Comparative Study of Leaves and Stem Methanolic Extract on Antioxidant and Antimicrobial Activity through Quantitative Evaluation of Phytoconstituents

Raja Majumder\textsuperscript{a}, Moonmun Dhara\textsuperscript{b}, Lopamudra Adhikari\textsuperscript{c}

\textsuperscript{a,b}Research Scholar, Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Siksha’O’ Anusandhan University, Bhubaneswar.
\textsuperscript{c}Assistant Professor, Department of Pharmaceutical Analysis, School of Pharmaceutical Sciences, Siksha’O’ Anusandhan University, Bhubaneswar.

ABSTRACT

Ethnobotany has gifted many plants to the medicinal society. It is an effective way to discover future medicines. Leaves of \textit{Olax psittacorum} of family \textit{Olacaceae} have proven anti-inflammatory and anti-oxidant property. The aim of this study is to compare the methanolic extracts of stem and leaves with respect to their antioxidant and antimicrobial activities in accordance with the quantity of phytoconstituents they possess. Both stem and leaves were extracted using methanol and the phytochemical screening was done followed by the quantitative estimation of total Phenolic content, total flavonoid content and total Tannin content taking Gallic acid, Quercetin and Tannic acid as standards, respectively. Their antioxidant activity was determined using DPPH and ABTS radical scavenging activity taking Ascorbic acid as standard and the antimicrobial activity was determined using the well diffusion method taking both gram positive and gram negative bacteria and fungi. % Yield of LME is more than % Yield of SME. In terms of total phenolic content (in mg Gallic acid equivalent/g of extract) and flavonoids (in mg Quercetin equivalent/g of extract) LME (95.68 and 713.33, respectively) is more than that of SME (30.95 and 205.33, respectively). While the total tannin content (in mg Tannic acid equivalent/g of extract) of LME (15.28) is less than that of SME (146.50). The antioxidant property of LME is seen to be more than that of SME which is attributed to more flavonoid presence in LME than SME. But the antimicrobial activity of SME is seen to be more than that of LME which is attributed to the high quantity of tannin present in SME than LME.

Keywords: Phytochemical screening, Antioxidant, Antimicrobial, Tannin, Flavonoids.

1. INTRODUCTION

From ancient time in India, numerous plants are being used for treating various diseases (1). Still there remains gaps in terms of deep information and thus remains the scope of filling these gaps with the information about the medicinal properties of various species. It is very interesting fact that even different parts of a plant shows different level of activities with respect to the phytoconstituents and their concentration. These requires thorough investigation which provide remedy for different ailments (2). Herbal remedy has always got an edge over their synthetic counter parts being cost-effective and mostly because of fewer side effects. In the scenario of increasing resistance of bacteria and fungi towards the conventional medications which are currently available, the contribution of herbal alternative with antimicrobial activity can be taken as an alternative (3). The inherent anti-oxidative mechanism in human body control the biological functions like the anti-mutagenic, anti-carcinogenic and anti-ageing responses in the body. Antioxidants work by stabilizing or deactivating the free radicals often before they target the biological cells. Herbal plants, vegetables and fruits have been proven to have the presence of antioxidants such as phenolics, flavonoids, tannins and proanthocyanidines, thus making the herbal sources a store house of antioxidants (4).

\textit{Olax psittacorum} (Willd.) Vahl. belonging to the family \textit{Olacaceae} is a flowering plant found throughout the topical region of the world. The whole plant has saponin, olaxoside which when given orally two mice shows anti-inflammatory properties and decreases oedema induced carragenin. Olaxoside also shows laxative action (5). The leaf of \textit{Olax psittacorum} also shows antioxidant activity (6).

Aim of the present study is to compare the phytoconstituents both qualitatively and quantitatively, the free radical scavenging activity and the antimicrobial properties of the leaves with the stem extract of \textit{Olax psittacorum} and to determine the correlation between the antioxidant and antimicrobial activity with phytoconstituents by statistical analysis and to detect a new source of antimicrobial agent.
2. MATERIALS
Leaves and stem of Olax psittacorum was collected from Andharua, Bhubaneswar, Odisha and authenticated by Regional Plant Resource Centre (RPRC), Bhubaneswar (Authentication Field Number, RMOP-1). All the chemicals and solvents used in the study were of Merck Specialities Private Limited.

3. METHODS
3.1. Extract Preparation: Freshly obtained leaves and stem of *Olax psittacorum* was dried in shade for 7 days after which they were coarsely grounded and defatted using Petroleum ether. 500 g each of defatted grounded leaves and stem was macerated with methanol for 72 hours. They were filtered by vacuum filtration technique and the filtrate was concentrated using rotary evaporator to obtain leaves methanolic extract (LME) and stem methanolic extract (SME). LME was obtained as greenish black sticky mass and SME as deep brown sticky mass. % yield of both the extracts were calculated and compared (7).

\[
\text{% Yield} = \frac{\text{Amount of concentrated extract obtained}}{\text{Amount of defatted grounded material introduced for maceration}} \times 100 \quad \ldots \text{Formula 1.}
\]

3.2. Phytochemical Screening: LME and SME are subjected to various chemical procedures for the detection of alkaloid, carbohydrates and reducing sugar, flavonoid, glycoside, proteins and amino acids, saponin, steroids and triterpenoids and tannin, present in them (8).

3.3. Quantitative estimation of Phytoconstituents
3.3.1. Estimation of Total Phenolic Content: Total phenolic content of LME and SME was determined using Folin-Ciocalteu reagent taking Gallic acid as standard. Different concentrations (10-90 µg/ml) of Gallic acid and 1mg/ml of LME and SME was prepared using methanol. 0.5 ml of each sample was taken in a test tube. To it 2.5 ml of 10 fold diluted FC reagent was added followed by 2 ml 7.5% Na₂CO₃. Tubes were allowed to stand for 30 minutes at room temperature keeping it covered with paraffilm. The yellow colour of FC reagent turns blue in presence of polyphenols and their absorbance was calculated at 760 nm spectrophotometrically (9).

3.3.2. Estimation of Total Flavonoid Content: Total flavonoid content of LME and SME was determined by Aluminium chloride method taking Quercetin as standard. Different concentrations (100-900 µg/ml) of Quercetin and 1mg/ml of LME and SME was prepared using methanol. 1 ml of each sample was mixed with 4 ml of distilled water followed by 0.30 ml of 10% NaNO₂ solution and kept for 5 minutes. Then 0.30 ml of 10% AlCl₃ solution was added to it followed by 2 ml of 1% NaOH solution. It was mixed well and their absorbance was measured at 510 nm spectrophotometrically (6).

3.3.3. Estimation of Total Tannin Content: Total phenolic content of LME and SME was determined using Folin-Denis reagent taking Tannic acid as standard. Different concentrations (100-900 µg/ml) of Tannic acid and 1mg/ml of LME and SME was prepared using methanol. 1 ml of each sample was taken in a test tube. To it 1 ml of FD reagent was added followed by 2 ml 7.5% Na₂CO₃ and their absorbance were calculated at 700 nm spectrophotometrically (9).

3.4. In vitro antioxidant assay: Most acceptable two assay method i.e. DPPH (2,2’-diphenyl-1-picrylhydrazyl) & ABTS (2,2’-azinobis 3-ethylenbenzthiozoline-6 sulphonic acid) was followed to determine the in vitro antioxidant capacity & 1mg/ml concentration of solution of extracts were prepared by using methanol for both method & then from it the serial dilution was obtained i.e. 50, 100, 150, 200, 250µg/ml for antioxidant assay.

3.4.1. DPPH radical scavenging activity: Free radical scavenging activity of the dilutions was obtained by following the previously described method making some modification in the protocol (4, 10). DPPH assay was carried out by plotting inhibition percentage against extract concentration graph from which 50% inhibition concentration obtained which expressed as IC₅₀. 4ml of DPPH (20µg/ml) solution was mixed with 1ml of various extract concentration (50-250µg/ml) & then the mixture was shaken & incubated for 30 minutes at room temperature. Inhibition of DPPH free radical was measured through spectrophotometry at 517nm & inhibition percentage was calculated by the formula given below (11):

\[
\text{Inhibition (%)} = \left[ \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right] \times 100 \quad \ldots \text{Formula 2}
\]

where,
\[
A_{\text{control}} = \text{the absorbance of DPPH solution without any sample}
\]
\[
A_{\text{test}} = \text{the absorbance of DPPH solution with sample in different concentration}
\]
3.4.2. ABTS radical scavenging activity: ABTS cation scavenging activity was performed with slide modification (12, 13). ABTS solution (7.4 mM) in 1:1 ratio & was kept in dark place at -18°C overnight to obtained a dark colored greenish solution containing ABTS radical cations. This stock solution was diluted with methanol in 1:1 ratio. Then 1ml of extract solution was mixed with 4ml of working solution and incubated for 2 hours. Absorbance was measured at 734nm spectrophotometrically and the inhibition percentage was calculated by the following formula:

\[
\text{Inhibition} \ (%) = \left[ \left( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100 \right] \quad \text{…..Formula 3}
\]

where,

\[ A_{\text{control}} = \text{the absorbance of ABTS solution without any sample} \]

\[ A_{\text{test}} = \text{the absorbance of ABTS solution with sample in different concentration} \]

3.5. Antimicrobial study: The antimicrobial activity was studied using the well diffusion method which depends on the diffusion of extracts from a cavity through the solidified agar layer of petriplates to an extent, so that the growth of the inoculated microorganisms is prevented in a circular area or zone around the cavity containing the extracts (14). The antibacterial activity was studied against Bacillus stearothermophilus (gram positive), Staphylococcus aureus (gram positive), Klebsiella pneumonia (gram negative), Escherichia coli (gram negative), and Pseudomonas aeruginosa (gram negative). The antifungal activity was studied against Aspergillus niger and Candida albicans fungi. All these are clinically isolated bacterium which was directly collected from Department of Microbiology, NICU SUM Hospital, Bhubaneswar using an appropriate medium. The test strains (0.1ml) was inoculated into the media and uniformly spread. Wells dugout in the agar layer with the aluminium bore of 8mm diameter. 0.1ml of extracts dissolved in DMSO (dimethyl sulphoxide) was incorporated in the well. Then the plates are incubated at 37°C for 24 hours for antibacterial activity and 48 hours for antifungal activity. After incubation period the mean diameter of zone of inhibition around well was measured (15).

4. RESULT

Table 1: Yield amount and % Yield of LME and SME

<table>
<thead>
<tr>
<th>Extract</th>
<th>Amount of defatted grounded material introduced for maceration (g)</th>
<th>Amount of concentrated extract obtained (g)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>LME</td>
<td>500</td>
<td>235.55</td>
<td>47.11%</td>
</tr>
<tr>
<td>SME</td>
<td>500</td>
<td>15.85</td>
<td>3.17%</td>
</tr>
</tbody>
</table>

Percentage yield in Table 1 is obtained by using Formula 1. Equal amount (500 g) of grounded defatted leaves and stem were extracted using methanol. Percentage yield of LME (Leaves Methanolic Extract) was found to be 47.11% which is clearly more than the percentage yield of SME (Stem Methanolic Extract), i.e., 3.17%. This is evident from Table 1.

Table 2: Phytochemical Constituents present in LME and SME

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Phytoconstituents</th>
<th>LME</th>
<th>SME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates and reducing sugar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Glycoside</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Proteins and amino acids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Steroids and Terpenoids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Tannin</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 2 emphasizes on the results of phytochemical screening. ‘+’ sign denotes the presence and ‘-’ sign denotes the absence of particular phytoconstituents. On phytochemical screening (Table 2), it is evident that both LME and SME are devoid of alkaloid. Both LME and SME have carbohydrates and reducing sugar, flavonoids, glycosides, proteins and amino acids, saponin and tannin present in them. The only difference in this aspect is that LME contains steroids and triterpenoids and SME do not.

Table 3: Quantitative estimation of Total phenolic content, Total Flavonoid Content and Total Tannin content in LME and SME

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total Phenolic Content (in mg Gallic acid equivalent/g extract)</th>
<th>Total Flavonoid Content (in mg Quercetin equivalent/g extract)</th>
<th>Total Tannin Content (in mg Tannic acid equivalent/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LME</td>
<td>95.68</td>
<td>713.33</td>
<td>15.28</td>
</tr>
<tr>
<td>SME</td>
<td>30.95</td>
<td>205.33</td>
<td>146.50</td>
</tr>
</tbody>
</table>

Figure 1: Comparison of the presence of different Phytoconstituents present in LME and SME: (A) Total Phenolic Content, (B) Total Flavonoid Content and (C) Total Tannin Content

Table 3 indicates the quantity of total phenolic compounds, total flavonoids and total tannin in both the extracts. Total phenolic content, total flavonoid content and total tannin content per g of LME was found to be 95.68 mg Gallic acid equivalent, 713.33 mg Quercetin equivalent and 15.28 mg Tannic acid equivalent respectively. While, total phenolic content, total flavonoid content and total tannin content per g of SME was found to be 30.95 mg Gallic acid equivalent, 205.33 mg Quercetin equivalent and 146.50 mg Tannic acid equivalent respectively. It is clear from Table 3 and Figure 1 that Total phenolic content and Total flavonoid content of LME is more than that of SME. While, total tannin content of SME is more than that of LME.

Figure 2 summarizes the antioxidant activity study performed using LME, SME and Ascorbic acid. Figure 2(A) indicates the % of DPPH scavenging at different concentrations of LME, SME and Ascorbic acid. Ascorbic acid is taken as a standard in antioxidant activity study. Figure 2(B) indicates the % of ABTS scavenging at different concentrations of LME, SME and Ascorbic acid. In both Figure 2(A) and 2(B), it is seen that LME shows high free radical scavenging activity than SME. LME at higher concentrations shows almost similar free radical scavenging activity as that of Ascorbic acid.
Figure 2: Anti oxidant activity of LME, SME and Ascorbic acid at various concentrations. Each value represents a mean ± SD (n=3) (A) DPPH Radical Scavenging activity and (B) ABTS Radical Scavenging activity.

Table 4: Comparison of values of IC\textsubscript{50} of LME and SME with DPPH Radical Scavenging activity study and ABTS Radical Scavenging activity study

<table>
<thead>
<tr>
<th>Extracts</th>
<th>DPPH Radical Scavenging activity</th>
<th>ABTS Radical Scavenging activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LME</td>
<td>42.73 µg/ml</td>
<td>4.28 µg/ml</td>
</tr>
<tr>
<td>SME</td>
<td>91.10 µg/ml</td>
<td>62.08 µg/ml</td>
</tr>
</tbody>
</table>

IC\textsubscript{50} (concentration at which 50% inhibition or free radical scavenging is seen) values were obtained. It is seen in Table 4 and Figure 3 that the IC\textsubscript{50} value of LME from both the procedures of obtaining free radical scavenging activity is less than that of SME suggesting that LME has more free radical scavenging activity than SME.

Figure 4 shows the zone of inhibition of LME and SME against 6 strains of bacteria. As seen in Table 5 zone of inhibition of LME and SME in two different concentrations in seen. At 100 mg/ml LME fails to show zone of inhibition i.e., antibacterial activity except against Klebsiella pneumoniae. But in 250 mg/ml it shows zone of inhibition against all strains of bacteria, both negative and positive. SME shows antibacterial activity in both the concentrations except for Escherichia Coli.
Table 5: Antibacterial activity of LME and SME.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Test Organism</th>
<th>Zone of Inhibition(mm)</th>
<th>Zone of Inhibition(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LME</td>
<td>SME</td>
</tr>
<tr>
<td>1</td>
<td><em>Bacillus stearothermophilus</em> Gram positive</td>
<td>0</td>
<td>6.67 ± 0.58</td>
</tr>
<tr>
<td>2</td>
<td><em>Staphylococcus aureus</em> Gram positive</td>
<td>0</td>
<td>7.33 ± 1.15</td>
</tr>
<tr>
<td>3</td>
<td><em>Klebsiella pneumoniae</em> Gram negative</td>
<td>5.67 ± 0.58</td>
<td>10.33 ± 0.58</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia Coli</em> Gram negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td><em>Pseudomonas aeruginosa</em> Gram negative</td>
<td>0</td>
<td>10.33 ± 1.53</td>
</tr>
</tbody>
</table>

Figure 5 shows the zone of inhibition of LME and SME against 2 strains of fungi. Table 6 shows zone of inhibition of LME and SME in 100 mg/ml. Both LME and SME show positive results against the fungi used for testing. Zone of inhibition obtained with SME against both the strains of fungi is more than that of LME.
5. DISCUSSION
Methanol serves as the most popular solvent for extraction. Being popular in nature it pulls out most of the phytoconstituents from plants. In maceration maximum percentage yield of the extraction is shown with methanol (7). Percentage yield of LME is higher than the percentage yield of SME probably because stem may contain less polar materials than LME. Since both the grounded leaves and stem were initially defatted with petroleum ether so the nonpolar materials from stem would have come out with it and thus yielding less amount with methanol than seen with leaves.
Some percentage of steroids and triterpenoids were found in LME. This may be because of some error during the defatting of grounded leaves or because of some polar steroids which is probably not present in SME.
Biological functions of flavonoids as antioxidants are due to their free radical scavenging activity. Flavonoids have anti-aging and anticancer properties. Moreover, flavonoids helps preventing cardiovascular and neurodegenerative diseases (16). The antioxidant properties of flavonoids depend mostly on its concentration (17). Thus more is the quantity of flavonoid in the extract; more is the probability of showing the antioxidant property.

DPPH approach is commonly used to determine the antioxidant properties of a sample. DPPH is organic nitrogen radical with a UV-visible absorption in the range 515-520nm. The colour of DPPH which is initially violet fades upon reduction (18). ABTS is a synthetic radical which has the ability to determine the scavenging activity of both polar and nonpolar samples. It has the ability to be solubilize in both aqueous and organic solvents over a large pH range (19).

From the Figure2 (A) and Figure2 (B) it is seen that the LME is showing high scavenging activity at all concentrations as compared to SME. This may be because of higher concentration of flavonoid present in LME compare to SME as evident from Table 3 and Figure1. LME at higher concentration i.e. 200 and 250 µg/ml (Figure 2) shows closer alikeness with the free radical scavenging activity of ascorbic acid one of the best known antioxidant, taken here as a standard. IC\textsubscript{50} is the concentration of the sample at which 50 percent inhibition of free radical scavenging activity is seen (20). Thus, the extract showing less value of IC\textsubscript{50} will have higher free radical scavenging activity. The IC\textsubscript{50}Values of LME is less than that of SME after evaluating antioxidant property through DPPH and ABTS (Table 4 and Figure 3).

*Staphylococcus aureus*, a gram positive bacterium is considered as pathogen whose major site of colonization in human body is anterior nares. *S. aureus* cause infection that affect blood stream, skin, soft tissue and lower respiratory tracts (21). *S. aureus* one of the major causes of hospital-acquired and community acquire infections which results into serious problems (22). *Klebsiella pneumoniae*, as the name suggests is well known for causing nosocomial infections like pneumonia and Urinary tract infections (UTI) normally to patients with diabetic mellitus and neuropathic disorders (23). *E. coli*, a gram negative bacteria is commonly found in the lower intestine of warm-blooded organisms. The pathogenic *E. coli* causes serious food poisoning in humans (24). *Pseudomonas aeruginosa* also causes nosocomial infections. Moreover, this organism has incredible capacity to resist antibiotics (25). Candidiasis, caused by *Candida albicans* is a fungal infection that can affect skin, genitals, throat, mouth and blood (26). *Aspergillus niger* is a fungi which is generally recognized as safe according to US-FDA but due to its presence in air, food stuffs and other stored eatables, people get exposed to *A. niger* may cause allergic problems to them. Moreover, mycotoxins produced by them may be hepatocarcinogenic and nephrogenic in nature (27). These are the few reasons as why these strains have been choosen for this study. The antifungal action on *Candida albicans* can also be attributed to condensed tannins (28). The antimicrobial mechanism of tannins is related to its action on the membranes of the microorganisms. Tannic acid has inhibitory action towards the growth of intestinal bacteria (29).
The antibacterial and antifungal activity of SME is more than that of LME. It is seen that LME in the concentration 100mg/ml with DMSO shows almost no impact on antibacterial activity yet the antifungal activity is shown at this concentration. To check weather LME at all has the antimicrobial activity or not the concentration was increased to 250mg/ml with DMSO which shows the positive results and it reveals that LME has antibacterial activity at higher concentration though it is not seen in 100mg/ml. The antimicrobial activity of LME & SME may be attributed to their content of tannin. SME has more antimicrobial activity than LME as shown in Figure 4 and 5 and Table 5 and 6. This may be because SME have more tannin content than LME (Table 3 and Figure 1).

6. CONCLUSION

The present study compares the stem and leaves methanolic extract of Olax psittacorum in terms of their phytoconstituents, antioxidant properties and antimicrobial properties. An already established report suggested that leaves of O. psittacorum possess antioxidant property (6). Here, in this study, the stem of the plant is being proved to have both antioxidant and antimicrobial activity. It is also studied that the methanolic extract of leaves (LME) shows better antioxidant activity than stem methanolic extract (SME) while SME has much better antimicrobial activity than LME. The antioxidant property of LME at higher concentrations resembles the antioxidant property of ascorbic acid in the same concentration. The higher antimicrobial activity of SME is attributed to the higher quantity of tannin present in it while the high antioxidant property of LME is supported by the fact that it has high flavonoid content. Further studies may be carried out for the information regarding the in vivo safety and potential of the extracts that may prove to be an useful contribution to the medicinal knowledge.

Acknowledgements

We sincerely acknowledge the support of School of Pharmaceutical Sciences, SOA University for providing us facilities and environment to work. Our sincere thanks to RPRC, Bhubaneswar who authenticated the plant used. Sincere thanks to the support of Department of Microbiology, NICU, SUM Hospital, Bhubaneswar for providing the bacterial and fungal strains for antimicrobial study.

Reference

1) McChesney J. D. et. al. (2007); Plant natural products: Back to the future or into extinction?, Phytochemistry; Vol. 68; PP 2015-2022.
2) Das P. K. et. al. (1995); Some medicinal plants used by the tribals of Koraput, Orissa; Ancient Science of Life; Vol. XIV; PP 191-196.
3) Silva N. C. C. and Fernandes J. A. (2010); Biological properties of medicinal plants: A review of their antimicrobial activity; The Journal of Venomous Animals and Toxins including Tropical diseases; Vol. 16; Issue 3; PP 402-413.
4) Saeed N. et. al. (2012); Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L.; BMC Complimentary and Alternative medicine; Vol.12; PP 1-12.
5) Forgacs P. and Provost J. (1981); Olaxoside, a saponin from Olax andromenesis, Olax glabrilora and Olax psittacorum; Phytochemistry; Vol. 20; No.7; PP 1689-1691.
6) Sahu R. K. et. al. (2013); DPPH free radical Scavenging activity of some leafy vegetables used by tribes of Odisha, India; Journal of Medicinal plantsstudies; Vol. 1; Issue 4; PP 21-27.
7) Murugan R. et. al. (2014); Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from OsbeckiaparvifoliaArn.- An in vitro approach; Journal of King Saud University-Science; Vol. 26; PP 267-275.
8) Tiwari P. et. al. (2011); Phytochemical screening and Extraction: A review; InternationalPharmaceuticaSciencea; Vol. 1; Issue 1; PP 98-106.
9) Sultana M. et. al. (2012); Quantitative analysis of Total Phenolic, Flavonoid and Tannin contents in acetone and n-hexane extracts of Ageratum conyzoids; International Journal of ChemTech Research; Vol. 4; PP 996-999.
10) Soni A. and Sosa S. (2013); Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts; Journal of Pharmacognosy and Phytochemistry; Vol.2; Issue 4; PP 22-29.
1) Kumbhare M. R. et. al. (2012); In vitro antioxidant activity, phytochemical screening, cytotoxicity and total phenolic content in extracts of Caesalpinia pulcherrima (Caesalpiniaceae) pods; Pakistan Journal of Biological Sciences; Vol. 15; Issue 7; PP 325-332.

2) Nguyen V. T. (2015); Phytochemicals and antioxidant capacity of Xiao tam phan (Paramignyatrimera) root as affected by various solvents and extraction methods; Industrial Crops and Products; Vol. 67; PP 192–200.

3) Skotti E. et. al. (2014); Total phenolic content, antioxidant activity and toxicity of aqueous extracts from selected Greek medicinal and aromatic plants; Industrial Crops and Products; Vol. 53; PP 46-54.

4) Valya G. et al (2009); In vitro antimicrobial study of root extract of Chlorophytum raronundineaeum Baker; Natural Product Radiance; Vol. 8; Issue 5; PP 503-506.


6) Pawlak K. et. al. (2010); Antioxidant activity of flavonoids of different polarity, assayed by modified ABTS cation radical decolorization and EPR technique; ActaBiologicaCorcoviensia Series Botanica; Vol.52; Issue 1; PP 97-104.

7) Prochazkova D. et. al. (2011); Antioxidant and prooxidant properties of flavonoids; Fitoterapia; Vol. 82; PP 513-523.

8) Roy P. et. al. (2011); Preliminary study of the antioxidant properties of flowers and roots of Pyrostegiavenusta (Ker Gawl) Miers; BMC Complementary and Alternative Medicine; Vol. 11; PP 1-8.

9) Haddouchi F. et. al. (2014); Antioxidant activity profiling by spectrophotometric methods of aqueous methanolic extract of Helichysumstoechas subsp. Rupestri and Phagnalonsaxatile subsp. saxatile; Chinese Journal of Natural Medicines; Vol. 12; Issue 6; PP 415–422.

10) Oliveira A. M. F. et. al. (2012); Total phenolic content and antioxidant activity of some Malvaceae family species; Antioxidants; Vol. 1; PP 33-43.

11) Plata K. et. al. (2009); Staphylococcus aureus as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity; ActaBiochimicaPolonica; Vol. 56; No. 4; PP 597-612.

12) Diekema D. J. et. al. (2015); Survey of infections due to Staphylococcus species: Frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and the Western Pacific region for the SENTRY antimicrobial surveillance program, 1997-1999; Supplement Article: Staphylococcal Infections and Resistance; PP S114-S132.

13) Podschum R. and Ullmann U (1998); Klebsiella spp. as NosomialPthogens; Epidermology, Taxonomy, Typing methods and pathogenicity factors; Clinical Microbiology Reviews; Vol. 11; No. 4; PP 589-603.

14) Kaper J.B. et. al. (2004); Pathogenic Escheichia coli; Nature Reviews: Microbiology; Vol. 2; PP 123-140.

15) Mesaros N. et. al. (2007); Pseudomonas aeruginosa: resistance and therapeutic options at the turn of the new millennium; Clinical Microbiol Infect; Vol. 13; PP 560-578.

16) Molero G. et. al. (1998); Candida albicans; Genetics, dimorphism and pathogenicity; Internital. Microbiol.; Vol. 1; PP 95-106.

17) Gautam A. K. et. al. (2011); Diversity, pathogenicity and toxicology of A. niger: An Important Spoilage Fungi; Research Journal of Microbiology; Vol. 6; Issue 3; PP 270-280.

18) Ishida K. et. al. (2006); Influence of tannins from Stryphnodendronadstringens on growth and virulence factors of Candida albicans; Journal of Antimicrobial Chemotherapy; Vol.58; PP 942-949.

19) Akiyama H. et. al. (2001); Antibacterial action of several tannins against Staphylococcus aureus; Journal of Antimicrobial Chemotherapy; Vol.48; PP 487-491.