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Investigations on the antimicrobial potential of *Abutilon fruiticosum* Gill & Perr.

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Key words: Antimicrobial activity, Crude extracts, Polar and nonpolar solvents, *Abutilon fruiticosum*.

Abstract

The present study is an investigation on the antibacterial and antifungal activity of the extracts of ethno botanically important plant *Abutilon fruiticosum* Gill & Perr. in different polar and non-polar solvents. The zone of inhibition and minimum inhibitory concentration (MIC) were recorded against four different bacterial (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*) and two fungal strains (*Aspergillus niger, Aspergillus oryzae*). The results revealed that the plant had reasonably good antimicrobial potential. The root and stem extracts showed maximum zone of inhibition against bacterial strains, i.e. 47±0.14mm, 40±0.11mm and 38±0.28mm while the n-Hexane fruit and flower extracts exposed the best zone against both of the fungal strains. The ethanol extracts when subjected to the qualitative minimum inhibitory concentration using six different extract concentrations, indicated the most resistant values, i.e. 1.25mg/ml against bacterial strains and 2.5mg/ml against fungal strains.

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Introduction

Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. Diseases can spread, directly or indirectly, from one person to another (Rashid *et al.,* 2000; Haque *et al.,* 2000). Resistance to antimicrobial agent is emerging in a wide diversity of the pathogens and multiple drug resistant (MDR) is becoming common in miscellaneous microorganisms (Ahmad and Beg, 2001). Over the past 20 years, there has been a lot of analysis of plants as sources of new antimicrobial agents. But still there is an instantaneous need to identify innovative substances active towards pathogens with high resistance (Cragg *et al.,* 1997; Samy and Ignacimuthu, 2000). A recent study has shown that numerous intoxicating extracts of various medicinal plants exhibit antimicrobial activity (Akinyemi *et al.,* 2005). Infectious diseases are the second principal cause of worldwide death. About one-fourth of all the medicines we use, come from tropical forest plants. However, scientific studies have been steered only to a limited extent with few medicinal plants (Rashid *et al.,* 2000; Haque *et al.,* 2000).

In the Past decades, research scientists focused on increasing human infections caused by pathogenic bacteria, fungi and virus. Microorganisms have shown adverse effects on the quality and safety of human beings life. A variety of synthetic chemicals are widely used against these microorganisms, sometimes they develop resistance to many antibiotics due to the indiscriminate uses of commercial antibiotics. In addition, these antibiotics sometimes cause allergic reaction and immunity suppression. Plant-mediated phytocompounds play an important role in the chronic disease caused by these microorganisms (Ranjitha *et al*., 2013).

Herbal medicines have received much attention as a source of new antibacterial drugs since they are considered as time-tested and comparatively safe both for human use and for environment.

Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (Chopra *et al.,* 1992; Bruneton, 1995).

Therefore, the present study was designed to assess the antimicrobial activity the leaves, stem, roots, flower and fruit of *Abutilon fruiticosum* Gill. & Perr. *Abutilon fruiticosum* belongs to family Malvaceae and is distributed in Pakistan, Ethiopia & Somaliland (Abedin, 1979). Various species of the genus *Abutilon* is used in indigenous medicines for the treatment of various ailments (Bagi *et al.,* 1985; Rahuman *et al.,* 2008, Land and Norton, 1973). This plant has been recently studied for the presence of the phytochemicals and antioxidants by Muahtaq and Khan (2016) which exhibited that their different parts contain specific phytoconstituents responsible for their biological activity. The previous studies showed that the other species of the genus *Abutilon* species are traditionally demanded for their varied pharmacological and medicinal activities. (Sikorska and Matlawska, 2008).

Materials and methods

Plant Material

Theall parts of fresh plant *A. fruiticosum* Gill. & Perr. were collected from District Okara in October, 2014 and identified with the help of Flora of Pakistan (Abedin, 1979). The plant specimen was deposited in Dr. Sultan Ahmad Herbarium GC University, Lahore, after posting the voucher number, GC. Herb. Bot. 2932.

Microorganisms

Gram +ve (*Escherichia coli* and *Pseudomonas aeruginosa*), Gram +ve (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria and two fungi (*Aspergillus niger* and *Aspergillus oryzae)* were obtained from Punjab Institute of Cardiology Civil Hospital Lahore, Pakistan.

Extraction

The Plant material was dried in shad at room temperature. The plant material was crumbled by using pestle and mortar to form course powder.

The plant parts were extracted successively with nonpolar and polar solvents, like*n*-hexane, chloroform, ethanol and distilled water by maceration for 8 days in each of the solvents respectively. The extracts were concentrated on rotary evaporator and the dried extracts thus obtained were used to prepare plant fractions of various concentrations to evaluate their *in vitro* antibacterial and antifungal activities.

The percentage yield of concentrated extracts was calculated;

$$\% \text{ Extraction yield} = \frac{\text{Weight of dried plant extract}}{\text{Weight of initial plant sample}} \times 100$$

Determination of antimicrobial activity

The antimicrobial potential of the *Abutilon fruiticosum* Gill & Perr. was evaluated for their antifungal and antibacterial potential using Agar well diffusion method after Cruick-Shank *et al*. (1975), Ferreira *et al*. (1996) and Oretega and Julian (1996). The measurement of zone of inhibition represented the antimicrobial activity. The standard antibacterial and antifungal discs were used for the comparison.

Minimum Inhibitory Concentration (MIC)

The ethanolic extracts of the plant *Abutilon fruiticosum* Gill & Perr. Were tested for the estimation of minimum inhibitory concentration (MIC), according to Hendriksen (2003) using agar diffusion method.

Results and discussion

Antibacterial Activity

Chloroform root extract of the *Abutilon fruiticosum* Gill & Perr. showed higher zone than any other extract of it i.e. 47±0.14mm against *S. aureus* (Fig. 1a) while the least zone by leaf *n*-Hexane extract, i.e. 15±0.46mm against *B. subtilis*.

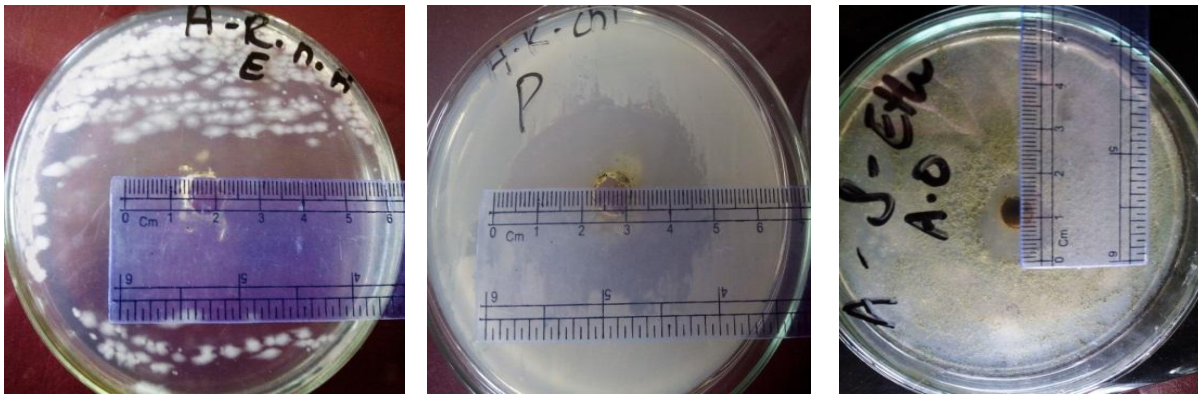

Fig. 1. a) zone inhibition of n-Hexane extract of root of *A. fruiticosum* against *E. coli,* b) Zone of inhibition of chloroform extract of Flower of *A. fruiticosum* against *P. aeruginosa,* c) Zone of inhibition of ethanol extract of stem of *A. fruiticosum* against *A. oryzae*.

Aqueous leaf extracts of the *A. fruiticosum* Gill & Perr. exhibited the significant zones against all the bacterial strains except *B. subtilis* while all the leaf extracts showed maximum zones against E. coli amongst the four used bacteria. Stem macerates in n-Hexane and ethanol showed the maximum zone against *E. coli* i.e. 38±0.28mm and 32±0.17mm respectively while all the leaf extracts against *B. subtilis* exposed the minimum zones (18±0.28mm, 17±0.17mm, 17±0.23mm and 19±0.11mm) and all others have remarkable zone inhibition. Amongst root extracts, chloroform and n-Hexane extracts presented the maximum zones in the extracts of all the plant parts i.e. 47±0.14mm against *S. aureus* and 40±0.11mm against *E. coli* (Fig. 1a & b). The ethanol extracts of the flower and fruit showed maximum zone of inhibition against all the bacterial strains (Fig. 2-5).

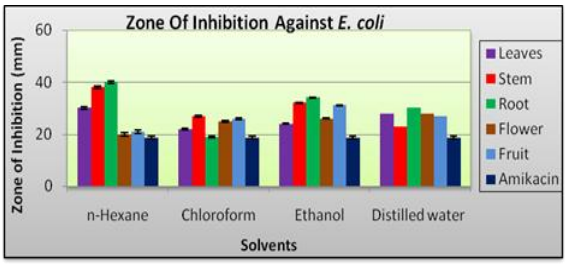

Fig. 2. Graphical representation of zone of inhibition produced by stem, leaf, root flower and fruit of *Abutilon fruiticosum* Gill & Perr. Against *E. coli*

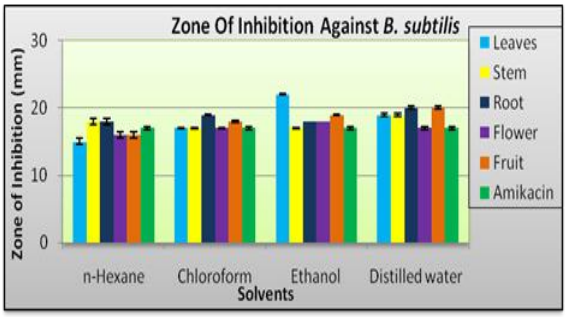

Fig. 3. Graphical representation of zone of inhibition produced by stem, leaf, root, fruit and flower of *Abutilon fruiticosum* Gill & Perr. Against *Bacillus subtilis*.

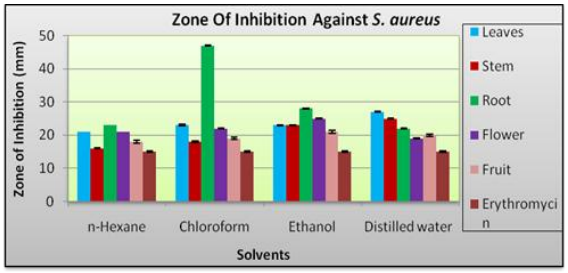

Fig. 4. Graphical representation of zone of inhibition produced by stem, leaf, root, fruit and flower of *Abutilon fruiticosum* Gill & Perr. Against *S. aureus*.

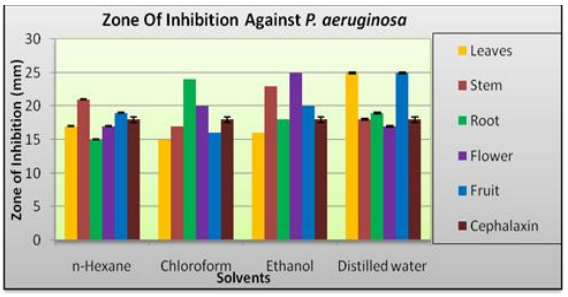

Fig. 5. Graphical representation of zone of inhibition produced by stem, leaf, root, fruit and flower of *Abutilon fruiticosum* Gill & Perr. Against *P. aeruginosa*.

Antifungal Activity

Abutilon fruiticosum Gill & Perr. showed good results against both of the fungal strains. The leaf extracts exposed the results that were nearer to each other against both of the fungal strains.

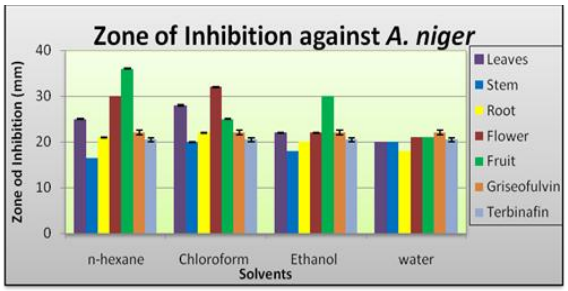

Fig. 6. Graphical representation of zone of inhibition produced by stem, leaf, root, fruit and flower of *Abutilon fruiticosum* Gill & Perr. Against *A. niger*.

The stem and root ethanol extracts of showed maximum results against *Aspergillus oryzae,* i.e. 35±0.28mm and 35±0.03mm (Fig.1c) correspondingly while minimum zone in *n*-hexane and ethanol extracts of stem (16.5±0.05mm & 18±0.01mm) whereas 18±0.01mm by aqueous extract of root against *Aspergillus niger* and stem in *n*-hexane (19±0.00mm) against *Aspergillus oryzae*.

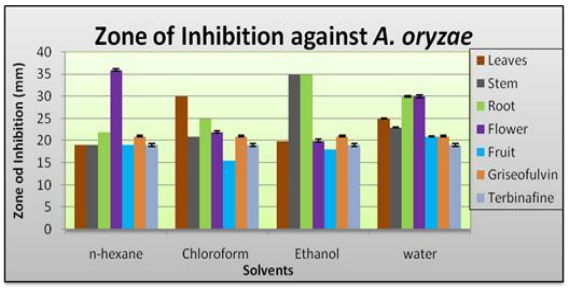

Fig. 7. Graphical representation of zone of inhibition produced by stem, leaf, root, fruit and flower of *Abutilon fruiticosum* Gill & Perr. Against *A. oryzae*.

Flower and fruit extracts exhibited maximum zone of inhibition against both the fungal strains i.e. 36±0.05mm, 30±0.02mm shown by the flower extracts in *n*-Hexane and water against *Aspergillus oryzae* and 30±0.3mm, 32±0.4mm in *n*-Hexane and chloroform against *Aspergillus niger* while fruit exposed maximum zone against *Aspergillus niger* i.e. 36±0.00mm, 30±0.5mm & 25±0.28mm in *n*-Hexane, ethanol and chloroform extracts, respectively and 28±0.09mm of aqueous extract was maximum zone against *Aspergillus oryzae* (Fig. 6 & 7).

Minimum Inhibitory Concentration (MIC)

Qualitative agar diffusion method was used to determine the Minimum Inhibitory Concentration (MIC) of ethanol extracts (leaf, stem, root, flower and fruit) *Abutilon fruiticosum* Gill & Perr. against bacterial and fungal strains. The growth of bacterial and fungal strains was noticed to estimate the Minimum Inhibitory Concentration (MIC) of ethanol extract of each part of the plants. The presence of the growth of microbes at which concentration of the ethanol extracts is the minimum inhibitory concentration of the plant macerates.

Discussions

The present study was executed in order to f evaluate the antibacterial and antifungal activity of the *Abutilon fruiticosum* Gill & Perr. through different methods i.e. zone of inhibition and minimum inhibitory concentration. Various parts of the plant were separated, shade dried, ground to fine powder and macerated in polar and non-polar solvents. The percentage yield of all crude extracts of *A. fruticosum* Gill & Perr. was calculated and the highest yield was documented by aqueous flower extract of *A. fruiticosum* Gill & Perr. i.e. 17.34%.

Antimicrobial potency of the extracts of the *A. fruiticosum* Gill & Perr. was estimated by measuring the inhibition zones produced to restrict the microbes growth. The standard discs were also used against microorganisms in order to make comparison between the inhibition zone produced by commercially available discs and extracts. Two Gram-positive, two Gram-negative bacteria and two fungal strains were employed in the study. The broad spectrum of potential was recorded by crude extracts of the plant parts against bacterial and fungal strains. The root extract of *A. fruiticosum* Gill & Perr. in chloroform showed highest zone i.e. 47±0.14mm against *S. aureus* (Fig. 4).

The different extracts of the *A. fruiticosum* Gill & Perr. showed maximum inhibition zone against fungal strains. The *n*-Hexane flower extracts of the plant exhibited maximum zone against the fungal strains, i.e. *Aspergilus oryzae* (36±0.05mm), *Aspergilus niger* (36±0.00mm) in comparison with standard drugs Griseofulvin and Terbenafine which showed minimum zones Fig 6 & 7).

The chloroform extract of flower also showed maximum inhibition zone (32±0.4mm) against *A. niger* while all other extracts of the plant were also shown appreciable zone for the microbes growth inhibition. The highest antifungal ability was noted in all the extracts of *n*-Hexane, chloroform ethanol and distilled water of the *A. fruiticosum* Gill & Perr. but the ethanol extracts as well as the *n*-Hexane extracts except stem *n*-Hexane macerates showed remarkable zones ranging from 18±0.01mm to 30±0.00mm and 19±0.00mm to 36a±0.00mm.

The qualitative Minimum Inhibitory Concentration (MIC) was carried out on ethanolic extracts of different parts of the *A. fruiticosum* Gill & Perr. the most resistant MIC value shown by all the extracts of all the plant parts was 1.25mg/ml against all the bacterial strains but the fruit extracts showed the inhibitory effect at the concentration of 0.625mg/ml against the bacterial strains except the *S. aureus*.

MIC value against fungal strains was also noted. The most resistant MIC value was recorded by the ethanol extracts of *A. fruiticosum* Gill & Perr. showed the most resistant value, i.e. 2.5mg/ml against both the fungal strains (Table 1).

This plant contains different phytoconstituents as explained in the recently published work of Mushtaq and Khan (2016) which showed that this plant contains the phenolic and flavonoid contents that show their activity against the microbes. The above mentioned results of the present work indicated that the targeted plant contained bioactive compounds although varied in solubility in polar and no*n*-polar solvents.

Conclusions

The stem, leaf, root, flower and fruit of *Abutilon fruiticosum* Gill & err. the ethnobotanically important plant, were investigated for their antimicrobial potential. The study concluded that *Abutilon fruiticosum* Gill & Perr. can be used to treat many fungal and bacterial diseases such as diarrhea, difficult urination, swelling, lockjaw and cancer. The antibacterial and antifungal potential of the *Abutilon fruiticosum* Gill & Perr. is more effective than the standard discs used for the antimicrobial and antifungal activities.

So, this plant can be used as an alternative of the synthetic standard medicine and also for treatment of many diseases, but after some studies on the cytotoxicity of this plant. Overall, *Abutilon fruiticosum* Gill & Perr. had significant antimicrobial activity, thus supporting its traditional medicinal practices.

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