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Evaluation of thrombolytic, antimicrobial and cytotoxicity potential of extract and its polar fractions of *Heliotropium strigosum*by *in-vitro* assay

Abdur Rehman[1], Sami Ullah[1], Raja Adil Sarfraz[2], Faisal Nawaz[3], Abdul Qayyum[*2], Muhammad Javid[4], Syed Muhammad Imran Bin Riaz[2], Khalil Ullah Khan[1]

[1]*Department of Chemistry, University of Lahore, Sargodha, Pakistan*

[2]*Department of Chemistry, University of Agriculture, Faisalabad, Pakistan*

[3]*Department of Basic Sciences, University of Engineering and Technology, Faisalabad, Pakistan*

[4]*Department of Botany, University of Lahore, Sargodha, Pakistan*

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Abstract

Medicinal plants are the major sources of natural product and are known as natural source of derived compounds which are being used in the safety of human life. In the early age of human life, the plants are used for the nutritional purposes but as the research is focus on the investigation of their medicinal potential then these are become a useful source of remedies for various diseases and health improvements. The present work was focus on evaluation of antimicrobial, cytotoxicity and thrombolytic potential of methanolic extract and its polarities based fractions of *Heliotropium strigosum*. The extraction was performed by methanolic solvent and fractionation by using chloroform, ethyl acetate and aqueous solvent sequentially on the basis of their polarities. Antibacterial activity and antifungal activity was performed by disk inhibition assay which showed significant ($p<0.05$) potential 36mm and 32mm was shown by against *Staphylococcus aurous* and *Escherichia coli* respectively. Highest antifungal activity was shown by 28mm by aqueous fractions whereas least 12mm by ethyl acetate fraction against the *Aspergilus nigerfungus* species. Cytotoxicity activity also showed significant results by hemolytic assay. Thrombolytic activity showed highest activity (34.54%) by methanolic crude extract while ethyl acetate fraction showed lowest (23.16%) activity. This study showed that the crude extract and polar fractions of crude methanolic extract of *H. strigosum* can be considered the potential sources of antimicrobial and thrombolytic agent not only in the food industry but also in cosmetics and pharmaceutical preparations.

* **Corresponding Author:** Abdul Qayyum ✉ abdulqayyum1106@gmail.com

Introduction

Thrombosis is a very complicated physiological system which interacts with clot components and surrounding plasma (Alkjaersig *et al*., 1959). It can develop in blood circulatory system due to homeostasis which causes vascular blockage that can be responsible to health threatening results (Collen *et al*., 1990). There are many factors such as aggregation, platelet adhesion and blood vessel injury which are the basic facilitators for the initial stage of formation of arterial thrombus that are followed through the blockage of blood that lead to cause the vein thrombus (Shao *et al*., 2013). The thrombolytic agents are anistreplase, streptokinase, alteplase, TPA (tissue plasminogen activator) and urokinase. All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and bleeding tendency (Gesler *et al*., 1992).

The plethora of microorganisms are also considering the major cause for the severe illness in humans and the biological damaging of useful materials. These microorganisms are getting potential to develop the resistance to synthetic antimicrobial agents for several diseases (Afzal *et al*., 2014). Many efforts have been made to discover new antimicrobial substances from different sources which have found to be useful in various traditional and folk medicines (Ivan *et al*.,2011). The medicinal plants which possess active potential against the growth of microorganisms and have least probability to get resistance against the plants extract (Shao *et al*., 2013).

The application of medicinal plants to remedy human diseases is being increase now a day due to the rich source of medicinally compounds (Qayyum *et al*., 2016). In folk and traditional medicinal system, the medicinal plants and their derivative products are used as medicine as these containing fewer side effects (Riaz *et al*., 2012). New scientific methodologies carried a revaluation in herbal medicine industry and all focus is concentrate on active principles (Javid *et al*., 2016). Plant extracts and their components have found consist of various biological activities such as antibacterial and antioxidant activities (Rasool *et al*., 2013), antifungal (Hamil *et al*., 2003), antimicrobial (Xue-Ting*et al*., 2009). The activities are due to presence of active phytochemical in the plants extract. Those phytochemical which have potential to inhibit the microorganisms with low toxicity level are considered more potentials to develop new toxic drugs (Barbour *et al*., 2004).

Generally the medicinal plants possess the phenolic compounds, flavonoids, tannins and alkaloids (Arslan*et al*., 2011). The usages of medicinal plants to treat the various diseases require the information on the activity of that plants and its toxicity which are growing interest in herbal medicine (Atawodi, 2005). This type of treatment is more preferred due to their economic feasibility, low toxicity, potent pharmacological activity and evaluation of medicinal potential of that plant (Rai *et al*., 2008).The present study was focus on the investigation of methanolic extract and its polar fractions regarding thrombolytic, antibacterial, antifungal and cytotoxicity.

Materials and methods

Collection of plant materials

The sample of *H. strigosum* was purchased from the market of Faisalabad, Pakistan. Whole dried plant sample was washed with distilled water for many times for the removal of dust particles and other impurities then grinding to form fine powder and stored in opaque screw-capped containers at room temperature.

Preparation of plant extracts

The plant sample in fine powder form (3 kg) of *H. strigosum* was macerated in methanol solvent for 15 days. After the maceration, the soluble fractions in methanol were filtered and the filtrate was concentrated at 40°C by using rotatory evaporator to give crude extract (yield=150 gm). The 50 gm crude extract was then dissolved in 150 mL distilled water and sequentially portioned with 150 mL of chloroform and 150 mL of ethyl acetate. The, chloroform, ethyl acetate and aqueous solutions yielded 1.9, 1, 0.5 and 8 gm of fractions respectively. All obtained methanolic crude extract and fractions were stored in refrigerator at 4°C (Qayyum *et al*., 2016).

Evaluation of thrombolytic activity of crude extract and its polar fractions of H. strigosum

Venous blood was drawn from healthy human volunteers without a history of oral contraceptive or anticoagulant therapy. The500 µl of blood was transformed to each of the previously weighed micro centrifuged tubes to form clots. Extract (100µL) and its fractions were mixed in 1 mL DMSO. This suspension was used as stock from which appropriate dilutions were made to observe the thrombolytic activity using the *in vitro* model developed in our laboratory.

This assay was carried out by taking venous blood drawn from healthy volunteers and was transferred in different pre weighed sterile micro centrifuge tube (500 µL/tube). Incubated this mixture at 37 °C for 45 minutes. After clot formation, serum was completely removed and each tube having clot was again weighed to determine the clot weight.

Clot weight= weight of clot containing tube – weight of tube alone.

Each micro centrifuge tube containing clot was properly labeled and 100 µL of crude extract and its polar fractions was added to the tubes. Water was also added to one of tube containing clot as it serves as a negative thrombolytic control. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis.

Determination of cytotoxic activity of crude extract and its polar fractions of H. strigosum

Stock solutions were prepared by dissolving extract and its polar fraction in DMSO solvent. The cytotoxicity studies of the plant extract and its polar fractions were analyzed by Hemolytic activity by Zuber *et al.* (2014). Three mL freshly obtained heparinized human blood was collected from volunteers after consent and counseling and bovine from the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad. Blood was centrifuged for 5 min at 1000x*g* plasma was discarded and cells were washed with three times with 5 mL of chilled (4°C) sterile isotonic Phosphate-buffered saline (PBS) pH 7.4. Erythrocytes were maintained 10^8 cells per mL for each assay. Hundred µL of each compound was mixed with human (10^8cells/mL) separately. Samples were incubated for 35 min at 37°C and agitated after 10 min. Immediately after incubation the samples were placed on ice for 5 min then centrifuged for 5 min at 1000x*g*. Supernatant 100 µL were taken from each tube and diluted 10 time with chilled (4°C) PBS. Triton X-100 (0.1% v/v) was taken as positive control and phosphate buffer saline (PBS) was taken as negative control and pass through the same process. The absorbance was noted at 576 nm using µ Quant (Bioteck, USA). The % RBCs lysis for each sample was calculated.

Antibacterial activity of crude extract and its polar fractions of H. strigosum by disc diffusion method

The strains *E. coli* and *S. aurous* were characterized from the Institute of Veterinary Microbiology, University of Agriculture Faisalabad.

These pathogenic strains were used to determine the antimicrobial activity of the crude extract and its polar fractions of selected medicinal plant. Pure cultures were maintained on nutrient agar medium in the petri plates. For the inoculums preparation 13g/L of nutrient broth (Oxoid, UK) was suspended in distilled water, mixed well and distributed homogenously. The medium was autoclaved at 121˚C for 15 min. Loop full of pure culture of a bacterial strain was mixed in the medium and placed in shaker for 24 hours at 37˚C. The inoculums were stored at 4˚C. The inoculums with 1×10^8 CFU/mL were used for further analysis.

Nutrient agar (Oxoid) 28 g/L was suspended in distilled water, mixed well and distributed homogeneously. The medium was sterilized by autoclaving at 121˚C for 15 min. Before the medium was transferred to sterilized Petri plates; inoculation (100 µL/100 mL) was added to the medium and poured in sterilized petriplates.

After this, small filter paper discs were laid flat on growth medium containing 100 µL of crude methanol extract and its polar fractions of crude methanol extract of selected plant.

The petriplates were then incubated at 37˚C for 24 hours, for the growth of bacteria. Crude methanol extract and its polarity based fractions of *H. strigosum* having antibacterial activity inhibited the bacterial growth and clear zones were formed. The zones of inhibition were measured in millimeters using zone reader (Afzal *et al*., 2014).

Investigation of Antifungal activity *by disc diffusion method*

The antifungal activity was performed according to Devi *et al*., 2014 with slight modification. Antifungal activity of the crude extracts and polar fractions of *H. strigosum* was determined by using the agar well diffusion assay method.

Stock cultures of *Aspergilus niger* were prepared and maintained in Sabouraud Dextrose Agar (SDA) slants at 4ºC. A positive control drug (Fluconazole) was also done parallel. The plates were examined for evidence of zone of inhibition, which appear as area around the wells. The diameter of such zones of inhibition was measured using a meter ruler.

Statistical analysis

The obtained results were statistically analyzed by one way analysis of variance by using statistica 8.1 software. The level of significant was set as $P<0.05$.

Results and discussion

This research work was conducted to investigate the antimicrobial, cytotoxicity and thrombolytic activity of methanolic extract and various fractionation of whole plant of *H. strigosum*. The fractionation was done in different solvents such as chloroform, ethyl acetate and water basis on their polarities.

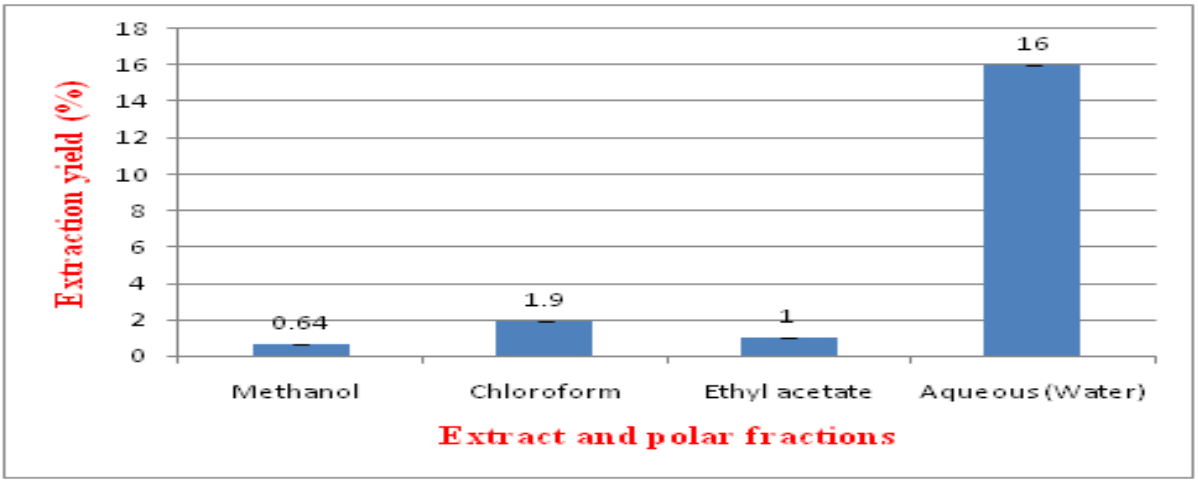

Fig. 1. Percentage yield (g/100g) of different extraction solvents for *H. strigosum*.

Yield of extracts

The methanol solvent was used for the extraction of the phenolic compounds. Then different solvents were used for fractionation depending upon their polarity. *H. strigosum* extract gave yield in g/100g at different fractionations solvents according to their polarityhad been shown in figure1. The methanol gave yield 0.64g/100g. While fractionation gave yields 1.9 g/100g, 1 g/100g and 16 g/100g by chloroform, ethyl acetate and aqueous solvents. The variation of obtained extraction yield is due to different parameters affect the yield of extract such as solvent used, method of extraction, amount and parts of the plant material and the nature of the plant material (Kumar *et al*., 2011).

Thrombolytic activity of H. strigosum

Generally thrombolytic substances are work through activating the plasminogen enzyme, that disturb the fibrin mesh that are cross-linked which lead to the improvement of the solubility of clot and restore of blood flow over occluded blood vessels.

So thrombolytic substances are more beneficial for the remedies of deep vein thrombosis, thromboembolic strokes, myocardial infarctions, damages of artery blockage and lead to the save permanently damage of perfused tissue (Gulcin *et al*., 2013). The present work includes *in-vitro* suspension of clots by *H. strigosum* crude methanolic extract and its polar fractions.

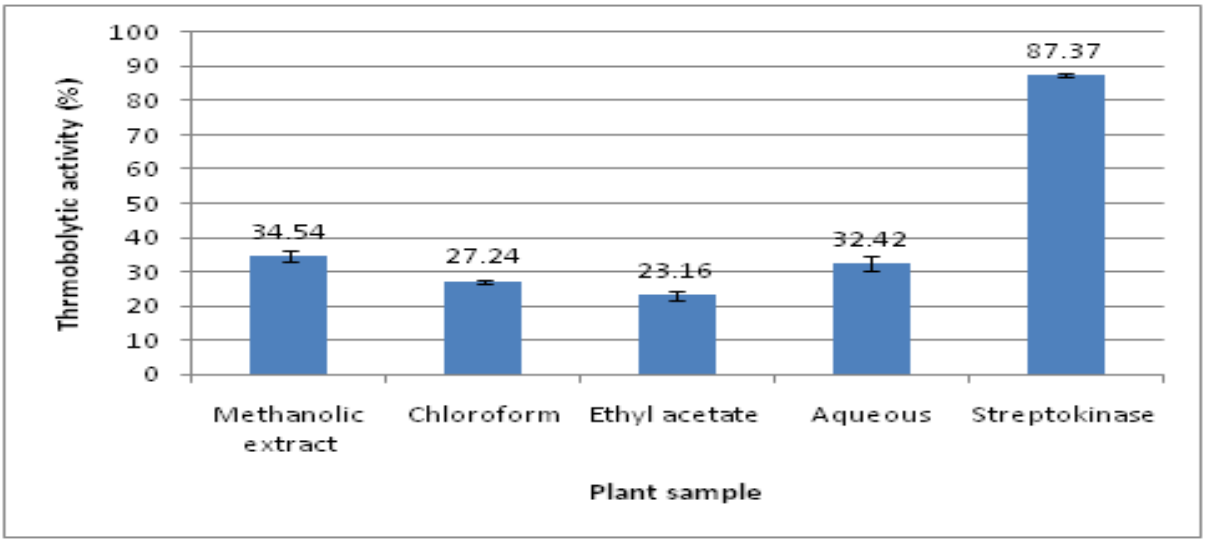

Fig. 2. Thrombolytic activity extract and its polar fractions of *H. strigosum*.

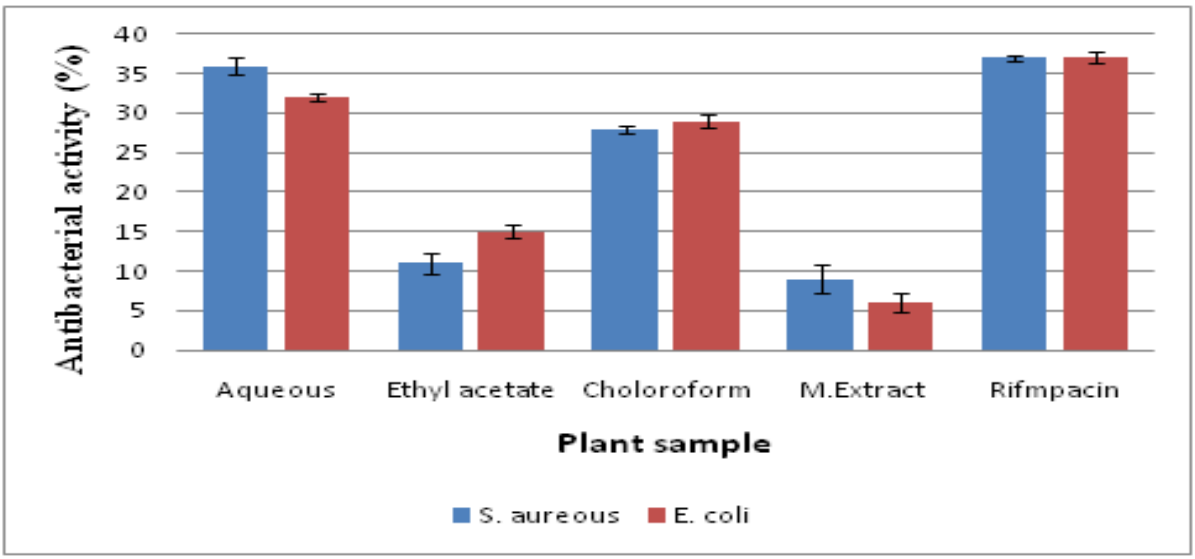

Fig. 3. Antibacterial activity of extract and its polar fractions of *H. strigosum*.

All the samples showed the significant results in clot lysis as presented in figure 2. The highest clot lysis was observed in methanolic extract 34.54 % while the lowest was observed in ethyl acetate fraction (23.16%) while moderate result 32.42% and 27.24% were shown by aqueous and chloroform solvents. The standard used for thrombolytic activity was streptokinase which showed the highest result (87.37%) than all the samples.

Antibacterial activity of H. strigosum

Antimicrobial activities of crude extract and polar fractions of *H. strigosum* were performed by using the disc diffusion method: against two bacterial strains. Results indicate that crude extract and polar fractions of *H. strigosum* showed both broad spectrum of activity by forming clear zones of inhibition or negligible zones of inhibition against these strains.

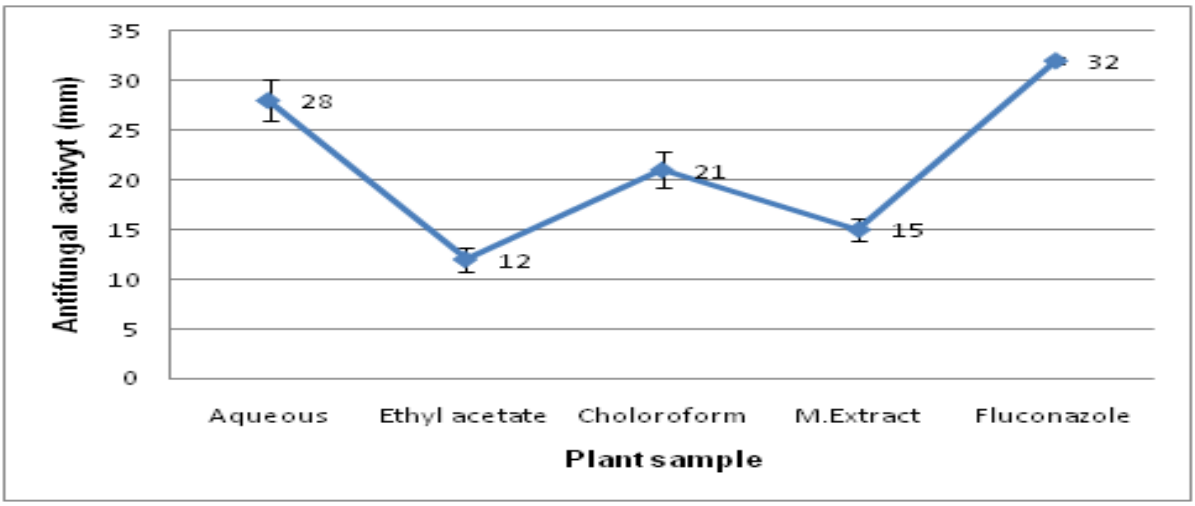

Fig. 4. Antifungal activity of extract and its polar fractions of *H. strigosum*.

Maximum antibacterial activity was shown by aqueous fraction 36mm and 32 mm against *S. aurous*and *E. colias shown in figure 3*.Methanolic crude extract showed minimum antibacterial activity 9mm and 6mm respectively. Chloroform and ethyl acetate fractions also showed significant antibacterial activity 28mm and 11mm against *S. aureous* bacterial strains while 29mm and 15 mm against *E. coil* bacterial strains respectively. The results of antibacterial activity was compared with Rifmpacin which is used as standard and it showed 37mm activity against both tested bacterial strains which is highest then the plant extract and its polar fraction.

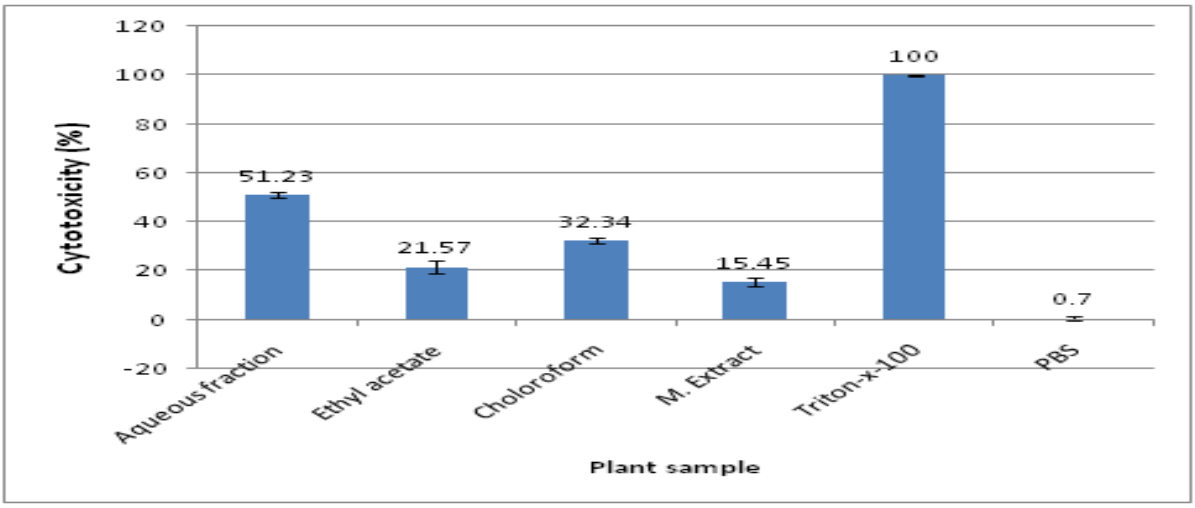

Fig. 5. Hemolytic activity of extract and its polar fractions of *H. strigosum*.

Antifungal activity of H. strigosum

In case of antifungal activity, the highest antifungal activity 28mm was shown by aqueous fractions followed by chloroform fraction which showed 21mm antifungal activity (Figure 4). Methanolic crude extract showed 15mm activity whereas ethyl acetate fraction showed 12 mm activity which least is shown by tested plant sample. Fluconazole is also performed parallel as standard antifungal agent which showed 32mm activity which is higher than the tested plants sample.

Cytotoxicity activityof H. strigosum

The hemolytic activities of methanolic crude extract, chloroform, ethyl acetate and aqueous fractions of *H. strigosum* were studied which showed that highest hemolytic activity 51.23% was shown by aqueous

fraction followed by 32.34 % and 21.5% shown by choloroform and ethylacetate fractions respectively. The lowest hemolytic activity 15.45% was shown by methanolic extract. Triton-x-100 and PBS were used as positive and negative control respectively. The obtained results of tested plant sample and its polartities based fractions were found lower hemolytic activity of Triton-x-100 (100%)as shown in figure 5. Negative control showed 0.7 % hemolytic activity. It was found that hemolytic activity increased in polar fractions of crude extract.

Conclusion

The present study was performed to examine the thrombolytic, antimicrobial and cytotoxicity activities of extract and its polar fractions *H. strigosum*. Thrombolytic activity was determined by *in vitro* clot lysis modal. Maximum clot lysis was observed in crude extract of *H. strigosum* with value of percentage lysis (34.54%) and minimum was observed in crude (23.16%). Cytotoxic activity was checked by hemolytic assay which showed aqueous fraction of *H. strigosum* showed maximum activity 51.23% while minimum cytotoxicity activity 21.57% was observed in ethyl acetate fraction. Crude extract and its polar fractions of *H. strigosum* were evaluated for the determination of antimicrobial activity by disc diffusion assay. Aqueous fraction showed maximum zone of inhibition against *B. subtilis* and *E. coli* (32mm and 32mm) while minimum zone of inhibition was observed in methanolic extract (9mm for *B. subtilis* and 6mm for *E. coli*).

Reference

Afzal M, Shahid M, Jamil A. 2014. Phytochemical spectrum of essential oil of *Paganum harmala* by GC-MS and antimicrobial activity using sequential solvents fractions and essential oil. Asian Journal of Chemistry **26,** 574.
http://dx.doi.org/10.14233/ajchem.2014.159988

Alkjaersig N, Fletcher AP, Sherry S. 1959. The mechanism of clot dissolution by plasmin. Journal of Clinical Investigation **38,** 1086.
http://dx.doi.org/10.1172/JCI1038855

Arslan R, Bor Z, Bektas N, Meriçli AH, Ozturk Y. 2011. Antithrombotic effects of ethanol extract of *Crataegus orientalis* in the carrageenan-induced mice tail thrombosis model. Thrombosis research, **127,** 210-213.
http://dx.doi.org/10.1016/j.thromres.2010.11.028

Atawodi SE. 2005. Antioxidant potential of African medicinal plants. African Journal of Biotechnology **4,** 128-133.

Barbour EK, Al Sharif M, Sagherian V. K., Habre AN, Talhouk RS, Talhouk SN. 2004. Screening of selected indigenous plants of Lebanon for antimicrobial activity. Journal of Ethnopharmacology **93,** 1-7.
http://dx.doi.org/10.1016/j.jep.2004.02.0277

Collen D. 1990. Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator?. Annals of internal medicine, **112,** 529-538.
http://dx.doi.org/10.7326/0003-4819-112-7-529

Devi JS, Bhimba BV. 2014. Antibacterial and Antifungal Activity of Silver nanoparticles Synthesized using *Hypnea muciformis*. Biosciences Biotechnology Research Asia, **11,** 235-238.

Gesler WM. 1992. Therapeutic landscapes: medical issues in light of the new cultural geography. Social science & medicine **34,** 735-746.
http://dx.doi.org/10.1016/0277-9536(92)90360-3

Gülçın İ, Oktay M, Kıreçcı E, Küfrevıolu ÖI. 2003. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. Food chemistry **83,** 371-382.
http://dx.doi.org/10.1016/S0308-8146(03)00098-0

Hamill FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, Soejarto DD. 2003. Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assays. Journal of Ethnopharmacology, **84**, 57-78.
http://dx.doi.org/10.1016/S0378-8741(02)00289-1

Hussain S, Jamil M, Ullah F, Khan A, Ullah F, Arfan M, Khatoon L. 2010. Antimicrobial and antioxidant activities of the plant *Heliotropium strigosum*. African Journal of Biotechnology **9,** 7738-7743.

Ivan S, Jozef F. 2011. Content of heavy metals in poppy seeds (*Papaver somniferum* L.). Advances in Environmental Biology **11,** 496-501.

Javid M, Sarfraz RA, Nawaz F, Qayyum A, Rehman A, Aziz S, Riaz SMIB. 2016. Effect of Solvents on the biological activities and phenolic composition of *Achyranthes aspera*. International Journal of Biosciences **9,** 145-153.
http://dx.doi.org/10.12692/ijb/9.6.145-153

Kumar BA, Lakshman K, Nandeesh R, Kumar, PA, Manoj B, Kumar V, Shekar DS. 2011. In vitro alpha-amylase inhibition and in vivo antioxidant potential of *Amaranthus spinosus* in alloxan-induced oxidative stress in diabetic rats. Saudi journal of biological sciences **18,** 1-5.
http://dx.doi.org/10.1016/j.sjbs.2010.08.002

Qayyum A, Sarfraz RA, Ashraf A, Adil S. 2016. Phenolic composition and biological (anti diabetic and antioxidant) activities of different solvent extracts of an endemic plant (*Heliotropium strigosum*). Journal of the Chilean Chemical Society **61,** 2828-2831.
http://dx.doi.org/10.4067/S07179707201600010016

Rai PK, Jaiswal D, Singh RK, Gupta RK, Watal G. 2008. Glycemic properties of *Trichosanthes dioica* leaves. Pharmaceutical biology **46,** 894-899.
http://dx.doi.org/10.1080/13880200802370167

Rasool N, Tehseen H, Riaz M, Komal R, Muhammad Z, Mahmood Y. 2013. Cytotoxicity studies and antioxidant potential of *Acacia nilotica* roots. Int J Chem Biochem Sci. **3,** 34-41.

Riaz M, Rasool N, Bukhari IH, Shahid M, Zubair M, Rizwan K, Rashid U. 2012. In vitro antimicrobial, antioxidant, cytotoxicity and GC-MS analysis of *Mazus goodenifolius*. Molecules **17,** 14275-14287.
http://dx.doi.org/10.3390/molecules171214275

Shao H, Huang X, Zhang Y, Zhang C. 2013. Main alkaloids of *Peganum harmala* L. and their different effects on dicot and monocot crops. Molecules **18,** 2623-2634.
http://dx.doi.org/0.3390/molecules180326233

Xue-Ting LIU, Yao SHI, Liang JY, Zhi-Da MI. N. 2009. Antibacterial ent-rosane and ent-kaurane diterpenoids from *Sagittaria trifolia* var. *sinensis*. Chinese Journal of Natural Medicines **7,** 341-345.
http://dx.doi.org/10.3724/SP.J.1009.2009.00345

Zuber M, Tabasum S, Jamil T, Shahid M, Hussain R, Feras KS, Bhatti KP. 2014. Biocompatibility and microscopic evaluation of polyurethane-poly (methyl methacrylate)- titnanium dioxide based composites for dental applications. Journal of Applied Polymer Science **131,** *1-9*.
http://dx.doi.org/10.1002/app.39806