled water. After that petri dishes were taken and 20 ml of stock solution was added to each petri dish. The bacterial strains were marked on the medium using a cotton swab. The plant extract of n-hexane was added and applied at 100,200,300 µg/ml concentration on the petri dishes. This step was carried out for chloroform, ethyl acetate, methanol fractions as well. The experiment is repeated for three times. After that, the petri dishes were incubated at 37 ºC and after 24 hours, the zone of inhibition (mm) was used to observe the anti-bacterial impact. Streptomycin was used as a reference standard to determine the anti-bacterial properties of different fractions.

RESULTS AND DISCUSSIONS

Phytochemical Screening of Caralluma Tuberculata

Different fractions were prepared from the dried plant (*Caralluma tuberculata*) by using Soxhlet apparatus. The purpose of extraction was to extract different soluble secondary metabolites in different solvents (n-hexane, dichloromethane, ethyl acetate, and methanol). These fractions were subjected to different phytochemical tests for each class of natural products. These fractions showed the presence of different phytochemicals as shown in table 2.

The phytochemical analysis of *Caralluma tuberculata* shows the presence of different bioactive secondary metabolites such as glycosides, phytosterols, alkaloids, flavonoids, terpenoids etc. These secondary metabolites are responsible for the medicinal importance of this plant (*Caralluma tuberculata).*

Phytochemical Screening of n-Hexane Fraction

The phytochemical screening of n-hexane fraction of *Caralluma tuberculata* shows the absence of flavonoids, tannins, phenolic compounds, reducing sugars, proteins and amino acids. As no colour change was observed for these compounds. Alkaloids were confirmed by the formation of reddish-brown precipitates. Terpenoids were identified by the formation of reddish-brown color. Saponin presence was

indicated by the formation of froth. Steroids presence were confirmed by a blue green mixture. Carbohydrates were confirmed by a violet ring (Molish test) and by appearance of dark green colour (Benedict's test). Glycosides were confirmed by formation of dark green and brown ring. Phytosterols were confirmed by the formation of a brown ring. These results were compared with a study carried out by Rauf et al. which were not in accordance with the present results except for the steroids [27].

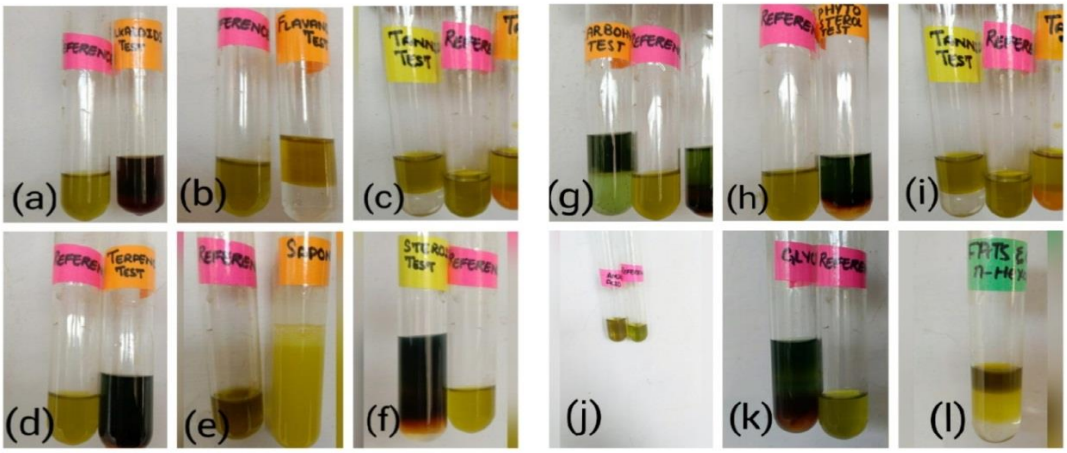

Figure 9. Qualitative tests of n-hexane fraction (a) Alkaloids (b) Flavonoids (c) Tannins (d) Terpenoids (e) Saponins (f) Steroids (g) Carbohydrates (h) Phytosterols (i) Phenolic compounds (j) Proteins and amino acids (k) Glycosides (l) Oils and fats.

Phytochemical Screening of Dichloromethane Fraction

The phytochemical screening of dichloromethane fraction shows the presence of alkaloids, flavonoids, tannins, terpenoids, phenolic compounds, steroids, carbohydrates, reducing sugars, glycosides and phytosterols. Alkaloids were identified by the formation of yellow precipitates. Flavonoids were confirmed when dark green color became colorless. The formation of white bulky precipitates indicates the presence of tannins compounds. Terpenoids were identified by formation of reddish-brown color. Steroids were confirmed by appearance of black color. Phenolic compounds were indicated by appearance of bluish-black color. The formation of Carbohydrates was identified by the formation of a ring. Reducing sugars were identified by the formation of dark green colour. Formation of a Brown ring indicates presence of glycosides. Formation of reddish-brown ring indicates presence of phytosterols. Saponins, Proteins and amino acid shows negative result in this fraction. These results were compared with a study carried out by Rauf et al. which is similar to the results except for alkaloids and flavonoids [27].

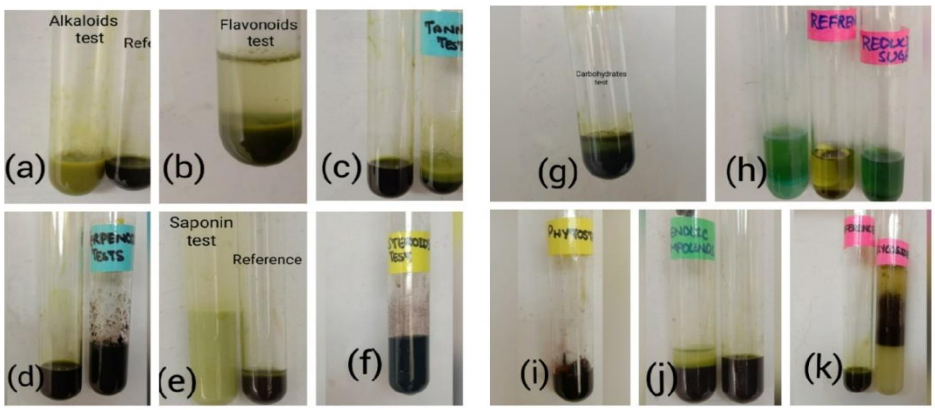

Figure 10. Qualitative Tests of dichloromethane fraction (a) Alkaloids (b) Flavonoids (c) Tannins (d) Terpenoids (e) Saponins (f) Steroids (g) Carbohydrates (h) Reducing Sugars (i) Phytosterols (j) Phenolic compounds (k) Glycosides.

Phytochemical Screening of Ethyl Acetate Fraction

Phytochemical screening of Ethyl acetate fraction shows absence of tannins, phenolic compound, proteins and amino acids. Alkaloids were identified by the formation reddish-brown precipitates. Flavonoids were

confirmed when the mixture turned colourless. Terpenoids were indicated by the formation of brown color. Saponins were indicated by the formation of foam. Formation of black colour indicates the presence of steroids. Carbohydrates are indicated by the formation of a rusty-brown colour. Reducing sugars were confirmed by the formation orange precipitates and dark green color (Benedict's test). Formation of a green and brown ring indicates the presence of glycosides. Phytosterols are indicated by the formation of a dark brown ring. In case of ethyl acetate fraction the present results were in accordance with the studies carried by Rauf et al 2013 except alkaloids, flavonoids and glycosides [27].

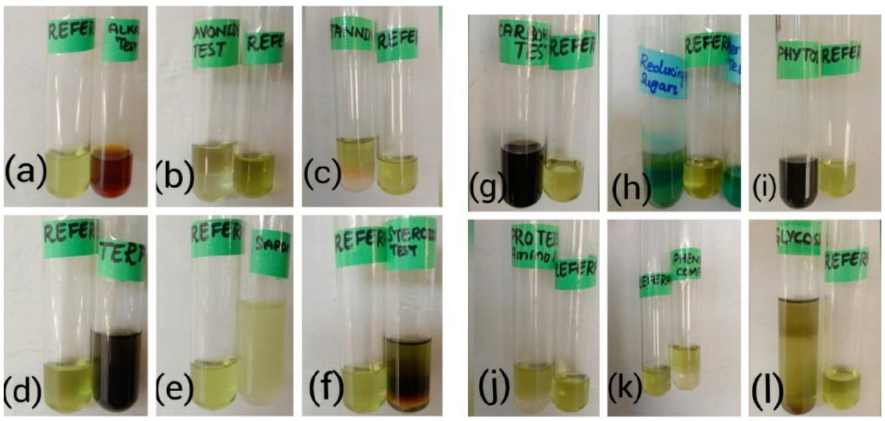

Figure 11. Qualitative tests of Ethyl acetate fraction (a) Alkaloids (b) Flavonoids (c) Tannins (d) Terpenoids (e) Saponins (f) Steroids (g) carbohydrates (h) Reducing sugars (i) Phytosterols (j) Phenolic Compounds (k) Proteins and Amino Acids (l) Glycosides.

Phytochemical Screening of Methanol Fraction

Extract of plant *Caralluma Tuberculata* were subjected to phytochemical analysis for the presence of various primary and secondary metabolites. Phytochemical screening of plant extracts reveals the presence of flavonoids, terpenoids, saponins, steroids, carbohydrates, reducing sugar, phytosterols, phenolic compounds, proteins and Amino acids. Terpenoids were observed by brown colour appearance. A mixture of light and dark brown colour shows the entity of phytosterols. Phenolic compounds were identified by formation of white precipitate (lead acetate) and appearance of bluish black colour (ferric chloride). Blue colour formation showed the presence of proteins and amino acid. The fraction was devoid of alkaloids, tannins, and glycosides. In case of methanol fraction the present results were similar to a study carried out by Rauf et al 2013 except for alkaloids and flavonoids.

All of the four fraction results were compared with a study carried out by Muhammad A.K et al 2019 shows similar phytochemicals obtained in the present results [24]. These results were compared with another study carried out by Mudrikah et al shows almost similar phytochemicals obtained from the present study [25].

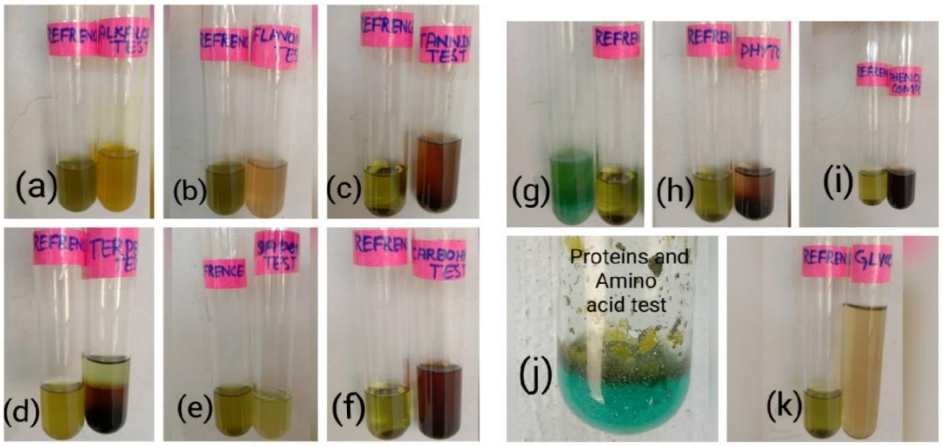

Figure 12. Qualitative tests of Methanol Fraction (a) Alkaloids (b) Flavonoids (c) Tannins (d) Terpenoids (e) Saponins (f) Carbohydrates (g) Reducing Sugars (h) Phytosterols (i) Phenolic compounds (j) Proteins and amino acids (k) Glycosides.

Table 2. Results of Phytochemical Screening of Different Fractions of *Caralluma tuberculata*

	n-Hexane Fraction	**Dichloromethane Fraction**	**Ethyl acetate Fraction**	**Methanol Fraction**
Alkaloids	+	+	+	-
Flavonoids	-	+	+	+
Tannins	-	+	-	-
Terpenoids	+	+	+	+
Saponins	+	-	+	+
Steroids	+	+	+	+
Carbohydrates	+	+	+	+
Reducing sugars	-	+	+	+
Phytosterols	+	+	+	+
Phenolic compounds	-	+	-	-
Proteins and amino acids	-	-	-	+
Glycosides	+	+	+	-

RESULTS OF ANTI-BACTERIAL ACTIVITIES

Results of Anti-Bacterial Activity of *Caralluma tuberculata* Against *Citrobacter freundi*

Different solvent extracts *of Caralluma tuberculata* were examined for their antibacterial activity against *Citrobacter freundii*. The results obtained shows that by increasing concentration and polarity the activity of the fractions increases. From the table it is clear that all the fractions show %inhibition to a certain extent which means that all the fractions have bacterial activity. The methanol fraction having 100µg/ml concentration shows the %inhibition value 92 which is close to standard (streptomycin) having %inhibition value 100. By increasing concentration, the methanol fraction %inhibition values are the highest among all the other fractions. Another factor is the polarity, by increasing polarity the activity increases as follows: methanol has highest %inhibition values. Ethyl acetate and dichloromethane shows moderate %inhibition while n-hexane shows least activity against *Citrobacter freundii* compared to the standard. This means that the presence of OH groups increase the ability of the compounds to enter the cell wall of the bacteria and interact with it to slow down it's activity. The compounds present in n-hexane and dichloromethane are less active compared to the methanol fraction. Hence methanol fraction is very effective against *Citrobacter freundii* and show high resistance against this bacterial strain. The result obtained was in accordance with previous studies carried out by Bashir. A et al which shows the same results for *Citrobacter freundii.* [29]

Table 3. Anti-Bacterial Properties against *Citrobacter freundii*

	Concentration µg/ml	Zone of inhibition (mm)			Mean	%Inhibition
		R1	R2	R3		
Methanol fraction	100	38	36	35	36.33	92
	200	45	47	44	45.33	114.29
	300	51	54	54	53	133.6
Ethyl acetate fraction	100	18	17	17	17.33	43.6
	200	23	24	23	23.33	58.9
	300	28	30	31	29.66	74.8
Dichloromethane fraction	100	16	14	17	15.66	39.5
	200	23	21	20	21.33	53.8
	300	28	27	25	26.66	67.32
n-hexane fraction	100	9	11	10	10	25.25
	200	13	14	13	13.33	33.6
	300	19	17	19	18.33	46.28
Streptomycin	100	42	38	39	39.66	100

Results of Anti-Bacterial Activity of *Caralluma tuberculata* against *Escherichia coli*

All fractions of *Caralluma tuberculata* were analyzed for their antibacterial activity against *Escherichia coli*. It is clear from the table that the %Inhibition value increases by increasing concentration as well as polarity. As methanol has the highest polarity among all the other solvents, it shows 96% inhibition at 100 µg/ml which is in accordance with the standard (streptomycin). Also, by increasing concentration the %inhibition increases as shown in the table below. Ethyl acetate shows the second highest value i.e., 76.62% of inhibition against *Escherichia coli* at 100 µg/ml concentration. While dichloromethane and n-hexane show moderate

values. Hence it is clear that methanol and ethyl acetate fraction compounds are more active than the other two fractions. Furthermore, if we compare these results with the results obtained against *Citrobacter freundii*, it is clear that all the fractions activity is more against *Escherichia coli* compared to *Citrobacter freundii*. Specifically, methanol is more effective against *Escherichia coli* compared to *Citrobacter freundii*. In case of *Escherichia coli*, the results were mismatched with previous study carried out by Bashir. A et al which shows similar results [29].

Table 4. Anti-Bacterial Properties against *Escherichia coli*

	Concentration µg/ml	Zone of Inhibition (mm)			Mean	%Inhibition
		R1	R2	R3		
Methanol fraction	100	34	35	34	34.33	96.27
	200	41	40	41	40.66	114.02
	300	43	43	42	42.66	119.62
Ethyl acetate fraction	100	26	28	29	27.66	76.66
	200	34	35	33	34	95.34
	300	38	39	41	39.33	110.29
Dichloromethane fraction	100	19	18	19	18.66	52.32
	200	23	25	23	23.66	66.34
	300	28	26	27	27	75.71
n-hexane fraction	100	13	10	12	11.66	32.69
	200	17	15	14	15.33	42.93
	300	21	19	18	19.33	54.20
Streptomycin	100	35	38	36	35.66	100

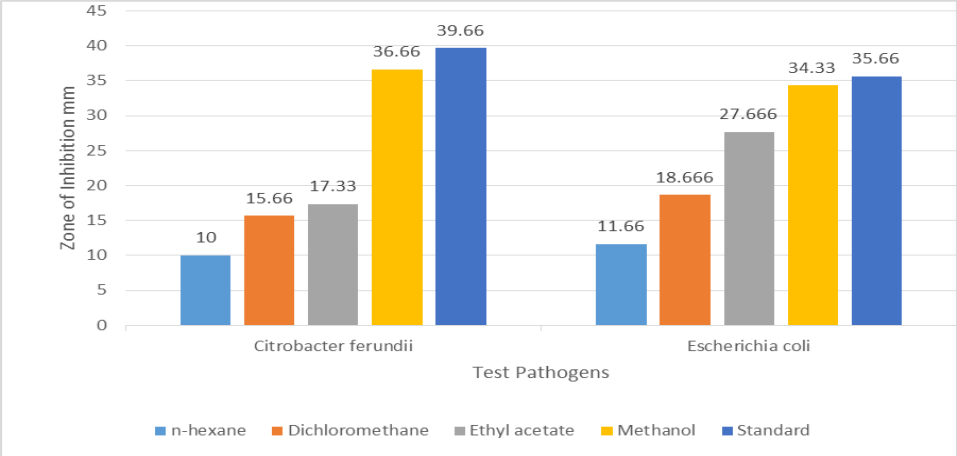

Figure 13. Antibacterial activities of *Caralluma tuberculata* against test pathogens

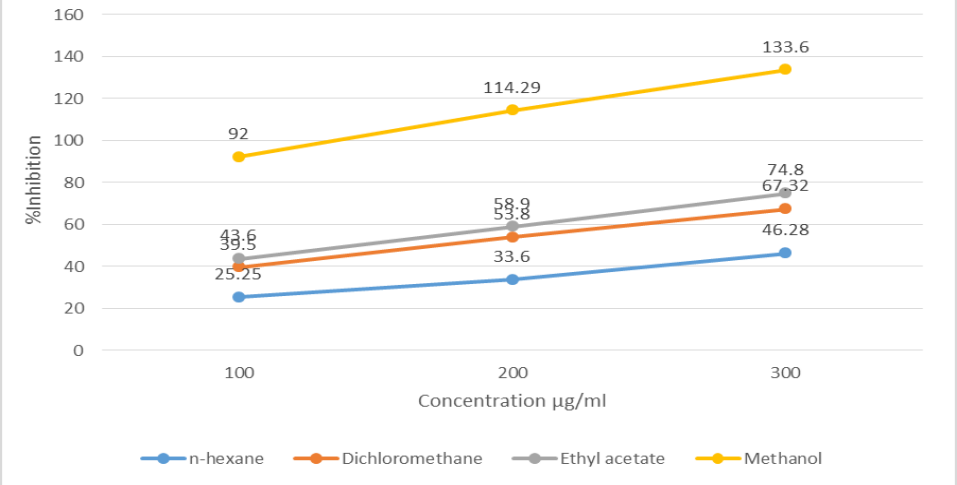

Figure 14. Graphs depicts the % inhibition of *Caralluma tuberculata* of all fractions again *Citrobacter freundii*

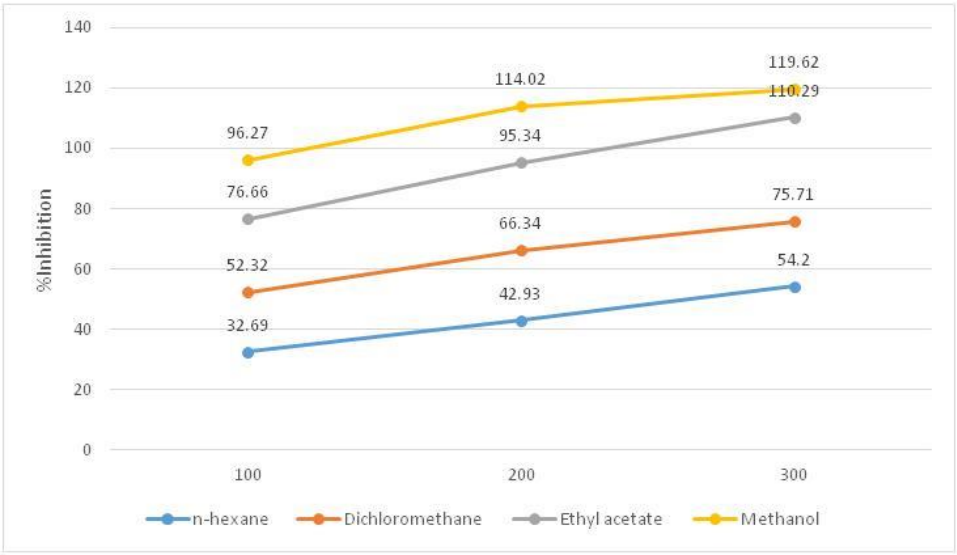

Figure 15. Graph depicts %Inhibition of *Caralluma Tuberculata* Extracts of all fractions against *Escherichia coli.*

CONCLUSION

Plant of *Caralluma Tuberculata* was extracted in different solvents of varying polarities i.e., n-hexane, dichloromethane, ethyl acetate, methanol and water. Phytochemical analysis was carried out for all the fractions. Phytochemical screening of these fractions showed the presence of alkaloids, flavonoids, tannins, terpenoids, saponins, phytosterols, phenolic compounds, glycosides, carbohydrates, reducing sugars, steroids. Phytosterols and steroids were present in all fractions. Glycosides were present in all fractions except methanol fraction. Saponins were present in all fractions except dichloromethane fraction. Antibacterial activities of the fractions were carried out against *Citrobacter freundii* and *Escherichia coli*. The activity of the fractions increases with increase in concentration and polarity. Plant extract of methanol fraction shows 96% inhibition against *Escherichia coli* which is close to standard Streptomycin (100%) inhibition value. In case of *Citrobacter freundii* the best result was obtained by methanol fraction showing 92% inhibition. From the result analysis it was also observed that increase in %inhibition occur by increasing in concentration. Methanol fraction shows 133% inhibition against *Citrobacter freundii* at 300 µg/ml concentration. It was observed that methanolic fraction shows best results against *Escherichia coli* compared to *Citrobacter freundii*.

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