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# Phytochemistry and Antibacterial activity of C*onvolvulus arvensis* Linn against Escherichia coli

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**Abstract:** In the present research work *convolvulus arvensis* was investigated for antibacterial activity and phytochemicals analysis. The results of antibacterial activity showed that the effect of different extract of *convolvulus arvensis* on the growth of *E.coli* is different in different solvents. Maximum zone of inhibition was shown by acetone and chloroform extract. Ethanol exhibited 2.1cm, Methanol 2.6 cm, while the lowest zone of inhibition was showed by aqueous extract i.e. 1.96cm. *Convolvulus arvensis* antibacterial activity result indicates that acetone and chloroform have highest antibacterial activity which indicates that parts of *convolvulus arvensis* have antibacterial chemicals which are dissolved in the solvent and the bacterial agent has diffused in agar medium and inhibited the growth of *E. coli*. The lowest antibacterial activity is shown by aqueous extract. The results of phytochemical analysis showed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, cardiac glycosides was found in methanolic and ethanolic extracts, while alkaloids, phlobatannins. Flavonoids, carbohydrates, saponins, phenols and terpenoids were found present in in the rhizome methanolic and ethanolic extracts.

**Key words:** Phytochemistry, Antibacterial activity, Convolvulus *arvensis* and AWKUM Pakistan

## INTRODUCTION

An antibacterial is an agent that kills or suppresses the growth of bacteria and also its ability of reproduction. “Antibacterial activity” is the ability of any compound which includes antibiotics, food antimicrobial agents, sanitizers, germicidal, and other substances that acts against bacteria and delay

their growth. Antibiotics on the other hand are antimicrobial agents produced by bacteria, fungi or of synthetic in nature[1]. In the developing countries 50% of death is cause by diseases. Antimicrobial agents are very important to decrease worldwide problems of infectious diseases. As the resistant pathogens multiply and increase its number, so the efficiency of the antibiotics is diminished or retard. The resistance of bacteria to the antibacterial agents is a very serious problem to human health, and for different types of antibiotics, including the major last-resort drugs, increasing the number of resistance universally[2]. Resistance of bacteria to antibiotics increases the death rate and also the probability of stay extant in the hospital[3]. E. coli is an enteral gram - negative, optimum grasped as noninvasive symbiotic and multitalented bacteria. In human *E. coli* breeds mostly in intestine and also in the gut lumen of animal in the form of culture, additional threatening microorganism. E. coil is opportunistic microbe of animals and humans[4]. . E. coli is bacterial groups commensally of people and warm blooded creatures. Nevertheless some strains have evolved the capability to cause both intestinal and extra intestinal illnesses[5], [6]. *E. coli* with an antihuman circulation is a major cause of loose bowels, regularly with high death rates, in developing nations[7]. In particular geographic regions, such as in some developing countries, infections with diarrhea genic *E. coli* transmitted by inter-human contacts, such as Enter aggregative *E. coli* [7, 8].

The medicinal plants are useful for human diseases as well as for curing of healing because of the presence of phytochemical constituents[9]. Phytochemicals are naturally occurring chemical, biologically active compounds found in plants, which be responsible for health benefits for humans further these recognized to micronutrients and macronutrients[10]. They protect plants from damage and disease and contribute to the plant's color, flavor and aroma. In common, the plant chemicals that defend plant cells from environmental threats such as stress, drought, pollution, pathogenic attack and UV exposure are called as phytochemicals[11]. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics[12]. In the medicinal plants Phytochemicals are naturally present in roots, vegetables and leaves protect from various diseases due to their defense mechanism. Phytochemicals have two types. Primary and secondary constituents. Proteins, chlorophyll, and common sugars are incorporated in primary and secondary phytochemicals have alkaloids, terpenoids and phenolic compounds [13].

Various important pharmacological activities exhibit by terpenoids i.e., anticancer[14], anti-malarial and anti-inflammatory[15, 16] inhibition of cholesterol production, anti-bacterial and anti-viral activities[17-20]. Terpenoids are very essential in attracting beneficial mites and ingest the herbivorous insects [21]. Convolvulus is derived from the Latin (convolere) meaning to entwine, and arvensis means (of fields). Belong to family Convolvulaceae. Common names of the plant were: Afrikaans: akkerwinde, klimop; Arabic: leblab elhokul. English: bindweed, common bindweed, field bindweed, lesser bindweed, small bindweed, white convolvulus, wild morning-glory; it was distributed in Africa: Algeria, Egypt, Libya, Morocco and Tunisia; Asia: Afghanistan, Cyprus, Iran, Palestine, Jordan, Lebanon, Syria, Turkey, Armenia, Azerbaijan, Georgia, Russian Federation, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan, Mongolia, China, India, Nepal and Pakistan [21].

## METHODS AND MATERIALS

**2.1 Plants collection and Botanical identification:** The present study the plant species *Convolulus arvensis L.* were collected from botanical garden Abdul Wali khan university Mardan Garden campus Pakistan during flowering stage and dried at room temperature, the collected plants were identified

botanically in department of Botany, Abdul Wali Khan University Mardan Garden Campus. For the phytochemical analysis and anti-bacterial activity plants were used.

**2.2 Plant extracts Preparation:** From the plants the leaves were removed and to remove dust then washed the selected plants under running tap water. For few days the plant samples were then air dried and into small pieces the leaves were crushed and stored in polythene bags. With the help of electric grinder the dried plant was powdered. The powder were kept in air tight plastic bottles for further phytochemical analysis. 10 gm of plant powdered was retained in distinct conical flask and 90 ml of solvent i.e. (methanol, ethanol, chloroform and acetone) was added to the powdered separately. With the help of aluminum foil the flask were covered and retained in shaker for 72 hrs for the shaking purposes. After 72 hrs the extracts were filtered with the help of Whatman filter paper and then through filtration process plant extracts were removed[22].

**2.3 Test Strains**: Bacterial strains were obtained from standard laboratory of microbial lab of Bach Khan Medical College and Hospital Mardan Pakistan. Evaluate the antibacterial activity of the plant extracts using strain of bacteria, the gram negative Bacteria Escherichia coli collected from laboratory. The typed cultures of bacteria was sub-cultured on Nutrient agar (Oxoid) and stored at 4o °C until required for study.

**2.4 Preparation of media:** For the study of antimicrobial activity of weed plant parts, Nutrient Agar Media was used as a growing medium for bacteria. Agar 1.5% and 0.8% Nutrient broth were taken in beakers. Mixed them and made up final volume to 500ml with distilled water in 500ml flask .plugged with cotton and autoclaved for 25 min. Media was poured in plates when solidified placed in refrigerator. Wells were made with the help of sterile Cork borer.

**2.5 Bacterial growth assays:** Well-diffusion method was used to evaluate the antibacterial activity of aqueous and organic solvents plant extracts of *Convolvulus arvensis.* 0.8% Nutrient broth taken in flask and distilled water was added until final volume is up to 100ml.Flask was covered with cotton and autoclaved. After sometime bacteria was added in the flask containing media with the help of sterilized loop and placed in shaker for 24 hrs.0.5% 0f inoculum was taken through micropipette in the plates and 1 to 2 drops of extract of sample was added in wells of each inoculated plate through micropipette. Triplicates were made for each sample. Each treatment was made in triplicate. Control plates received similar amount of solvent. Such Petri plates were incubated overnight at 37 ºC. This action was performed for all of the five extracts i.e. methanol, ethanol, chloroform, acetone and water. Antibacterial activity was evaluated by measuring the inhibition zones of bacterial growth (if present) around the wells in cm and expressed as the mean of three triplicates ± SE (standard error). Each set of experiment was repeated twice.

**2.6 Determination of antibacterial activity:** To screen the antibacterial activity of the selected medicinal plants chloroform, methanolic and ethanolic, acetone and aqueous extracts the agar well diffusion method was used[12]. With crude extracts of all parts of plants the assay was performed.

**2.6.1 Extract Preparation for activity:** In 1 ml of DMSO 20mg crude extracts of plant samples were completely dissolved. Solution of a standard antibiotic (2 mg/ml of Cefotaxime) was used as positive control. Negative control was used pure DMSO.

**2.6.2 Measurement of zone of inhibition and Pouring of test solution incubation:** Using micropipette, 75 µl of plant samples solution were poured in labeled wells. Each of the labeled plate was provided with samples of extracts, as positive standard cefotaxime and as negative standard di methyl sulphoxide (DMSO) was used. At 37ºC incubation was done. After 24 h of incubation, the

diameter of clear zones, showing no bacterial growth around each well was measured. Three times activity was repeated and average of zone of inhibition with standard deviation was calculated.

**2.7 Phytochemical analysis :** The plant extract i.e. methanol, ethanol and aqueous were tasted for the absence or presence of phytochemical constituents' like alkaloids, tannins ,Phlobatannins, flavonoids, carbohydrates, phenols, saponin and terpenoids[23] .

**2.7.1 Tests for Alkaloids:** For detection of alkaloids, a few drops of Wagner's reagent (Potassium iodine) are add to 2 ml of all three methanol, ethanol and aqueous extracts. The formation of reddish brown precipitate showed the presence of alkaloids[23].

**2.7.2 Tests for Tannins:** For the detection of tannins Ferric chloride test was done. Ferric chloride ($FeCl_3$) solution was mixed with all three extracts separately. Formation of blue green coloration indicated the presence of tannins[24]..

**2.7.3 Tests for Phlobatannins:** In test tubes 0.5 ml of all the three extracts was taken separately, added 3ml distilled water and shaken for a few minutes then 1% aqueous hydro chloride (HCl) was added and boiled on water both. The presence of phlobatannins is indicated by the formation of red color[13] .

**2.7.4 Tests for Flavonoids**

For flavonoids detection, all the three extracts were treated with sodium hydroxide (NaOH) solution. Red precipitation formation of indicate the presence of flavonoids[25].

**2.7.5 Tests for Carbohydrates:** For detection of carbohydrates, 0.5 ml of all three extracts were treated with 0.5 ml of Benedict's regent. The solution were heated for 2 minutes on a water bath. By the formation of reddish brown precipitate the presence of carbohydrate was confirmed [26].

**2.7.6 Tests for Phenols:** For phenol detection, 2 ml of ferric chloride ($FeCl_3$) solution was added to 2 ml of all the three extracts in a test tube separately. Formations of deep bluish green solution showed the presence of phenol[27].

**2.7.7 Tests for Saponins:** For the detection of saponin, in test tube 5 ml of all three extracts were shaken vigorously. The formation of froth indicated the presence of saponins [28].

**2.7.8 Tests for (Cardiac) Glycosides:** For cardiac glycosides detection, 2 ml of all three extracts solution were shaken with 2 ml of glacial acetic acid than added few drops of concentrated sulphuric acid ($H_2SO_4$) and iron tri chloride ($FeCl_3$). The formation of a brown ring indicated the presence of glycosides[23].

**2.7.9 Tests for terpenoids:** One ml of Adiantum capillus-veneris Hook plant extracts (methanol, ethanol and aqouse) was added with 2 ml of chloroform and carefully added concentrated sulphuric acid ($H_2SO_4$) along the sides of tube to form a layer. The formation of reddish brown coloration indicated the presence of terpenoids[27].

**2.8 Statistical analysis:** All the tests were performed as individual triplicate experiment. All the data are expressed as mean ± standard deviation.

## RESULTS AND DISCUSSION

**3. Phytochemical analysis and antibacterial activities:** In the present research work the phytochemical investigation of methanolic, ethanolic and aqueous extracts of *Convolvulus arvensis* and antibacterial activity of methanolic, ethanolic, acetone and aqueous extracts was carried out.

**3.1: Effect of ethanol extract on the growth of E.coli:** Fig.4.1Showed the effect of ethanol extract of convolvulus arvensis on the growth of E.coli by well diffusion method. It is very clear from the figure that ethanol extract of the plant has very good antibacterial activity as compared to control (contain only the solvent i-e ethanol).The ethanol extract have very clear bigger zone of inhibition (2.1cm) as compared to control (0.4cm).It is might due to the fact that the convolvulus arvensis has antibacterial chemical which are dissolved in the solvent and showed clear zone of inhibition.

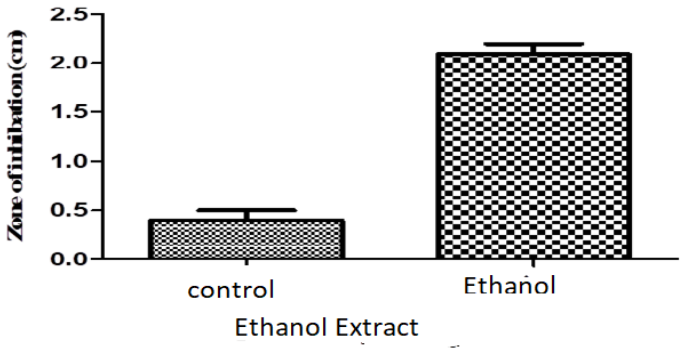


**Fig. 3.1:** Effect of Ethanol extract of *convolvulus arvensis* on the growth of *E. coli*.

**3.2. Effect of acetone extract on the growth of E.coli:** Fig.3.2 showed the effect of acetone extract of convolvulus arvensis on the growth of E.coli by well diffusion method. It is very clear from the figure that acetone extract of the plant has very good antibacterial activity as compared to control (contain only the solvent i-e acetone).The acetone extract have clear bigger zone of inhibition (2.8cm) as compared to control (0.86cm).It is might due to the fact that the convolvulus arvensis has antibacterial chemical which are dissolved in the solvent and showed clear zone of inhibition.

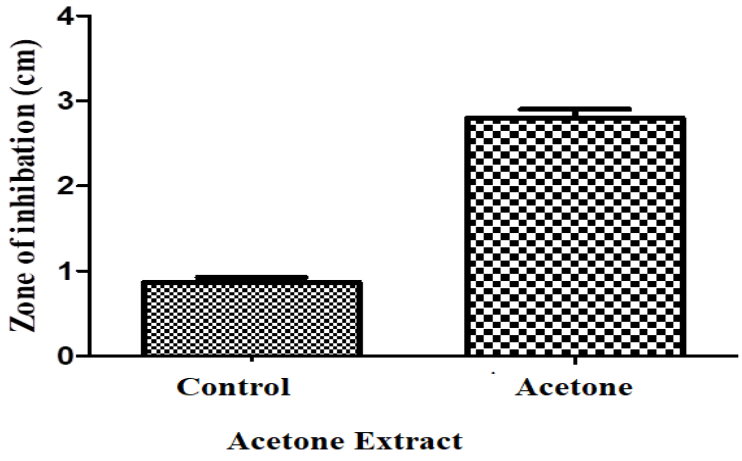


**Fig. 3.2:** Effect of Acetone extract of *convolvulus arvensis* on the growth of *E.coli.*

**3.3. Effect of methanol extract on the growth of E.coli:** Fig.3.3 showed the effect of methanol extract of convolvulus arvensis on the growth of E.coli by well diffusion method. It is very clear from the figure that methanol extract of the plant has very good antibacterial activity as compared to control (contain only the solvent i-e methanol).The methanol extract have clear bigger zone of inhibition (2.6cm) as

compared to control (o.93cm).It is might due to the fact that the convolvulus arvensis has antibacterial chemical which are dissolved in the solvent and showed clear zone of inhibition.

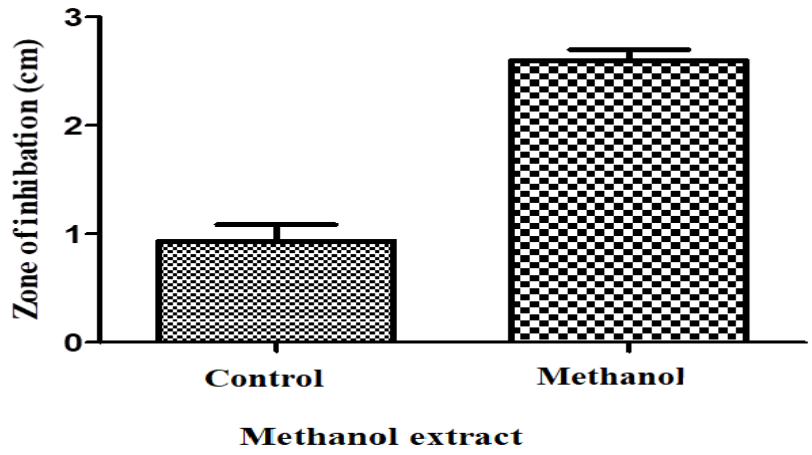


**Fig. 3.3:** Effect of Methanol extract of *convolvulus arvensis* on the growth of *E. coli*.

**3.4: Effect of Chloroform extract of *convolvulus arvensis* on the growth of *E. coli:*** Fig.3.4 showed the effect of chloroform extract of convolvulus arvensis on the growth of E.coli by well diffusion method. It is very clear from the figure that chloroform extract of the plant has very good antibacterial activity as compared to control (contain only the solvent i-e chloroform).The chloroform extract have clear bigger zone of inhibition (2.8cm) as compared to control (0.63cm).It is might due to the fact that the convolvulus arvensis has antibacterial chemical which are dissolved in the solvent and showed clear zone of inhibition.

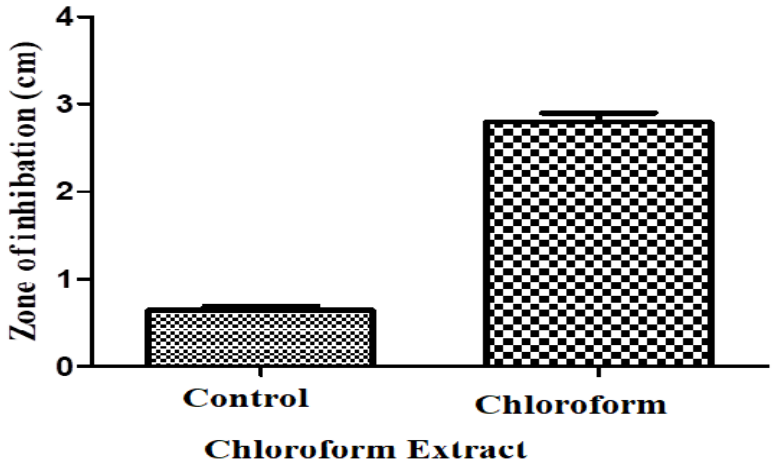


**Fig. 3.4:** Effect of chloroform extraction on the growth of E.coli

**3.5 Effect of distil. Water extraction on the growth of E.coli:** Fig.3.5 showed the effect of water extract of convolvulus arvensis on the growth of E.coli by well diffusion method. It is very clear from the figure that water extract of the plant has very good antibacterial activity as compared to control

(contain only the solvent i-e distils. Water) .The distils. water extract have clear bigger zone of inhibition (1.96cm) as compared to control (0.23cm).It is might due to the fact that the convolvulus arvensis has antibacterial chemical which are dissolved in the solvent and showed clear zone of inhibition.

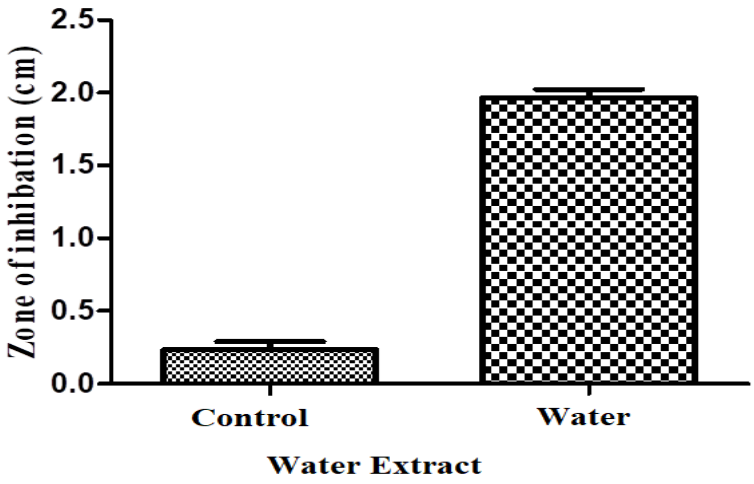


**Fig. 3.5:** Effect of Water extract of convolvulus arvensis on the growth of E. coli.

**3.6 Effect of different extracts of convolvulus arvensis on the growth of E. coli:** Fig 4.6 showed the effect of different extract of convolvulus arvensis on the growth of E.coli. Maximum zone of inhibition is shown by acetone and chloroform extract. Ethanol exhibited 2.1cm, Methanol 2.6cm, while the lowest zone of inhibition is shown by Water extract i.e. 1.96cm.Result indicates that acetone and chloroform have highest antibacterial activity which indicates that parts (roots, stem, leaves) of convolvulus arvensis have antibacterial chemicals which are dissolved in the solvent and the bacterial agent has diffused in agar medium and inhibited the growth of E. coli. The lowest antibacterial activity is shown by water.

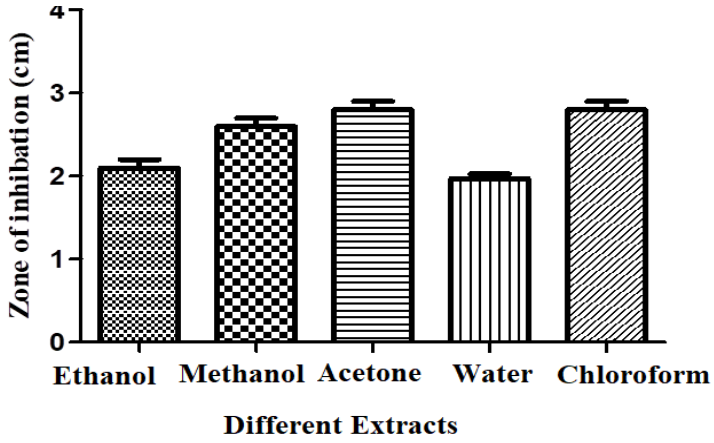


**Fig. 3.6:** Effect of different extracts of *convolvulus arvensis* on the growth of *E. coli*.

**3.7 Phytochemical detection in the whole plant of *Convolvulus arvensis:*** Qualitative analysis of *Convolvulus arvensis* was carried out for the detection of alkaloid, flavonoids, carbohydrate, phlobatannins, glycosides, saponins, phenol, terpenoids and tannins. The results showed that alkaloids,

flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, cardiac glycosides was found in methanolic and ethanolic extracts, while alkaloids, phlobatannins. Flavonoids, carbohydrates, saponins, phenols and terpenoids were found present in in the rhizome methanolic and ethanolic extracts. In these results +++ indicate that the secondary metabolites present in highest amount, the ++ indicated that the moderate level of phytochemicals' are present and the + indicated that low level of phytochemicals are present and – indicated that the phytochemicals are absent in all these three extracts plants (**Table 1,).**

**Table 1:** Qualitative Detection of Bioactive compound in the *Convolvulus arvensis* in methanolic, ethanolic and aqueous extracts

| S.NO | Phytochemical test | Methanolic | Ethanolic | aqueous |
|---|---|---|---|---|
| 1 | Alkaloid | +++ | ++ | _ |
| 2 | Flavonoids | + | +++ | + |
| 3 | Carbohydrate | +++ | + | + |
| 4 | Phlobatannins | +++ | ++ | _ |
| 5 | Glycosides | + | +++ | + |
| 6 | Saponins | + | ++ | + |
| 7 | Phenol | +++ | ++ | + |
| 8 | Terpenoids | ++ | +++ | _ |
| 9 | Tannins | +++ | ++ | + |

**Key: +++: present highest level, ++ showed moderate level, + showed low level - absent**

## DISCUSSION

In the present research work the *convolvulus arvensis* was investigated for antibacterial activity and phytochemicals analysis. The results showed that the effect of different extract of *convolvulus arvensis* on the growth of *E.coli*. Maximum zone of inhibition is shown by acetone and chloroform extract. Ethanol exhibited 2.1cm, Methanol 2.6 cm, while the lowest zone of inhibition is shown by Water extract i.e. 1.96cm. Result indicates that acetone and chloroform have highest antibacterial activity which indicates that parts of *convolvulus arvensis* have antibacterial chemicals which are dissolved in the solvent and the bacterial agent has diffused in agar medium and inhibited the growth of *E. coli*. The lowest antibacterial activity is shown by water. The results of phytochemical analysis showed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, cardiac glycosides was found in methanolic and ethanolic extracts, while alkaloids, phlobatannins. Flavonoids, carbohydrates, saponins, phenols and terpenoids were found present in in the methanolic and ethanolic extracts. Diseases caused by bacteria are still a main threat to public health and their effect is particularly large due to comparative unavailability of medicine in developing countries[29]. Resistance to antibiotics is one of the major problems that face public health[29]. This problem is a natural importance of the adaption of infectious pathogens to antimicrobials used in several areas, including medicine, food animals, crop production and disinfectants in farms, hospital and households[30]. Bacteria show resistance to all known antibiotics. In order to find new antimicrobial agents plants have been explored as sources for the detection of new and effective antimicrobials. Plants are an important source of antimicrobial products, most of them with effectiveness against diverse organisms including fungi, yeasts and

bacteria, insects, nematodes and other plants[31]. The antimicrobial property is claimed to be conferred by phytochemicals present in the plant. To prevent the development of microorganisms, tannins have been reported by precipitating microbial protein and making nutritional proteins unavailable for them[32]. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins[33]. Wide variety of pharmacological activities showed by different phytochemicals, which may help in protection against chronic diseases. Tannins, flavonoids, saponins, glycosides, and amino acids have anti-inflammatory and hypoglycemic activities. Steroids and terpenoids shows central nervous system (CNS) activities and analgesic properties. Because of their antimicrobial activity saponins are involved in plant defense system[34]. These phytochemicals showed antimicrobial activity through different mechanisms. With proline-rich protein tannins have been found to form irreversible complexes [35] resulting in the inhibition of cell protein synthesis[36] Reported that tannins are known to react with proteins to deliver the typical tanning effect which is essential for the treatment of ulcerated or inflamed tissues. Herbs that have tannins as their key components are astringent in nature and are used for treating intestinal disorders such as dysentery and diarrhea[37]. Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers[38]. These observations therefore support the use of Convolvulus arvensis in herbal cure remedies, thus suggesting that Convolvulus arvensis has potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of tannins in *Convolvulus arvensis* supports the traditional medicinal use of this plant in the treatment of different ailments. Alkaloid was another phytochemicals constituent's observed in the extract of *Convolvulus arvensis.* One of the best common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the reduction and elimination of human cancer cell lines[39].

## CONCLUSION

The result of present study showed that convolvulus arvensis a medicinal plant demonstrated highly inhibition to a pathogenic bacteria i-e E.coli. The E.coli and other pathogenic bacteria can lead to serious infection and also causing death and diseases in humans. The potential activity of the plant may be due to the presence of phytochemical constituents. Some of these compounds possess antibacterial activity. Further studies involving the purification of the chemical constituents of the plant and investigation in the biochemical pathway may results in the development of a potent antibacterial agent with low toxicity and better therapeutic index.

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