Antimicrobial activity and phytochemical analysis of selected medicinal plants species

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ABSTRACT

Pathogenic disorders are increasing day by day. To escape these diseases, it is compulsory to explore bioactive compounds present in medicinal plants which are effective against these pathogenic diseases. The main objective of the present study was to investigate the phytochemical compounds and antimicrobial activity of the extracts of selected medicinal plants. Bioactive compounds such as Saponin, Tannins, Quinones, Alkaloids, Flavonoids, Phenols, Coumarin, Terpenoids, Glycosides and Cardiac glycosides were screened from selected seven medicinal plants with variation between individual plants. The bioactive compounds were obtained from the leaves of selected medicinal plants species (Cymbopogon citrates Stapf (DC), Thymus vulgaris L., Lavandula angustifolia Miler., Rosmarinus officinalis L., Carica papaya L., Ocimum americanum L. and Ocimum basilicum L) to screen the antimicrobial activity of selected pathogens such as Escherichia coli, Xanthomonas oryzae and Staphylococcus aureus using disc diffusion method. The Ethanolic extract of C. citratus presented the highest anti-X. oryzae activity among all the tested plants and was effective against all bacterial strains tested. Thereafter, all the remaining plants extracts of T. vulgaris, L. angustifolia, O. basilicum, O. americanum, R. officinalis and C. papaya showed also a valuable activity against X. oryzae, S. aureus and E. coli. We conclude from this that all these extracts exhibit amazing antimicrobial properties that support their traditional use as antiseptics.

Key words: Medicinal plants, bacterial strains, antimicrobial activity, phytochemical analysis, ethanolic extracts, bioactive compounds.

INTRODUCTION

In all over the world, large proportion of population is depending on plant biodiversity for herbal medicine and still about 60 to 80 percent is depending on them for traditional fitness and health care system since the prehistoric ages. Demonstration of phytochemicals in these medicinal plants produce decisive effect on human being and also form the base for medicinal drugs currently (Edegoa et al., 2005; Akinmo-laudn et al., 2001; Rout et al., 2009). For the treatment of stomach-aches and kidney problems, Lavandula angustifolia (lavender) from the family of Lamiaceae is used as Arabic medicine (Ghazanfar, 1994), while for the cure of arthritis and normalizing digestive system, Carica papaya (papaya) from the family of Caricaceae is used, which contains chymopapain and papain (Arvind et al., 2013). The phytochemical properties and antimicrobial activities of several medicinal plants were investigated in recent few eras. Ocimum basilicum (basil) from the family of Lamiaceae is used as anti-viral, anti-microbial, antioxidant, and anticancer agent (Chiang et al., 2005; Bozin et al., 2006); however, Cymbopogon citratus (Lemongrass) from the family of Poaceae is best used as anti-oxidant, antimicrobial and anti-fungal agent (Nikos and Costas, 2007; Oloyede et al., 2010). Thymus vulgaris (Thyme) from the family of Lamiaceae has
antispasmodic, bactericides, antisepsics, antioxidants, anthelmintic properties and also as cancer prevention agent (Monira et al., 2012). Although Rosmarinus officinalis (Rosemary), also from the family of Lamiaceae, has many herbal properties such as antioxidant due to the presence of many phenolic compounds (Fecka and Baranowska, 2007). For allelopathic actions, it has been specified that phenolic acids are the most frequently stirring natural products (Singh et al., 2003). Phytochemicals are important bioactive compounds, that is, alkaloids, tannins, flavonoids and phenolic compounds. Approximately, indigenous medicinal plants are used as food plants and also added to foods for the treatment of many ailments related to females (Okwu, 1999, 2001; Hill, 1952). In Pakistan and in several countries of the world, medicinal plants are used as a source of many powerful drugs and have many antimicrobial possessions (Srivastava, 1996). In all over the world and also in many unindustrialized countries, numerous persons died due to many infectious diseases (Nathan, 2004). The phytochemicals existing in numerous medicinal plants are extremely important for healing and treating most human diseases (Nostro et al., 2000). Unembellished infections in humans caused by the bacterial creatures counting Gram positive and Gram negative species such as Bacillus, Staphylococcus, Salmonella and Pseudomonas which have the ability to survive in harsh condition due to their multiple environmental surroundings (Ahameethunisa and Hoper, 2010). Since ancient times, plant kingdom has obtained a variety of compounds of known beneficial properties, such as analgesics, anti-inflammatories, medicines for asthma, and many others. In recent years, from diverse parts of the world, the antimicrobial properties of plant extracts have been described with growing frequency (Cowan, 1999). Some diseases caused by pathogenic bacterial strains are shown in Table 1.

Massive diversity of bioactive plants in Pakistan is grown naturally. In the present study, we explored the bio-activity of the following seven naturally growing plants: T. vulgaris (Thyme) L. angustifolia (Lavender) R. officinalis (Rosemary), C. citratus (Lemon grass), O. basilicum (Italian Basil) and O. americanum (Lime Basil). In this study, the presence of phytochemical bioactive constituents in these medicinal plants was examined. The phytochemicals used for many ailments were determined for therapeutic uses. We also demonstrated the antibacterial screening of crude extracts of these medicinally important plant species and their extract fractions were carried out using disc diffusion method. The biological activity of plant extracts was tested against Gram positive and Gram negative clinical isolates.

METHODOLOGY

Collection of medicinal plant

From the field of National Agricultural Research Center (NARC), Islamabad, Seven restoratively authoritative or medicinal plants such as T. vulgaris, L. angustifolia, R. officinalis, C. citratus, O. basilicum and O. americanum were gathered with the end goal of experimentation.

Extraction of solvent

The chosen seven therapeutic plants were washed and put under shade at room temperature for two weeks. At the point when the plants were dried, they were kept in plastic hermetically sealed sacks. Each of the leaves was separated from the plants and crushed. Unrefined concentrate was set up by the procedure of extraction (Reddy and Mishra, 2012). In 150 ml of ethanol, 30 gm of dried powder of each plant were splashed and put on shaker for 48 h (Figure 1). Under fume hood, the concentrates were separated with channel or filter paper and again with muslin filter paper and put away at 4°C in the wake of spinning on rotary evaporator where concentrated concentrates were remaining.

Phytochemical screening

The ethanolic therapeutic plants extricates were tested for Flavonoids, Coumarin, Glycosides, Cardiac glycosides, Terpenoids, Quinones, Saponins, Alkaloids, phenols and Tannins. The subjective or qualitative screening was executed using the standard methodology (Sofawara, 1931; Trease and Evans, 1989).

Test for tannins

To 1 ml of each concentrated extract, about 2 ml of 5% iron chloride were placed in a test tube. Green shading appearance demonstrated the nearness of tannins in test trial.

Test for saponins

About 2 ml of autodaved distal water were added to each 2 ml of plants extricates and shacked the long way lengthwise for 15 min. Development of stable foam indicated the occurrence of saponin.

Test for flavonoids

To the 5 ml of dilute ammonia (weaken smelling salts (NH₃) was added a segment separate 1.2 ml took after by option of concentrated sulphuric acid (H₂SO₄) in a test tube. Entry of yellow shading indicates the frequency of flavonoids.

Test for alkaloids

2 ml of plant extracts were added to 2 ml of concentrated hydrochloric acid (HCL) and then couple of drops of
Table 1: Diseases caused by bacterial strains under study.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Diseases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Xanthomonas oryzae</em></td>
<td>affects the rice crop in its severe form</td>
<td>(Goto, 1992)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Affects brain and cause septicemia</td>
<td>Lowy, 1998</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Affect urinary tract, cause Hemolysis and Travelers Diarrhea and also other clinical infections pneumonia neonatal meningitis.</td>
<td>Kaper <em>et al.</em>, 20004</td>
</tr>
</tbody>
</table>

Figure 1: Shaking of extracts on shaker.

Mayer's reagent were included in a test tube. Green shading assigned the nearness of alkaloids.

**Test for quinones**

1 ml plant unrefined concentrate was blended in concentrated sulphuric acid (1 ml). Appearance of red shading demonstrates the imminence of quinones.

**Test for terpenoids**

5 ml of each plant extricate was added to 2 ml of chloroform took after by some expansion of 3 ml concentrated H2SO4. A layer of the reddish-brown colouration was framed at the limit, demonstrating positive outcomes for the presence of terpenoids.

**Test for phenols**

With 1 ml concentrate, 2 ml of refined water diagrammed by few drops of 10% iron chloride brought about the production of green shade that showed the nearness of phenols.

**Test for coumarins**

1 ml of every unrefined crude concentrated extract was treated with 1 ml of 10% Sodium hydroxide (NAOH).

Formation of yellow shading indicated the event of coumarin.

**Test for glycosides**

2 ml of concentrate and 3 ml of chloroform with 10% ammonia solution were included. Pink shading arrangement displayed the nearness of Glycosides.

**Test for cardiac glycosides**

5 ml of every unrefined crude concentrate was added with 2 ml of glacial acidic acids and a few drops of 5% iron chloride, and 1 ml of concentrated sulphuric acid was added. A darker ring creation at the interface demonstrated the presence of cardiac glycosides.

**Bacterial strains collection**

Three distinctive distinguished pathogenic bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, and *Xanthomonas oryzae*) were gathered from various storehouse of NARC Islamabad, Pakistan. After implantation was achieved, the chosen strains at 4°C utilized supplement agar and thereafter, were sub-refined or cultured for 24 h before testing.
Method used

Disc diffusion technique was utilized by discovering the antibacterial action of chose therapeutic plants species of ethanolic crude extracts (Bakht et al., 2011).

Study design

Analysis was performed by utilizing or using complete randomize design (CRD) and each investigation was rehashed or repeated three times.

Preparation of agar plates and tests

Positive and negative tests were performed, while for positive control unadulterated pure DMSO (dimethyl sulfoxide) was used and for negative control immaculate pure Ethanol was utilized. For food and development of microscopic organisms, supplement nutrient agar media were utilized for refining. With a specific end goal to plan media, 20 g of supplement agar was added and totally disintegrated by consistent shaking in 400 ml of autoclaved refined distal water. pH was kept at 7.0. After the procedure, the media was kept in autoclave at 121°C for 15 min and afterward, 25 ml filled the Petri dishes in the laminar flow hood and kept in icebox or refrigerator at 2 to 3°C (Bakht et al., 2011).

Inoculation and extract loading

From the refined or cultured plates of known microbes, 1 to 2 colonies were picked by sterile swab technique and streaks three times on the agar surface while, the swab was disposed of in the wake of utilizing (Bakht et al., 2011). Autoclaved forceps were used for putting circle on plates. Approximately 50 μl of plants crude extracts with concentration of 100 mg/ml were applied on each disc using micropipette while for negative and positive control another plate was used by applying 25 μl each for specific duration.

Statistics analysis

Statistix 8.1 programming was used for recording the information obtained and the ANOVA, while for making the Graphs, Ms Excel programming was utilized after descriptive statistics.

RESULTS

Phytochemical analysis

After the experiment, the presence or absence of phytochemicals, that is Terpenoids, Flavonoids, Alkaloids, Tannins, Saponins, Phenols, Quinones, Coumarins, Glycosides and Cardiac glycosides was checked, and the result showed that Alkaloids had positive outcome for L. angustifolia extract but showed negative effect for T. vulgaris and C. citratus, while alkaloids showed positive results for O. americanum, O. basilicum, C. papaya and in R. officinalis. Additionally, all the chosen medicinally important test plants have Coumarin except L. angustifolia and the extract of C. papaya which have no coumarins. Outcomes also showed positive effect of tannins and saponins in entirely the seven test plants in high amount. Flavenoids have top application in all the test plants except in O. basilicum which have little concentration and were totally absent in C. papaya and in L. angustifolia. Quinones presented optimistic results for all test plant except C. papaya and C. citratus which showed negative results. Terpenoids were encouraging in entire plants while undesirable result was found in O. americanum and C. papaya. With high concentration, phenols were progressive in all the selected medicinal plants extracts. Glycosides and Cardiac glycosides were existing in all the test plants although both were absent in L. angustifolia and slightly in low concentration in C. papaya (Table 2). Results obtained for the antimicrobial activity showed that the tested seven medicinal plants possess antimicrobial activity against X. oryzae, S. aureus and E. coli. When tested using disc diffusion method, ethanolic extract of C. citratus showed significant activity against C. citratus with 12 mm of inhibitions zone and minimum action were against S. aureus with zone of inhibition of 0.33 mm, while E. coli have intermediate activity with zone of inhibition of 0.53 mm. Maximum zone recorded in T. vulgaris is in contradiction with Xanthomonas with a value of 7.66 mm, while lowest activity was observed against S. aureus and standard activity was 0.46 mm of E. coli. O. americanum showed supreme inhibition zone against X. oryzae (6 mm), whereas minimum bustle was recorded against S. aureus (0.16 mm) and average activity of E. coli (0.20 mm). O. basilicum showed all-out inhibition (5 mm) against X. oryzae and showed minute activity against E. coli (0.46 mm) and transitional activity against S. Aureus with zone of inhibition of 0.50 mm. Rosmarinus officinalis showed significant activity against X. oryzae with zone of inhibition of 4 mm. Beside, least action was shown against E. coli (0.63 mm) though intermediate activity were recorded (1.33 mm) against S. Aureus. L. angustifolia showed active performance against X. oryzae with zone of inhibition of 4.33 mm and least activity verified against S. aureus 0.16 mm and middling activity was 0.50 mm against E. coli. C. papaya showed maximum activity against X oryzae with inhibition recorded zone of 4.33 mm, while minimum activity was observed in S. aureus (0.23 mm) and moderate activity in E. coli (0.60 mm). Significant activity was recorded in X. oryzae (p≤0.05) (Table 3 and Figures 2 and 3).

DISCUSSION

Chemical tests were performed on crude extracts of seven
Table 2: Qualitative analysis of the phytochemicals.

<table>
<thead>
<tr>
<th>Phytochemicals/Plants</th>
<th>Thymus vulgaris</th>
<th>Lavandula angustifolia</th>
<th>Ocimum americanum</th>
<th>Ocimum basilicum</th>
<th>Cymbopogon citratus</th>
<th>Carica papaya</th>
<th>Rosmarinus officinalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Saponin</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Coumarin</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+++</td>
<td>_</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>


Table 3: All pair wise comparison test for all strains.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Treatments</th>
<th>Xanthomonas oryzae</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive control</td>
<td>0.0000d</td>
<td>0.0000c</td>
<td>0.0000c</td>
</tr>
<tr>
<td>2</td>
<td>Negative control</td>
<td>0.6667d</td>
<td>0.0367c</td>
<td>0.1333ab</td>
</tr>
<tr>
<td>3</td>
<td>Cymbopogon citratus</td>
<td>12.000a</td>
<td>0.3333bc</td>
<td>0.5333ab</td>
</tr>
<tr>
<td>4</td>
<td>Rosmarinus officinalis</td>
<td>4.0000c</td>
<td>1.3333a</td>
<td>0.6333a</td>
</tr>
<tr>
<td>5</td>
<td>Thymus vulgaris</td>
<td>7.6667b</td>
<td>0.3000bc</td>
<td>0.4667bc</td>
</tr>
<tr>
<td>6</td>
<td>Carica papaya</td>
<td>4.3333c</td>
<td>0.2333bc</td>
<td>0.6000a</td>
</tr>
<tr>
<td>7</td>
<td>Ocimum americanum</td>
<td>6.0000bc</td>
<td>0.1667bc</td>
<td>0.2000bcd</td>
</tr>
<tr>
<td>8</td>
<td>Ocimum basilicum</td>
<td>5.0000c</td>
<td>0.5000b</td>
<td>0.4667abc</td>
</tr>
<tr>
<td>9</td>
<td>Lavandula angustifolia</td>
<td>4.3333c</td>
<td>0.1667bc</td>
<td>0.5000abc</td>
</tr>
<tr>
<td>10</td>
<td>LSD(0.05)</td>
<td><strong>2.2148</strong></td>
<td><strong>0.3906</strong></td>
<td><strong>0.3556</strong></td>
</tr>
</tbody>
</table>

Figure 2: Effect of ethanolic extracts of medicinal plants against tested strains.
medicinal plants to scrutinize phytochemicals (Figure 4). The tests conducted during research were on Tannins, Saponins, Flavonoids, Alkaloids, Quinones, Terpenoids, plants material will be helpful for the augmentation of various crude drugs (Gurumurthy et al., 2008). Phytochemical analysis of Tannins of Ethanolic extracts were positive in all tests plants which were previously supported by Akrayi and Abdulrehman (2013), Oancea et al. (2013), Okere et al. (2014) and Adham (2015), while the result of the present study is not in line with the findings of Asressu (2013) and Rasha-Saad et al. (2014). Tannins contained in plants can be used to treat diarrhoea (Yoshida et al., 1991). It is also used as anti-inflammatory and antiseptic (Haslam, 1989). The presence of coumarin in plants may be helpful for antitumor activity in humans (Weber et al., 1998). This is not in line with the finding of Akrayi et al. (2013). In the present study, all the selected plants have saponin, and this is in line with the finding of Rashed et al. (2013). It is helpful for antibody production and the ability to stimulate immune system (Oda et al., 2000). The presence of alkaloids in all the tested plants is in line with the finding of Geetha and Geetha (2014), but contrary to the finding of Asressu (2013). Alkaloids have the best qualities in terms of muscle relaxant properties (Booj, 2000). Our recent work on flavonoids is in line with the finding of Okere et al. (2014). Flavonoids present in plants have the activity against microbes (Okwu, 2004). Study quinones is in disagreement with the finding of Jiju et al. (2013). The presence of quinones maybe helpful for recorded against tested microbial strains such as S. aureus, E.coli and X. oryzae. The present study showed the Phenols, Coumarins, Glycosides and Cardiac glycosides. Various activities have been shown by previous different researches on phytochemicals. Phytochemicals present in trauma (Kalayci et al., 2011). In the present study, the phenols concentration in the selected plants is in line with that reported by Haddouchi et al. (2011) but contrary to that reported by Arakayi and Abdulrehman (2013). They serve as anti-allergic, anti-inflammatory, anti-tumour, antibacterial, anti-tumour, anti-oxidant and anti-viral agents (Hanasaki et al., 1994; Stefani et al., 1999). The various concentrations of terpenoids in our plants are in line with those reported by Rashed and Fouche (2013), but contrary to those reported by Rasha-Saad et al. (2014). They have the best anti-inflammatory, anti-malarial, antibacterial activities (Mahato and Sen, 1997). The findings of the present study on glycosides and cardiac glycosides are in line with the results obtained by Rashed et al. (2013) and Ayoola and Adeyeye (2010), but contrary to that of Neer (1995). Glycosides will be helpful for different biological function and cardiac glycosides may be helpful for contractile cardiac forces (Neer, 1995; Neer, 1995). Plant harvesting period may be responsible for the variation between our and previous studies. According to Ciulei et al. (1995), the amount of bioactive compounds present in plants is influenced by extraction procedure, geographic and climatic conditions.

The effects of selected medicinal plants, such as C. citratus, L. angustifolia, C. papaya, O. americanum, O. basilicum, T. vulgaris and R. officinalis were checked and significant activity of C. citratus among all the tested plants which is in agreement with the results of Hindumathy

**Figure 3:** (a) Activities of all tested plants against *Staphylococcus aureus* and *Escherichia coli* (b) *Thymus vulgaris*. (c) *Carica papaya*. (d) *Ocimum basilicum*. (e) *Rosmarinus officinalis*. (f) *Ocimum americanum*. (g) *Cymbopogon citratus* and (h) *Lavandula angustifolia*, showing activity against *Xanthomonas oryzae*. 
The C. citratus may showed the antimicrobial activity due to the presence of some bioactive components (Hamza et al., 2009). In the present study, Inhibition zone was shown by T. vulgaris against all the selected bacterial strains which is in line with previous studies of Dababneh (2007). Also in the present study, Ocimum species showed maximum inhibition zone against X. oryzae and also showed activity against S. aureus and E. coli and this is similar with the finding of Pawar and Pandit (2014) who found that the variety of basil Ocimum sanctum showed optimistic activity beside Xanthomomas. The leaf extract is Bio-ecologically compatible for the management of various strains of Xcmi (Pawar and Pandit, 2014). R. officinalis showed supreme activity against the selected strains and this is in agreement with the finding of Mousavi et al. (2014). The antimicrobial property of many plants may be due to the presence of alkaloids (Batista et al., 1994; Goji et al., 2006). L. angustifolia in our present study showed activity against selected three bacterial strains. This result is similar with the observations of Skwirzynska et al. (2014). On other hand, in varieties of lavender, the found zone of inhibition recorded was due to the lavender essential oils present in their flowers. In recent observation, C. papaya showed maximum activity against all the tests strains and similar finding was observed by Nirosha and Mangalanayaki (2013). The activity of its leaves extract may be due to some chemical compounds present in their leaves because according to Gracelin et al. (2012), its activity may be due to the presence of wide range of antibiotic compounds existing in diverse parts of the flowers.

Conclusion

The result of the current study showed the presence of bioactive compounds present in the seven medicinal plants studied. The present studies and previous studies proved that the plants have medicinally important properties and numerous physiological activities and as such, can be used to treat many ailments. The selected plants may be seen useful for drugs preparation. Therefore, it is suggested that further studies should be carried out to isolate the active compounds responsible for many activities of these plants. The results of the present investigation clearly indicate that the antibacterial activity vary with the species of the plants and plant material used. Thus, the study discovers the value of plants used in various traditional systems such as Indian medicine (Ayurveda), Chinese medicine, and various other traditional medicine which could be of extensive attention to the expansion of new drugs.

REFERENCES


