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Screening of Crude Phytochemicals and Antimicrobial Activities of Selected Medicinbal Plants of Peshawar Region Khyber Pakhtoon Khawa Pakistan

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Abstract: Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacology. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological action on human body. The selected medicinal plants are used by local community to treat various diseases and also recommended by local medical practitioners.The crude phytochemicals and antimicrobial activities of the selected medicinal plants namely *Acorus calamus, Artemesia annua, Cupressus sempervirens , Chenopodium foliosum, Euphorbia helioscopia, Lipedium sativum, Nerium oleander, Rananculas repens, Tecoma stans and Urtica dioca* were carried out in the study. The trend of phytochemicals during analysis showed very less concentration of alkaloids, flavonoids, saponins, tannins and phenols in all the ten plants while the antimicrobial activity shown by *Euphorbia helioscopia* is comparatively high.

Key words: Antimicrobial activities · Ten medicinal plants · 2 · 2-Diphenyl-1-picryl-hydrazyl radical · Peshawar Region

INTRODUCTION

The world is fertile with medicinal plants. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacology. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological action on human body [1]. Some of the most significant bioactive phytochemicals are alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds and many more [2]. These natural compounds form the foundation of modern prescription drugs as we know today [3]. Phytochemicals are natural compounds occur in medicinal plants, vegetables and fruits, that work with nutrients and fibers to act against diseases or more specifically to protect against diseases. Phytochemicals are mainly divided into two groups, which are primary and secondary constituents according to their activity in plant metabolism. Primary constituents contain common sugars, amino acids, proteins and chlorophyll while secondary constituents comprise of alkaloids, flavonoids, saponins, tannins, phenolic compounds and many more [4].

Infectious diseases are the main cause of death in 8% of the 9 deaths occurring in United States [5]. Nowadays, multiple drug resistance has been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometime associated with adverse effects on the host including hypersensitivity, immuno suppressant and allergic reactions. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [6]. Antimicrobial agents of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [7].

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Therefore there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action for new and re emerging infectious diseases [9]. Keeping in view the importance of medicinal plants the present study was aimed to investigate the phytochemicals and antimicrobial activity of ***Acorus*** *calamus, Artemesia annua, Cupresus sempervirens , Chenopodium foliosum, Euphorbia helioscopia, Lipedium sativum, Nerium oleander, Rananculas repens, Tecoma stans and Urtica dioca*.

MATERIALS AND METHODS

Post Harvest Treatment of Plant Materials: All the ten selected specimens were collected from Mardan, Peshawar, Nowshera and Kohat districts of Khyber Pakhtoon khwa, Pakistan. Botanical identification was done by a botanist at the PCSIR laboratories complex (Peshawar), Ministry of Science and Technology, Government of Pakistan. Voucher specimens have been kept at the PCSIR laboratories complex (Peshawar), for reference purposes. The selected specimens were chopped into small pieces and dried under shad. The dried specimens were then crushed into fine powder. The powdered form of these plants were then stored in air-tight glass containers and protected from sun light till required for analysis.

Phytochemical Analysis

Sample Prepration: The aqueous extract of each sample was prepared by soaking 10 g of powdered samples in 200 mL of distilled water for 12 h. The extract was then filtered using Whattman filter paper. The phytochemicals in each sample was determined qualitatively and quantitatively according to the literature [10-12].

Qualitative Analysis

Alkaloids: The extracts were evaporated to dryness and the residues were heated with 2% HCl solution on a boiling water bath. The extracts were cooled, filtered and then treated with the Mayer's reagent. The sample was then observed for the presence of yellow precipitation or turbidity [10-12].

Flavonoids: 1.5 mL of a 50% aqueous methanol was added to 4 mL of plant extracts. Warmed the solution and metal Mg was added. Then 5-6 drops of concentrated HCl was added to that solution and observed for red coloration [10-12].

Tannins: To 0.5 mL of extract solution, added 1 mL of distilled water and 1-2 drops of ferric chloride solution to it, observed for blue or green black coloration [10-12].

Saponins: 2 mL of distilled water was added to 2 mL of the test solution and shake well till frothing was observed [10-12].

Phenols: Added ethyl alcohol to 2 mL of the test solution and few drops of ferric chloride solution and observed for coloration [10-12].

Quantitative Analysis

Alkaloids: 5 g of each plant sample was prepared in a beaker and 200 mL of 10% CH3COOH in C2H5OH was added to the plant sample. The mixture was covered and allowed to stand for 4 h. The mixture then filtered and the extract was allowed to become concentrated by heating on a water bath until it reaches ¼ of the original volume. Concentrated NH4OH was added until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue obtained was alkaloids, which was then dried and weighed [10-12].

Flavonoids: Extracted 10 g of the plant sample with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through a filter paper and the filtrate was dryed by evaporation using a water bath. The solution was then evaporated to dryness and weighed until a constant weight [10-12].

Tannins: 500 mg of powdered plant sample was weighed and transferred to 50 mL flask. Then added 50mL of distilled water and stirred for 1h. Filtered the sample into a 50 mL volumetric flask and made up volume to the mark with same distilled water. Pipette out 5 mL of the filtered sample into test tube and then mixed with 2 mL of 0.1M ferric chloride. The absorbance was measured using spectrophotometer at 395 nm within 10 min [10-12].

Saponins: 20 g of each grounded plant samples were put into a conical flask and 100 mL of 20% aqueous ethanol was added to the plant samples. The said samples were heated on a water bath for 4h at about 55°C with continuous stirring. The extracted mixture was then filtered and the residue was then re-extracted again with 200 mL of 20% aqueous ethanol. The collective residue was reduced to 40 mL over a hot water bath.

The concentrated residue was then transferred to a separating funnel and 20 mL of diethyl ether was added shaked well. The aqueous layer was recovered while the organic layer was discarded and the process of purification was repeated. 60 mL of n- butanol was added and combined n- butanol extract were washed twice with 10 mL of 5% NaCl solution. The remaining solution was then heated on a water bath and after evaporation; the samples were dried in oven to a constant weight [10-12].

Phenols: Boiled the plants sample for 15 min with 50 mL of $(CH_3CH_2)_2O$. Pipette 5 mL of the sample into 50 mL flask and 10 mL of distilled water was added. Then 2 mL of NH_4OH solution and 5 mL of concentrated $CH_3(CH_2)_3CH_2OH$ to the mixture was added. The sample was made up to the mark and left to react for 30 min for color development. The absorbance of the resultant colored product was measured at 505 nm using a spectrophotometer. From the calibration plot, the amounts of phenols were determined. [10-12].

Antimicrobial Activity

Preparation of Crude Extract: 100 g of each of the coarsely powdered plant material were taken and extracted separately with ethanol, water and n-hexane. The extracts were filtered and then few crystals of NaCl were added to the filtered extract to form precipitates. The precipitates were then separated through filter paper; air dried and transferred to air tight amber glass container. The crude extract was dissolved in chloroform and water to make the final concentration, which was kept in refrigerator till used [13].

Preparation of Standard Bacterial Suspension: The average number of viable, *Bacillus subtilis* (NCTC8236), *Escherichia coli* (ATCC25922), *Proteus vulgaris* (ATCC6380), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhi* (ATCC0650) and *Staphylococcus aureus* (NCTC25953) organism per mL of the stock suspension was determined by means of the surface viable counting technique. About 10^8-10^9 colony forming units (CFU) per mL were used. A fresh stock suspension was prepared each time [14,15].

Antibacterial Activity: The antimicrobial activity of the prepared extracts was determined by using well agar diffusion method. The standard bacterial stock suspension 10^8-10^9 CFU/ mL was mixed with 60 mL of sterile nutrient agar thoroughly. 20 mL inoculated nutrient agar was poured into sterile petri dishes. Left the agar to set and four well (10mm in diameter) were made in each of these plates using sterile cork borer No. 8 and then agar discs were removed. The entire well were filled with 0.1 mL of each extracts using microtiter-pipette and allowed to diffuse at room temperature for 2 h. The plates were incubated at 37°C for 24 h. Two replicates were also performed for each extract against each of the test organism. Simultaneously, addition of the respective solvent instead of extract was carried out as controls. After incubation, the zones of inhibition was measured (in mm) and mean value was calculated [14, 15].

Preparation of Standard Fungal Suspension: The fungal cultures, *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC7596) were maintained on saboraud dextrose agar, incubated at 25ºC for four days. The fungal growth was harvested and washed with sterile normal saline and the suspension was stored in refrigerator till used [14, 15].

Anti Fungal Activity: The antifungal activities of the prepared extracts were determined by using well agar diffusion method. The 0.6 mL standard fungal stock suspension 10^8-10^9 CFU/ mL was mixed with 60 mL of sterile yeast and mould extract agar thoroughly. 20 mL inoculated yeast and mould extract agar was poured into sterile petri dishes. Left the agar to set and four wells (10 mm in diameter) was made in each of these plates using sterile cork borer No 8. and then agar discs were removed. The entire well were filled with 0.1 mL of each extracts using microtiter-pipette and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 25°C for 04 days for *C. albican* and three days for *A. niger*. Three replicates were also performed for each extract against each of the test organism. Simultaneously, addition of the respective solvent instead of extract was carried out as controls. After incubation, the zones of inhibition (in mm) were measured and mean value was calculated [14, 15].

RESULT AND DISCUSSION

Phytochemicals and antimicrobial activity were determined in selected medicinal plants namely *A. calamus, A. annua, C. foliosum, C. sempervirens, E. helioscopia, L. sativum, N. oleander, Rananculas repens, T. stans and U. dioca.* The antimicrobial activity and phytochemicals were determined quantitatively using the available literature methods.

Table 1: Qualitative Analysis of crude Phytochemicals of Selected Medicinal Plants.

S.No	Plant Name	Alkaloids	Flavonoids	Tannins	Saponins	Phenols
1.	*A. calamus*	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
2.	*A. annua*	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
3.	*C. foliosum*	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
4.	*C. sempervirens*	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
5.	*E. helioscopia*	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
6.	*L. sativum*	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
7.	*N. oleander*	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
8.	*R. repens*	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
9.	*T. stans*	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
10.	*U. dioca*	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve

Table 2: Quantitative Analysis of crude Phytochemicals of extracts of Selected Medicinal Plants (%)

S.No.	Sample Code	Alkaloids	Flavonoids	Tannins	Saponins	Phenols
1.	*A. calamus*	0.3	0.71	0.20	2.3	0.06
2	*A. annua*	1.5	0.99	0.26	1.7	0.04
3.	*C. foliosum*	1.8	0.52	0.28	1.3	0.07
4.	*C. sempervirens*	1.4	0.22	0.31	1.9	0.07
5.	*E. helioscopia*	2.7	0.53	0.02	1.4	0.05
6.	*L. sativum*	0.4	0.42	0.61	2.01	0.045
7.	*N. oleander*	2.9	0.91	0.01	1.20	0.039
8.	*R. repens*	0.8	0.39	0.01	0.8	0.056
9.	*T. stans*	2.6	0.53	0.19	2.1	0.048
10.	*U. dioca*	0.5	0.60	0.21	0.7	0.037

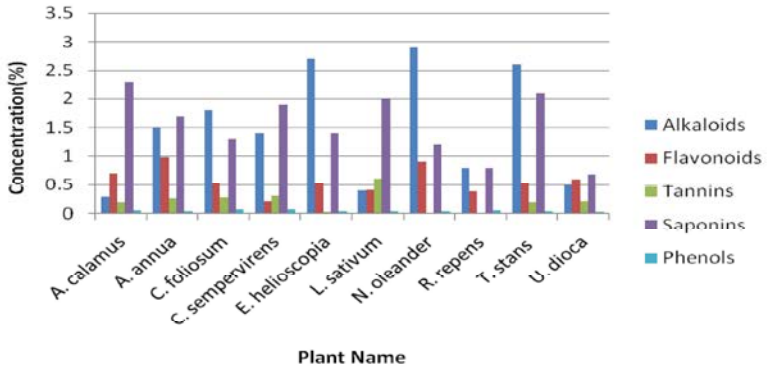

Fig. 1: Graphically Representation of Quantitative Analysis of Phytochemicals of Selected Medicinal Plants.

Phytochemicals

Qualitative Analysis: The Qualitative Phytochemicals analysis revealed the presence of all the mentioned phytochemicals i.e. Alkaloids, flavonoids, tannins, saponins and phenols. On the basis of this the quantitative analysis was carried out.

Quantitative Analysis: The analytical results are summarized in Table-2 while the graphical representation is shown in Fig No 1.

Alkaloid: The term alkaloids have been defined as a cyclic organic compound having nitrogen in a negative oxidation state, which has limited distribution in living organism [16]. Based on their structure, alkaloids are divided into several subgroups: Non heterocyclic alkaloids and heterocyclic alkaloids which are again divided into 12 major groups according to their basic ring structure. The use of alkaloids containing plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization [17]. They are well known for their CNS activities [18] Yamamoto *et al*, 1980]. High concentration 2.9% of alkaloids was found in *N. oleander* followed by 2.7% in *E. helioscopia* and 2.6% in *T. stans.* The concentration of Alkaloids was found low 0.4% in *L. sativum*. The concentrations of alkaloids in the rest of samples are as follows1.5% in *A. annua*, 1.4% in *C. sempervirens*, 0.8% in *U. dioca* and 1.8% in *C. foliosum.*

Flavonoids: Flavonoids are a group of polyphenolic compounds possessing low molecular weight that exhibit a common benzo-pyrone structure. They are categorized into various subclasses including flavones, flavonols, flavonones, isoflavonones, isoflavonoids, anthocyanidins and catechins [19, 20] Cushnie *et al*, 2005 and Yaqin *et al*, 2005]. They show anti-allergic, anti-inflammatory [21], antimicrobial [22] and anticancer activities. Flavonoids also referred to as bioflavonoids, are polyphenol antioxidants found naturally in plants. They are secondary metabolites, meaning they are organic compounds that have no direct involvement with the growth or development of plants. The effect of flavonoids on plants growth, which is known, is at least partly indirect and associated with the action of auxins. It was reported that flavonoids can improve the blood circulation and lower the blood pressure [23]. High amount of flavonoids 0.99% was detected in *A. annua* while 0.91% was determined in *N. oleander* followed by 0.71% in *A. calamus,* 0.60% in *U. dioca,* 0.53% in *E. heloiscopia* and *T. stans,* 0.42% in *L. Sativum* while the amount of flavonoids was found low in *R. repens* and *C. sempervirens* which is 0.39% and 0.22% respectively.

Saponins: The saponins are naturally occurring surface-active glycosides. Many pharmacological activities have been reported about saponins such as antibiotic antifungal, antiviral, hepatoprotective, anti-inflammatory and anti-ulcer [24, 25]. Saponins have been reputed to have important biological activities in humans including hypocholesterolaemic, haemolytic, immunostimulatory and anti-tumourigenic activities [26] as well as chemoprotective activities [27]. Steriod and triterpenoid saponins with a single sugar chain were found to have strong haemolytic activity, whereas those with two sugar chains showed less activity [28, 29]. Some saponins and sapogenins have been shown to be capable of deactivating viruses for example purified saponin mixture from maesa lanceolata [30]. The percentage of saponins 2.3% was found very high in *A. calamus* followed by 2.1% in *T. stans* and 1.2% in *N. oleander*. *A. annua* contains 1.7% of saponins while 1.4% was noted in *E. helioscopia*. While the concentration of saponins in rest of samples were noted low that is 0.8%, 0.7%, 2.01%, 1.9% and 1.3% were observed in *R. repens*, *U. dioca*, *L. sativum*, *C. sempervirens* and *C. foliosum* respectively.

Tannins: Tannins are basically use for the treatment of inflammation, leucorrhoea, gonorrhea, burn, piles, diarrhea and as antidote in the treatment of alcaloidal poisoning [31]. They are also used for tanning of animal hides to convert them to leather. High concentration 0.61% was detected in *L. sativum* followed by 0.31% in *C. sempervirens,* 0.28% in *C. foliosum*, 0.26% in *A. annua,* 0.21% in *U. dioca*, 0.20% in *A. calamus*, 0.19% in *T. stans.* The concentration of tannins was recoded low 0.01% in both *N. oleander and R. repens*.

Phenols: Phenols are very wide spread in nature. They range from simple structures having a simple aromatic ring to highly complex polymeric structures and often exist in glycosidic forms [7]. Capsaicin is found in the dried ripe fruit of different species of Capsicum. It has been used internally for dyspepsia and flatulence. Externally it is frequently used as counterirritant [8]. The Table-6 shows a very less concentration of phenols in the whole plant samples. The concentration of phenols was recorded high 0.07% in *C. sempervirens*, while nearly equal amount of phenols was noted 0.048% and 0.06% in *T. stans and A. calamus*, also equal but low concentration of phenols were observed 0.0.04% in *A. annua*, 0.07% in *C. foliosum, 0.045%* in *L. sativum* and 0.037% in *U. dioca* while in case of *N. oleander* and *R. repens* the amount was also noted very low which was 0.039% and 0.056%.

Antimicrobail Activities: Sustainable amount of new antibiotic available in the market are obtained from natural or semi synthetic resources [32]. Which are obtained from about 20% of the plants present in world which were submitted to pharmaceutical or biological tests. The chemical compounds with antimicrobial activities isolated from plants have vast remedial power and are useful in the cure of infectious diseases. Reports have been exists on the use of several plants by products which posses antimicrobial properties on same pathogenic bacteria and fungi.

Antibacterial Activity

Water Extract: The Zones of inhibitions of water extracts in millimeters of Bacterial strains are summarized in Table-3 while the graphical representation is shown in Fig. No 2.

As can be seen from Table-3, high activity 21 mm was recorded in *E. helioscopia* against *Bascillus subtillis* while less activity 9 mm was seen against *Bascillus subtillis* in *A. annua*. The Table-3 also showed high activity of *E. helioscopia* against *Proteus vulgaris* which is 16 mm while 14 mm was recorded against the same bacteria in *C. sempervirens* of water extract. *A.annua* and *L. sativum* have the same activity 13 mm against

Table 3: Zones of inhibitions of water extracts of selected medicinal Plants in millimeters of Bacterial strains

		Zones of inhibitions in millimeter					
S.No	Medicinal Plants	*B. subtillis*	*P. vulgaris*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
1.	*A. calamus*	13	10	8	5	2	2
2.	*A. annua*	9	13	19	4	3	1
3.	*C. foloisom*	12	7	14	6	3	4
4.	*C. sempervirens*	9	14	11	4	6	3
5.	*E. helioscopia,*	21	16	19	13	10	9
6.	*L. sativum*	11	13	8	2	4	1
7.	*N. oleander*	14	9	12	6	3	2
8.	*R. repens*	12	10	7	5	2	4
9.	*T. stans*	10	8	12	4	2	6
10.	*U. dioca.*	15	11	13	6	2	0
11.	Standard	26	25	25	24	23	24

Gram positive bacteria: *Bs-Bacillus subtillis Pv-Proteus vulgaris Sa-Staphylococcus aureus,*

Gram Negative Bacteria: *Ec-Escherichia coli Pa- Pseudomonas aeruginosa St-Salmonella typhi*

Standard: Ofloxacin.

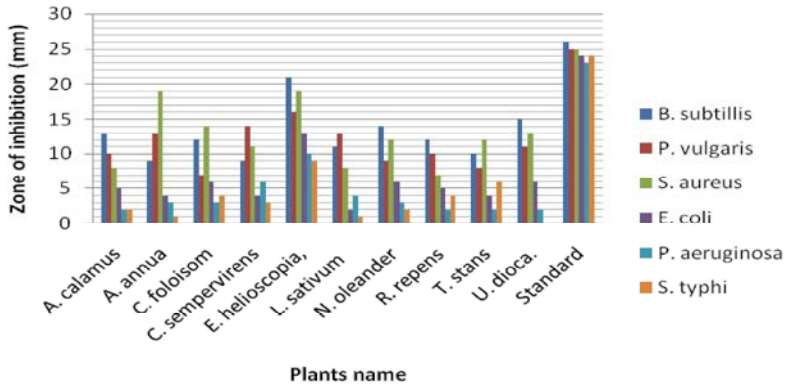

Fig. 2: Graphical Representation of Zones of inhibitions of water extracts of selected medicinal Plants in millimeters of Bacterial strains.

the *Proteus valgaris*. The activity of the other water extract were less different from each other i.e. 11 mm in *U. dioca* 10 mm in both *R. repens* and *A. calamus* while 9 mm, 8 mm and 7 mm were found in *N. oleander, Tecoma stans* and *C. foloisom. A. annua* and *E. helioscopia* showed same activity 19 mm against *Staphylococcus aureus* while less activity 7 mm recorded in *R. repens*. High activity 13 mm was observed in *E. heliscopia* against *Escherichia coli* followed by 6 mm, 6 mm, 6 mm, 5mm, 4mm, 4mm, 4mm and 2mm in *C. foloisom, N. oleander, U. dioca, A. calamus, A. annua, C. sempervirens, T. stans and L. sativum*. High activity 10mm was shown by *E.helioscopia* against *pseudomonas aeruginosa* while 6mm activity recorded in *C. sempervirens* against same bacteria. The activities showed by other plants were less than 5mm. *E.helioscopia* was found more active 9mm against *Salmonella typhi* while 6mm activity observed in *T. stans* and same 4mm activity in both *C. foloisom* and *R. repens*.

Chloroform Extract: The Zones of inhibitions of Chloroform extracts in millimeters of Bacterial strains are summarized in Table-4 while the graphical representation is shown in Fig. 3.

High activity 19mm was determined in *E. helioscopia* of chloroform extract against *Bacillus subtillis* while the activity shown by the rest of the plant extracts were in between 9mm- 17mm. The activity 16mm was recorded in *L. sativum* against *Proteus vulgaris* and 14mm, 13mm, 12mm, 11mm, 10mm, 10mm, 9mm, 7mm and 7mm activities were found in *E.heliscopia, C. foloisium, N. oleander* and *T. stans*, respectively. *E. helioscopia* was also more active 21mm against *staphylococcus aureus* while less activity was found 2mm in *L. sativum*. The activity 7mm was recorded high in *T. stans* against *Escherichia coli* and 6mm in *A. calamus*. 5mm activity was recorded in most of the plants while 3mm and 2mm activity were also observed. *E. helioscopia* was also more

Table 4: Zones of inhibitions of Chloroform extracts of selected medicinal Plants in millimeters of Bacterial strains.

		Zones of inhibitions in millimeter					
S.No	Medicinal Plants	*B. subtillis*	*P. vulgaris*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*
1.	*A. calamus*	8	12	9	6	2	4
2.	*A. annua*	14	9	11	4	3	5
3.	*C. foloisom*	10	13	8	5	2	4
4.	*C. sempervirens*	12	10	9	5	3	1
5.	*E. helioscopia,*	19	14	21	3	5	6
6.	*L. sativum*	9	16	11	2	4	7
7.	*N. oleander*	11	7	12	5	4	2
8.	*R. repens*	13	10	15	3	0	5
9.	*T. stans*	15	7	14	7	3	0
10.	*U. dioca.*	17	11	9	5	3	1
11.	Standard	26	25	25	24	23	24

Gram Positive Bacteria: *Bs-Bacillus subtillis Pv-Proteus vulgaris Sa-Staphylococcus aureus,*
Gram Negative Bacteria: *Ec-Escherichia coli Pa- Pseudomonas aeruginosa St-Salmonella typhi*.
Standard: Ofloxacin.

Table 5: Zones of inhibitions of water and Chloroform extracts of selected medicinal Plants in millimeters of fungal strains

		Zones of inhibitions in millimeter			
		Water Extract		Chloroform Extract	
S.No	Medicinal Plants	*A. niger*	*C. albicans*	*A. niger*	*C. albicans*
1.	*A. calamus*	2	2	0	0
2.	*A. annua*	2	0	2	2
3.	*C. foloisom*	1	2	1	2
4.	*C. sempervirens*	1	0	0	3
5.	*E. helioscopia,*	3	4	3	2
6.	*L. sativum*	3	2	2	1
7.	*N. oleander*	0	1	2	2
8.	*R. repens*	2	1	1	0
9.	*T. stans*	2	2	2	4
10.	*U. dioca.*	3	1	2	1
11.	Standard	8	12	---	---

Fungi: *An-Aspergillus niger, Ca-Candida albicans.*
Standard: Grasioflavin

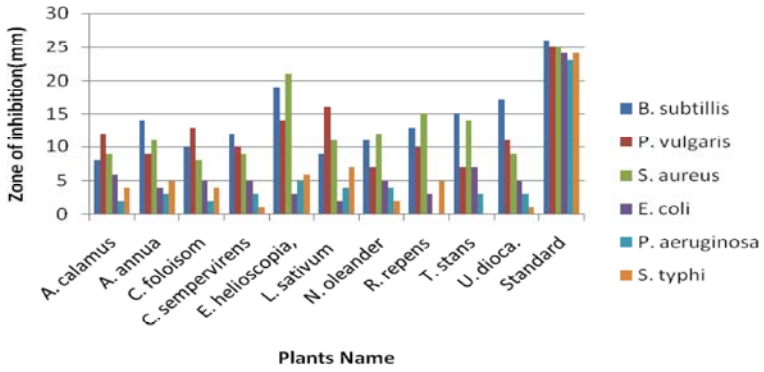

Fig. 3: Graphical Representation of Zones of inhibitions of Chloroform extracts of selected medicinal Plants in millimeters of Bacterial strains.

active 5mm against *Pseudomonas aeruginosa* and *R.repens* showed no activity. High activity 7mm was shown by *L. sativum* against *Salmonella typhi*, 6mm by *E. Helioscopia*, 5mm by *A. annua* and *R. repens*, 4mm by *A. calamus* and *C.foloisom*, 2mm by *N. oleonder*, 1mm by both *C. semperverins* and *U. dioca* and no activity by *T. stans* against *Salmonella typhi*.

The activity shown by the standard (Ofloxacin) against Gram positive and Gram negative bacteria nearly 24.5 mm zone of inhibition which is very close result as compared to *E. helioscopia* which can be used as a source of antimicrobial agent but still need further research and clinical trials.

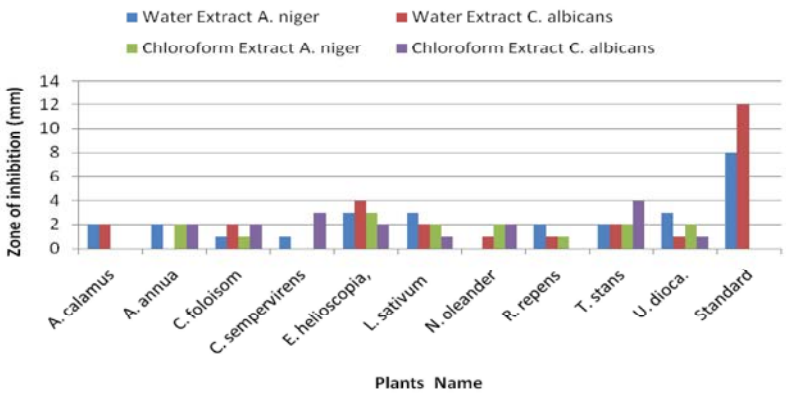

Fig. 4: Graphical Representation of Zones of inhibitions of water and Chloroform extracts of selected medicinal Plants in millimeters of Fungal strains.

Antifungal Activity: The Zones of inhibitions of Water and Chloroform extracts in millimeters of fungal strains are summarized in Table 5 while the graphical representation is shown in Fig. 4.

The activity of water extract against fungi can be seen from Table 5 which shows less activity 2mm against *A. calamus* and *N. oleander* while 1mm was recorded in *A. annua, L. sativum. E. helioscopia, L. sativum* and *U. dioca* showed high activity 3 mm against *Aspergillus Niger* while 2 mm activity were found in *A. calamus, A. annua, R. repens* and *T. stans* while *N. oleander* shows no activity against *Aspergillus niger*. High activity 4mm of *E. helioscopia* was noted against *Candida albicans* while same activity 2mm, 1mm and no activity was recorded in rest of the plant extracts. While in chloroform extract antifungal activity shown by *E. helioscopia* was determined high 3mm against *Aspergillus niger* while the activity of other plant extracts were in range from 0-2mm. *T. stans* was noted more active 4mm against *Candida albicans* fallowed by 3mm, 2mm and 1mm in rest of the plants while no activity was shown by *R. repens*.

In case of fungi the Grasioflavin was used as a standard and by comparing with the results of both extracts against all plants shows very less activity and can not be used as a source of antifungal agent; however these extracts required to be tested against some other strains of fungi to achieve the final conclusion.

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