Antiglucosidase Activity and Phytochemical Evaluation of *Tanacetum parthenium*

*Saugata Dutta*, Sujit Biswas, Mohabbat Ullah, Shuvra Debnath, Jamilur Rahman Bhuiya, Amdadul Huque

1*Department of Pharmaceutical, Chemical & Environmental Sciences, School of Science, University of Greenwich, Chatham Maritime, Kent ME4 4TB, United Kingdom.
2Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.
3Department of Pharmacy, University of Development Alternative, Dhaka-1209, Bangladesh.
4Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh.
5Department of Pharmacy, East West University, Aftabnagar, Dhaka-1219, Bangladesh.

Available Online: 29*th November, 2014

**ABSTRACT**

*Tanacetum parthenium* is a medicinal plant of multifarious implications. Its application on the human being is increasing day by day, but still a lot of its beneficial properties are to be disclosed. The aims of this study were to probe the antiglucosidase activity of *T. parthenium* and to evaluate the phytochemical properties of this plant. We observed whether there is any color change after termination of a chromogenic reaction. Then we measured the mean of the absorbance reading for the water extract, ethyl acetate extract, petroleum ether extract of *T. parthenium* and the crude *Tanacetum parthenium* of different concentrations. We analyzed different plant parts (leaf and root), DCQA (Dicaffeoyl quinic acid), plant part together with DCQA (Dicaffeoyl quinic acid), petroleum ether extract, ethyl acetate extract and water extract of that plant part and the standard by gradient HPLC at two wavelengths- 215 nm and 280 nm. The color of the water extract, ethyl acetate extract, petroleum ether extract of *T. parthenium* and the crude *T. parthenium* remained unchanged. So, we can say that there is no antiglucosidase activity at all neither in the extracts of *T. parthenium* nor in the crude *T. parthenium*. At both the wavelengths, all the samples clearly indicated that the leaf sample of *T. parthenium* has the desired compound. The other samples like root, DCQA, combination of DCQA and root did not have the certainty that the desired compound will exist in these samples. In case of petroleum ether, there is no probability to get the desired compound in the extract whereas there is a probability to get the standard compound in the water extract. At both the wavelengths, ethyl acetate extract retained a strong probability for the existence of the desired compound. *T. parthenium* does not possess any type of antiglucosidase property. Moreover, out of different studied samples leaf and ethyl acetate extract of *T. parthenium* root showed the best results. So, more effective and optimized drugs can be developed from leaves and ethyl acetate extract of *T. parthenium*.

**Key Words:** *Tanacetum parthenium*, feverfew, antiglucosidase, phytochemical, HPLC, extract.

**INTRODUCTION**

*Tanacetum parthenium* (L.) Schultz Bip. is an aromatic perennial plant which is basically found in the northern hemisphere. The plant is also been cultured in gardens1. It is commonly known as feverfew2. This plant has long been used to treat headache, stomach-ache, menstrual irregularities etc. by the western herbal experts3. Feverfew can be used in anti-bacterial treatments. *T. parthenium* can also be used to be cured from fever, toothache and insect bite4. Whole dried leaf of feverfew can prevent migraine5. Feverfew is also been used as a traditional medicine for arthritis6. Compounds found in *T. parthenium* and alcoholic extract of *T. parthenium* have metal chelating and free radical scavenging property4. In the United States of America, *T. parthenium* is now one of the 50 best-selling supplements. At present *T. parthenium* can be found in capsules, bag tea3 and also as extracts and powder form5. It also has its application in preparing different dishes1.

Antiglucosidases are highly potential to treat diabetes type II. They are also very potential in treating HIV infection, metastatic cancer, obesity, glycosphingolipid lysosomal storage disease etc. Now more complicated diseases have been tried to be cured by the antiglucosidases7. Since late 1970s, extensive research work has been done on *T. parthenium*. But still no research work has been done to examine the existence of antiglucosidase activity of this plant. If there is any antiglucosidase activity found in feverfew, it can be used to treat some threatening diseases. Furthermore, from the phytochemical evaluation, potential

*Author for correspondence*
plant-part and extract of *T. parthenium* can be specified and that can be used to prepare more effective and optimized drugs. The aims of our study were to find out whether there is any antiglucosidase activity in this plant and to evaluate the phytochemical properties of the plant.

**MATERIALS AND METHODS**

Plant: *Tanacetum parthenium* collected from market.

Chemicals:
- Methanol - HPLC grade (Fisher Scientific, Leicestershire, UK)
- Petroleum ether - Laboratory reagent grade (Fisher Scientific, Leicestershire, UK)
- Ethyl acetate - Laboratory reagent grade (Fisher Scientific, Leicestershire, UK)

Equipments:
- Multiskan EX (ThermoLabsystems)
- HPLC 1200 series (Agilent Technologies)
- ZORBAX Eclipse XDB- C18 column (Agilent Technologies)

Preparation of Extracts:
15.73 mg of *Tanacetum parthenium* root extract was taken. It was fractioned by 300 ml petroleum ether for 3 times. Then again the remaining was fractioned by 300 ml ethyl...
Fig. 2: Chromatogram of T. parthenium standard at 215 nm wavelength

Fig. 3: Chromatogram of T. parthenium standard at 280 nm wavelength

Fig. 4: Chromatogram of T. parthenium leaf (5mg/ml) at 215 nm wavelength (Sequence 1)

Fig. 5: Chromatogram of T. parthenium leaf (5mg/ml) at 280 nm wavelength (Sequence 1)

Fig. 6: Chromatogram of T. parthenium leaf (5mg/ml) at 215 nm wavelength (Sequence 2)
acetate for 3 times. Anhydrous sodium sulphate was added with both petroleum ether and ethyl acetate to absorb water from there. The remaining was water extract. In this way, we got three extracts from the crude extract- petroleum ether extract, ethyl acetate extract and water extract of *T. parthenium*. Then we made three solutions from them- petroleum ether extract (1mg/ml), ethyl acetate extract (1mg/ml) and water extract of *T. parthenium* (1 mg/ml).

α-Glucosidase Inhibition Assay: In this study, we evaluated the antgliucosidase activity according to Kazem et al (2013) with minimal modifications [8]. There a reaction mixture was taken in a plate. The mixture was containing 25 µL of 250 mM phosphate buffer (pH 6.8; it contained 0.12 M Na₂HPO₄ and 0.13 M NaH₂PO₄), 25 µL of 1.2 unit/ml α-glucosidase, 25 µL of test sample, 25 µL of 2.5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) was added. 25 µL of 1.2 unit/mL α-glucosidase was basically added to make a full concentration of 0.3 unit/mL α-glucosidase. The plates were incubated at 37°C for 10 minutes. Then the reaction was terminated by adding 100µl of 0.2 M Na₂CO₃. We measured the absorbance reading 4-nitrophenol at 405 nm for different test samples as water extract, ethyl acetate extract, petroleum ether extract of *T. parthenium* and the crude *T. parthenium* at different concentrations: 66.66 µg/ml, 22.22 µg/ml, 7.40 µg/ml, 2.46 µg/ml, 0.82 µg/ml, 0.27 µg/ml. We also measured the absorbance reading of a control and a blank sample. We know that in analysing there is always a possibility of error in report. So, to minimize the error we measured all the absorbance readings for three times and then took the average of those readings.

HPLC Analysis: Here in all the cases we took the chromatogram at two wavelengths- 215 nm and 280 nm. 9 mg of root of *Tanacetum parthenium* was dissolved in 1.8 ml methanol. Then 8 mg of leaf of *T. parthenium* was dissolved in 1.6 mg of methanol. In both the cases, the concentration was 5mg/ml solution in methanol using gradient HPLC system. The solutions were analyzed by HPLC. Then *T. parthenium* standard and DCQA (0.5 mg/ml) were analyzed respectively by HPLC. After that, combinedly *T. parthenium* root (5 mg/ml) and DCQA (0.5 mg/ml) was analyzed by HPLC. Then we analyzed petroleum ether extract (1mg/ml), ethyl acetate extract (1mg/ml) and water extract of *T. parthenium* (1 mg/ml) by HPLC.

**RESULTS AND DISCUSSION**

The color was unchanged throughout 24 hours of

| Table 4: Data analysis of petroleum ether extract of *T. parthenium* |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Control     | 66.66µg/ml | 22.22µg/ml | 7.40µg/ml | 2.46µg/ml | 0.82µg/ml | 0.27µg/ml | Blank  |
| Average     | 0.594      | 0.568      | 0.559667   | 0.463333   | 0.521333   | 0.532333   | 0.541667 | 0.036333 |
| Standard Deviation | 0.02   | 0.012124  | 0.005508   | 0.038553   | 0.031262   | 0.018502   | 0.033081 | 0.001258 |
| Square Root  | 1.732051   | 1.732051   | 1.732051   | 1.732051   | 1.732051   | 1.732051   | 1.732051 | 1.732051  |
| Standard Error| 0.011547   | 0.007      | 0.00318    | 0.022259   | 0.018049   | 0.010682   | 0.019099 | 0.000882  |

termination of the reaction for all the individual test samples. As we did not notice any color changes for any of the test samples- water extract, ethyl acetate extract, petroleum ether extract of *Tanacetum parthenium* and the crude *T. parthenium*, we can firmly say that there is no antgliucosidase activity in our tested samples, that is, neither in the extracts nor in the crude sample.

The report that we got from the Multiskan EX has been given in Table 1. In case of water extract of *Tanacetum parthenium* (Table 2), we noticed the highest average absorbance (0.631667) in the control and the lowest average absorbance (0.038667) in the blank sample. We also measured the standard deviation of water extract of *T. parthenium* in all different concentrations. Then we calculated the standard error value of water extract of *T. parthenium* at all different concentrations dividing the standard deviation value by the square root value of times of analysis. Here we did all the analyses for three times, so in all the cases the square root value will be 1.732051. At 0.27 µg/ml concentration it showed the highest standard error (0.023667) and at the blank sample it showed the lowest standard deviation (0.002333). We incorporated the readings of the average absorbance and standard error in the Figure 1. In case of ethyl acetate extract, petroleum ether extract of *T. parthenium* and crude *T. parthenium*, we again saw the highest average absorption in control samples (0.617333, 0.594 and 0.603333 respectively) and the lowest average absorption in blank samples (0.037667, 0.036333 and 0.037 respectively). In the same way as previous, we measured the readings of standard deviation and standard error of all the samples at all different concentrations. We showed the readings of average absorbance, standard deviation and standard error in Table 2, 3, 4 and 5. But in case of ethyl acetate extract of *T. parthenium* we saw the highest standard error (0.019519) at 2.46 µg/ml concentration and the lowest standard error (0.001667) at the blank sample. In case of petroleum ether extract of *T. parthenium*, it showed the highest standard error (0.022259) at 7.40 µg/ml concentration and the lowest standard error (0.000882) at the blank sample and in case of crude *T. parthenium*, it showed the highest standard error (0.01618) at 2.46 µg/ml concentration and the lowest standard error (0.000577) at the blank sample. We incorporated all the readings of average absorption and standard error in the Figure 1.

The HPLC analysis report of the standard solution and the sample solutions were collected. It is not always possible to achieve full precision in laboratory works. As a reason, the experimental reading can vary from the predicted one. The variation in result can be due to man, machine,
Fig. 7: Chromatogram of T. parthenium leaf (5mg/ml) at 280 nm wavelength (Sequence 2)

Fig. 8: Chromatogram of T. parthenium leaf (5mg/ml) at 215 nm wavelength (Sequence 3)

Fig. 9: Chromatogram of T. parthenium leaf (5mg/ml) at 280 nm wavelength (Sequence 3)

Fig. 10: Chromatogram of T. parthenium root (5mg/ml) at 215 nm wavelength (Sequence 1)

Fig. 11: Chromatogram of T. parthenium root (5mg/ml) at 280 nm wavelength (Sequence 1)
materials etc. Here in every case we took the chromatograms in two wavelengths- at 215 nm and 280 nm. We took the chromatograms of the standard and the sample, that is, \textit{T. parthenium} leaf, \textit{T. parthenium} root, DCQA, \textit{T. parthenium} root and DCQA, petroleum ether extract, ethyl acetate extract and water extract of \textit{T. parthenium}.

From the chromatogram of the \textit{Tanacetum parthenium} standard at 215nm wavelength (Figure 2) we can clearly notice that at there was a large peak having retention time of 36.625 minutes. Its width was 0.2446 minutes, height was 1471.88428mAU and it covered 58.9014\% of the whole area under the curve. This was the most obvious peak in that chromatogram. In case of 280 nm wavelength (Figure 3), a large peak was noticeable whose retention time was 40.215 minutes having 0.3925 minutes width, 304.95422 mAU area, 12.08264 mAU height and 32.855\% of the whole area under the curve.

By comparing these chromatograms with the chromatograms of the samples, we can see that at 215 nm wavelength at the first chromatogram of \textit{Tanacetum parthenium} leaf (5 mg/ml) (Figure 4) there was a long peak having 36.749 minutes of retention time. Here we can say that the particular compound showing the highest peak at the standard was also present in the leaf sample. The second chromatogram of \textit{T. parthenium} leaf sample (Figure 5) had the highest peak at 36.595 minutes of retention time and the third chromatogram of the same sample (Figure 6) had the highest peak at 36.657 minutes of retention time. These three peaks represented the highest peak of the standard at the same wavelength, i. e., the little variation occurred in between them might be due to the reason that different factors effected the predicted reading. So, the specific compound representing the highest peak at the standard was also present in the second and third leaf sample. Overall, at 215 nm wavelength, all three \textit{T. parthenium} leaf samples showed the particular compound which was present in the \textit{T. parthenium} standard.

In case of 280 nm wavelength, the first chromatogram of \textit{Tanacetum parthenium} leaf sample (Figure 7) possessed a large peak whose retention time was 40.455 minutes. The second and third sample of \textit{T. parthenium} leaf also exhibited large peaks having retention time of 40.651 minutes (Figure 8) and 39.804 minutes (Figure 9) respectively. These three peaks indicated the highest peak of the standard. These three chromatograms also expressed that these samples had the same compound as the \textit{T. parthenium} standard. So, all the samples of both the wavelengths clearly indicated that the leaf sample of \textit{T. parthenium} had the same compound as the standard of \textit{T. parthenium}.

For \textit{Tanacetum parthenium} root (5 mg/ml) sample, we did the HPLC analysis for three times (Figure 10, 11 and 12 respectively), too. But at 215 nm wavelength, we did not see any noticeable peak at any of these three chromatograms at or around the retention time where the standard had the highest peak. At 280 nm wavelength, at the first (Figure 13) and second chromatogram (Figure 14), we noticed two little but clear peaks having 40.017 minutes and 40.248 minutes of retention time respectively which were indicating the highest peak at 280nm wavelength of \textit{T. parthenium} standard. At the third time (Figure 15) though there was a peak at 40.291 minutes of retention time, it was not that much clear like the peaks of earlier chromatograms. Here, in brief, we can say that the compound existing in the standard might be available at the root sample, but we cannot guarantee about the existence of that compound in the root sample.

In case of DCQA (0.5 mg/ml), at 215nm wavelength the chromatogram (Figure 16) did not possessed a clear peak at or around 36.625 minutes of retention time as the 215 nm signal of standard had. But at 280 nm signal, the DCQA chromatogram (Figure 17) exhibited a clear peak having retention time of 40.055 minutes which represented the highest peak of \textit{Tanacetum parthenium} standard at 280 nm wavelength. That peak of the DCQA sample at 280nm wavelength indicated the probability of the existence of same compound as the standard of \textit{T. parthenium}, but as at the 215 nm wavelength it did not showed any similar peak as the standard one, so we are not sure about the presence of the same compound in the DCQA as the \textit{Tanacetum parthenium} standard.

When we analyzed the \textit{Tanacetum parthenium} root (5 mg/ml) and DCQA (0.5 mg/ml) together, at 215 nm wavelength (Figure 18) there was no peak at or around the retention time of the highest peak of the standard, but there we had a clear peak at 40.161 minutes of retention time at 280nm wavelength (Figure 19) which was close to the highest peak of \textit{T. parthenium} standard.

The chromatogram of petroleum ether extract of \textit{Tanacetum parthenium} (1 mg/ml) showed no clear peaks at or around 36.625 minutes and 40.215 minutes of retention time at 215 nm (Figure-20) and 280 nm wavelength (Figure 21)respectively. As there was no noticeable peaks at all at or around those mentioned retention time, we can say that the compound available in \textit{T. parthenium standard} was not present in the petroleum ether extract of \textit{T. parthenium}.

<table>
<thead>
<tr>
<th>Crude</th>
<th>Control 66.66\mu g/ml</th>
<th>22.22\mu g/ml</th>
<th>7.40\mu g/ml</th>
<th>2.46\mu g/ml</th>
<th>0.82\mu g/ml</th>
<th>0.27\mu g/ml</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>0.60333</td>
<td>0.570333</td>
<td>0.536667</td>
<td>0.550333</td>
<td>0.561333</td>
<td>0.541667</td>
<td>0.551667</td>
</tr>
<tr>
<td>Standard</td>
<td>0.027683</td>
<td>0.017039</td>
<td>0.008145</td>
<td>0.004163</td>
<td>0.028024</td>
<td>0.014572</td>
<td>0.019296</td>
</tr>
<tr>
<td>Deviation</td>
<td>1.732051</td>
<td>1.732051</td>
<td>1.732051</td>
<td>1.732051</td>
<td>1.732051</td>
<td>1.732051</td>
<td>1.732051</td>
</tr>
<tr>
<td>Square</td>
<td>0.015983</td>
<td>0.009837</td>
<td>0.004703</td>
<td>0.002404</td>
<td>0.01618</td>
<td>0.008413</td>
<td>0.011141</td>
</tr>
<tr>
<td>Root Error</td>
<td>0.011141</td>
<td>0.00577</td>
<td>0.002404</td>
<td>0.028024</td>
<td>0.014572</td>
<td>0.019296</td>
<td>0.000577</td>
</tr>
</tbody>
</table>

Table-5: Data analysis of crude \textit{T. Parthenium}
Fig. 12: Chromatogram of T. parthenium root (5mg/ml) at 215 nm wavelength (Sequence 2)

Fig. 13: Chromatogram of T. parthenium root (5mg/ml) at 280 nm wavelength (Sequence 2)

Fig. 14: Chromatogram of T. parthenium root (5mg/ml) at 215 nm wavelength (Sequence 3)

Fig. 15: Chromatogram of T. parthenium root (5mg/ml) at 280 nm wavelength (Sequence 3)

Fig. 16: Chromatogram of DCQA (0.5mg/ml) at 215 nm wavelength
For ethyl acetate extract of *Tanacetum parthenium* (1 mg/ml), there were two peaks showing 36.185 minutes and 36.831 minutes of retention time at 215nm wavelength (Figure 22). These two peaks indicated the probability of the existence of the same compound as the standard of the *T. parthenium*. At 280nm wavelength (Figure 23), we noticed two peaks at 40.135 minutes and 40.433 minutes of retention time. These two peaks were corresponding to the highest peak of *T. parthenium* standard at 280nm wavelength. Any of those two peaks can indicate the same compound of *T. parthenium* standard. So, at both the wavelengths the ethyl acetate extract of *T. parthenium* indicated the existence of same as the standard of *T. parthenium*.

At the chromatogram of water extract of *T. parthenium* (1 mg/ml) at 215nm wavelength (Figure 24) there was no clear peak at or around 36.625 minutes of retention time and at 280 nm wavelength (Figure 25) there was a peak at 40.440 minutes of retention time respectively. As only the
chromatogram at 280nm wavelength showed a peak corresponding to the peak of the *T. parthenium* standard, so there was an uncertainty of whether same the compound of the standard was present in that sample or not. Overall, we can say that there is no antiglucosidase activity at all neither in the extracts of *T. tanacetum parthenium* nor in the crude *T. parthenium*. Moreover, if we compare in between different parts of *T. parthenium*, leaf is that part of that plant that had the same compound as the standard, because at 215nm wavelength in all three trials, it showed noticeable peaks at 36.749 minutes (Figure 4), 36.595 minutes (Figure 5) respectively and 36.657 minutes of retention time respectively (Figure 6) and at 280 nm wavelength it also showed large peaks in all three trials at 40.455 minutes (Figure 7), 40.651 minutes (Figure 8) and 39.804 minutes (Figure 9) respectively. The highest peaks of the leaf represented the highest peaks of the standard with great similarity. These peaks assure the presence of the same compound as the standard. The other samples like root, DCQA, combination of DCQA and root did not have the certainty that the standard compound will exist in these samples. There is no distinct resemblance in between the
peaks of these samples and those of the standard. By comparing the extracts of *Tanacetum parthenium* we can say that, ethyl acetate was the best solvent for extraction of *Tanacetum parthenium*, as the ethyl acetate extract retained a strong probability for the existence of the same compound as the standard. It showed two peaks showing 36.185 minutes and 36.831 minutes of retention time at 215nm wavelength (Figure 22) which indicated the probability of the existence of the same compound as the standard of the *Tanacetum parthenium*. As a solvent, water was better than petroleum ether for *Tanacetum parthenium* extraction. Because at 280nm wavelength (Figure 25) there was a peak at 40.440 minutes of retention time in case of water extract. In water extract, there was at least a probability to get the standard compound in the extract, but in case of petroleum ether, there is no probability to get the standard compound in the extract, because there was no noticeable peaks at all at or around the peaks of the standard in the petroleum ether extract of *Tanacetum parthenium*.

So, in future, in developing a new drug or a new dosage form, we can apply what we have learnt from this study. There is no hope of having any antiglucosidase activity in *Tanacetum parthenium* or in its extracts. Researchers can keep in mind that leaf is a potential part of *Tanacetum parthenium* and if somebody wants to use the extract, ethyl acetate will be the best solvent for it. Water can also be a solvent of choice in this regard.

**CONCLUSION**

From our study we can clearly say that, *Tanacetum parthenium* does not possess any type of antiglucosidase property. Moreover, out of different studied samples, leaf and ethyl acetate extract of *Tanacetum parthenium* showed the best results. So, for drug development or other research concern, we can apply the concept that leaf is such a part of the plant, *Tanacetum parthenium*, that has a great potential. Furthermore, out of different extracts, ethyl acetate extract of *Tanacetum parthenium* has the highest potential. Water extract of *Tanacetum parthenium* is also having some potential. It is undoubtedly true that *Tanacetum parthenium* is a plant of great potential. So, further research works should be carried on *Tanacetum parthenium* so that this incredible herbal plant can be applied in more various ways for the well-being of mankind.

**REFERENCE**


