

Research Article

Phytochemical screening and antimicrobial activity of selected medicinal plant species

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Abstract

Phytochemicals are essential compounds, utilized worldwide for curing of various human disorders. The present study comprised of 12 different medicinal plant species i.e. *Withania coagulans*, *W. somnifera*, *Cannabis sativa*, *Medicago sativa*, *Achyranthes aspera*, *Convolvulus arvensis*, *Solanum nigrum*, *Mentha longifolia*, *Mentha spicata*, *Tagetes erecta*, *Fagonia cretica* and *Acacia nilotica*. These species were used by the local inhabitants for treating various ailments. Methanolic extract of leaves of these plant species were investigated for cardiac glycosides, alkaloids, tannins, saponins, flavonoids, terpenoids, anthraquinones and reducing sugars. Among the reported medicinal plant species, Leaves of *Acacia nilotica* and *Withania somnifera* were tested for antibacterial activity which showed significant activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas fluorescens*.

Keywords: Medicinal plants; Phytochemical screening; Antimicrobial activity

Introduction

There are some diseases in the world which cause death, almost of 40,000 people. Disease like diarrhea cause huge mortality among childrens [1]. Bacteria like *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* are most common species which are pathogen on children [2]. In recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world [3]. A large number of drugs are obtained from plant and used for many serious disorders [4]. In East

Asia, many plants are considered to have significant medicinal features i.e anti-inflammatory, anti-bacterial and analgesic function because they contain a large variety of phytochemical i.e monoterpenoids, sesquiterpenoids, and curcuminoids [5, 6]. *Withania somnifera* (family Solanaceae) is a medicinal plant utilized for large number of diseases. Every part of these plant have a big sources of many chemicals and widely used for curing ulcers, fever, cough, dyspnoea, dropsy, rheumatism, toxicosis and leucoderma [7]. *Acacia nilotica*. Is very

common small tree it also known as kikar belongs to the family Mimosaceae [8]. *Acacia nilotica* is considered to be antidiarrheal and antispasmodic. The pod and leaves are also utilized for medicinal purpose i.e. diarrhea. *Acacia nilotica* part extract has been shown to exhibit antibacterial, antiplatelet and anti-oxidant [9]. The stem bark of *Acacia nilotica* is well-known for its diuretic properties [9].

These medicinal plants are important because of their uses for curing disorder and for healing as well [10]. These plants are important because of their phytochemical and large number of significant features. There are many other reports which show importance of these plants for their antimicrobial activity and phytochemicals like [10-12]. In Pakistan 6000 plant species were present in which about 180 plants were used for medicinal. The phytochemicals are two types including primary phytochemical which have not such a strong action in medicine while the secondary phytochemical are worldwide used for many ailments, the most important secondary phytochemical are Alkaloids, Terpenoids [12]. Secondary phytochemicals are compounds which show no function to be incorporated in primary metabolism, they act as accessory role. Rather than medicinal uses these phytochemicals are used by the plant for their defense as well [12].

Terpenoids play a vital role in most pharmacological work and activities, medicinally it plays an important role in anti-cancer, anti-malarial and anti-fever as well [3]. Alkaloids are also important secondary chemical which play a vital role as anesthetic agent. Alkaloids also create variation in physiology [13]. Phenol and its compounds are distributed in many plants, phenolic compounds are used for the treatment of many disorders and also due to its toxic action prevent the growth of many pathogens [14]. Flavonoids, sterols, and

saponins are essential secondary chemicals present in above 85 families, due to their multipurpose and specific chemistry they are used for the treatment of many disorders [15]. All these phytochemicals give a strong immunity in the form of resistance against many insects and herbivores as well [16]. In this universe about 4,44,000 flowering plant species are present, in which 40,000 plant species are utilized due to their phytochemical importance.

Materials and methods

Collection of plants

For the collection of plant species field surveys were conducted throughout the research site during 2014. The listed plant species (Table 1) were collected and then dried. These plant species were identified at the department of Botany Islamia College University, Peshawar.

Materials extracted from plants

The dried material was obtained from these plants by keeping them at room temperature 28 °C for 18 days, they were ground into fine powder. Extract of ethanol was made through soaking 70g of powder from plant specimen in 1.3 L ethanol. For making a viable extract of ethanol 42 hours were required at room temperature.

Extraction of solvent

The dried leaves of two plants i.e. *Withania somnifera* and *Acacia nilotica* were washed and the selected plant material was dried. The parts of these plants were kept in an oven at 40 °C then changed into powder with mortar and pestle. 80 grams of each part of plant and 200 ml of methanol were kept in mortar and with the help of pestle it was ground up. This extract was placed at room temperature for 30 hours. This extract was filtered through filter paper and dried at a temperature below 48 °C for methanol removal to obtain the dense extract and then they were kept in sterile bottles under refrigerated conditions until use.

Anti-bacterial testing

Antibacterial activity was measured using agar dilution technique. Briefly, the methanol extracts were dissolved in dimethyl sulfoxide (DMSO, Merck) and serially diluted in molten Mueller Hinton Agar (MHA, Sigma) in petridishes (100 mm×15 mm) to obtain final concentrations: 100, 50, 25 and 12.5 µg/ml. The solvent did not exceed 1% concentration and did not affect the growth of the organisms. All bacterial strains were grown in Mueller Hinton Broth (MHB, Sigma) for 4 h at 37°C. Bacterial suspensions with 0.5 McFarland standard turbidity, which is equivalent to 10⁸ cfu/ml were prepared by dilution with Mueller Hinton broth. The diluted inoculum was added to a Steer's replicator calibrated and incubated for 24 h at 37 °C. After incubation, all dishes were observed for microbial inhibition by the disc diffusion method (Table 2).

Rotary Evaporator

By using rotary evaporator the extract become concentrated. The solutions were also passed through filter paper to make it impurities free and the analyses of phytochemicals are occurred by some procedures which are standrad.

Cardic glycosides test

Cardic glycosides test needs chemical like Ferric chloride, glacial acetic acid and distal water as well. Extract of 0.7 gm diluted through 10 ml of water, 3 ml of glacial acetic acid and also few drop of feric chloride were also included. Specific colors were responsible for sugar indicator and light brownish spots were indicative feature of deoxysugar, also some green spots were present as well.

Flavonoids test

For the determination of flavonoids dilute amonia was introduced. 3ml of dilute amonia were added to extract, sulphiric acid were also used. By the appearance of yellow

colour showed that presence of flavonoids [17, 18].

Alkaloids test

For the indication of phytochemical like alkaloids, 0.6 g of extract were mixed to 7ml acidified alchole, after mixing they were boiled and then their filtration occurred. 4ml after filtration was mixed with 2ml of dilute amonia, 3ml chlorofome were introduced and then mixed, after mixing gently the chlorofome appearance occurred in the form of layer with 6ml of acitic acid. At last the formation of brownish red precipate appear which clearly indicate alkaloids [19, 20].

Terpenoid test

Take 0.3g of the sample extract and add to 2ml of chloroform. A small amount of concentrated H₂SO₄ (2ml) were introduced for obtaining the phytochemicals. The appearance of reddish brown colour showed the presence of terpenoids.

Tannins test

Boiled extract about 0.3g in 7ml of water were mixed in a test tube and then filter. Ferric chloride was also add about few drops 0.2% after addition blue black or brownish green colour occurred [19, 20].

Anthraquinones test

Boiled extract about 0.3 g were taken and mixed with 8ml of sulphuric acid after mixing then filtered. Mixed 4ml of chloroform with filtered and then shaken a layer of chloroform were formed which was pipette into test tube at last 2ml of ammonia were add and wait for colouration.

Reducing sugar test

Take 0.3g of sample and add 7ml of water. This mixture was add to boiling fehling's solution after reaction colour was observed.

Saponins test

Test for saponins required oil of olive, 0.4g of extract was taken and then mixed with 7ml of water and make a solution, this solution was mixed with a few drop of olive oil. Observed the colour [21, 22].

Test sample preparation for antimicrobial test

For the antimicrobial tests, ethanolic extracts were diluted in dimethylsulfoxide (DMSO):

methanol (1/1: v/v) solvent to a concentration of 20 mg/ml.

Table 1. List of medicinal plant species and their part used for phytochemical analysis

S. No	Plant Species	Local name	Part used
1	<i>Acacia nilotica</i>	Kikar	Leaves
2	<i>Acyranthus aspera</i>	Baikand	Leaves
3	<i>Cannabis sativa</i>	Bhang	Leaves
4	<i>Convolvulus arvensis</i>	Perwatai	Leaves
5	<i>Fagonia cretica</i>	Azgakay	Leaves
6	<i>Medicago sativa</i>	Alfa alfa	Leaves
7	<i>Mentha spicata</i>	Podina	Leaves
8	<i>Mentha longifolia</i>	Weenalay	Leaves
9	<i>Solanum nigrum</i>	Kachmacho	Leaves
10	<i>Tagetes erecta</i>	-	Leaves
11	<i>Withania coagulans</i>	Ashtoshbooti	Leaves
12	<i>Withania somnifera</i>	Ashwagandha	Leaves

Table 2. Antibacterial activity of some medicinal plant methanol extracts (50 µg mlG1) and fungicide (60 µg mlG1) against fungal species tested by disc diffusion Zone of inhibition (mm)

<i>Botanical species</i>	<i>Acacia nilotica</i>	<i>Withania somnifera</i>
<i>Bacteria species spp</i>	<i>Bark</i>	<i>Leaves</i>
<i>Bacillus subtilis</i>	16±0.34	10±0.32
<i>Escherchia coli</i>	13±0.20	8±0.33
<i>Stphaylococcus aureus</i>	11±0.13	6±0.01
<i>Pseudomonas fluorescense</i>	9±0.13	4±0.01

Results and discussion

The present work showed presence of phytochemicals which are considered as an active medicinal constituent. This work presented 12 different plants which are medicinally important because of the presence of such important phytochemicals as show in (Table 3 & Figure 1). The result also showed that two medicinal plant *Withania somnifera* and *Accacia nilotica* are utilized for antimicrobial activity. These plant also contain important medicinal phytochemical, include such as terpenoids, reducing sugar, alkaloid etc were found in the test species sample. [23] also reported the phytochemical anaylasis of the arial

parts of various plant i-e Flavonids, glycoside alkaloids same is true in the present rearseach. . *Withania coagulans* contain all the important phytochemical except the reducing sugar, *cannabis sativa*, *Mentha spicata*, *Mentha longifolia*, *Achyranthus asper*, *Medicago sativa*, *Tagetis erecta*, *Solanum nigrum*, *Convolvulus arvensis*, *Withania sommifera*, the specimen of these plant were showed the presence of all the phytochemical. The test of the *Fagonia cretica* showed the presences of terpenoids and flavonoids, while the tested part of the *Acacia nilotica* show the presences of reducing sugar, terpenoids and flavonoids. [24] also described various

medicinal flora which were used due to presences of phytochemicals which are in line with present research.

The presence and absence of medicinal phytochemical in plant are related to various activity in plants such as physiological and biosynthetic reaction. Ecological effect should not be neglected like temperature, soil nature, availability of water and soil fertility, these factor also play a vital role in plant activity. *Accacia nilotica* and *Withania somminfera* these two medicinal plant extrat are used for inhibition the growth of bacteria both of ths plant showed a significant result against *Bacillus subtilis*, *Escherchia coli*, *Stphaylococcus aureus* and *Pseudomonas fluorescense*. [25]. [26] also reported

evaluation of antibacterial properties of medicinal plant which were utilized in making many medicines that's what the present report show. [26-28] also reported the antimicrobial activity of of these flora which were utilized in making medicine same is true in the present finding. *Withania somminfera* is another important medicinal plant species containing many important alkaloids which are used in medicine [29]. [30, 31] also reported *Acacia nilotica* and *Withania somnifera* showed significant antibacterial activity against *Bacillus subtilis*, *Escherchia coli*, *stphaylococcus aureus* and *pseudomonas fluorescense*. Same is true in the present finding.

Table 3. Below shows phytochemical analysis of different medicinal plants

Plant specie	Reducing sugar	Anthraquinone	Terpenoids	Flavonoids	Saponins	Tannin	Alkaloids	Cardicglycocids
<i>Acacia nilotica</i>	+		+	+				
<i>Achyranthus asper</i>	+	+	+	+	+	+	+	+
<i>Cannabis sativa</i>	-	+	+	+	+	+	+	+
<i>Convolvulus arvensis</i>	+	+	+	+	+	+	+	+
<i>Fagonia cretica</i>	-	-	+	+	-	-	-	-
<i>Medicago sativa</i>	+	+	+	+	+	+	+	+
<i>Mentha longifolia</i>	+	+	+	+	+	+	+	+
<i>Mentha spicata</i>	+	+	+	+	+	-	+	+
<i>Solanum nigrum</i>	+	+	+	+	+	+	+	+
<i>Tagetis erecta</i>	+	-	+	+	+	+	+	+
<i>Withania somminfera</i>	+	+	+	+	+	+	+	+
<i>Withania coagulans</i>	+	+	+	+	+	+	+	+



Figure 1. Represent important plant species

Conclusion

The plant which are selected in this report provide important and medicinal secondary metabolites. These plants are big source of secondary metabolites including, Alkaloids, reducing sugar, anthraquinone, terpenoids, flavonoids, saponins, tannin, and cardiac glycosides. These secondary metabolites are useful in preventing different diseases. These phytochemical are used for many ailments like as antidiuretic, pain killer, anti-cancer, anti-viral, anti-fungal and anti-bacterial as well. Screenings of the phytochemical constituent are very important for the synthesis of new medicine and drugs.

Authors' contributions

Conceived and designed the experiments: S Ali, K Ali & Z Hussain, Performed the experiments: MS Khan, WM Khan, Analyzed the data: S Wali & M Shuaib, Contributed reagents/ materials/ analysis

tools: S Ali and K Ali, Wrote the paper: S Ali.

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