Phytochemical profiling and evaluation of modified resazurin microtiter plate assay of the roots of *Trillium govanianum*

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ABSTRACT

Trillium govanianum Wall. ex D. Don (Melanthiaceae *alt.* Trilliaceae), is native to the Himalayas**.** The present study, for the first time, was undertaken to explore the antimicrobial potential, to determine the minimum inhibitory concentration (MIC) values of the methanol extract of the roots of *Trillium govanianum* and its solid phase extraction (SPE) fractions by using resazurin microtiter assay (REMA) against Gram positive and Gram negative bacterial registered strains and to carry out phytochemical analysis. The remarkable amount of gallic acid equivalent phenolic and quercetin equivalent flavonoid content was manifested by MeOH extract (20.27±3.03 mg GAE/ g DW and 9.25± 0.50 mg QE/ g DW respectively). The GC/MS analysis revealed the presence saturated and unsaturated components. Considerable level of antibacterial potential against Gram-positive bacteria (MIC: 2.5-0.009 mg/mL) than against Gram-negative bacteria (MIC: 2.5-0.165 mg/mL) were observed. The use of microtiter plates has the advantage of lower cost, fast and quantitative results.

Keywords: *Trillium govanianum; Melanthiaceae*; *GC/MS*; SPE; REMA; MIC.

1. Introduction

Trillium govanianum Wall. ex D. Don (Melanthiaceae *alt.* Trilliaceae), commonly known as "nagchhatry" in India and "teen patra" or "matar zela" in Pakistan, distributed from Pakistan to Bhutan about 2500-3800 m altitude is indigenous to Himalayas ranges. The reported studies on different species of the genus *Trillium* have revealed that this genus is ample in steroidal saponins, e.g., steroidal saponins were found in *T. erectum* L. (Hayes et al., 2009), *T. kamtschaticum* Pall. (Wei et al., 2012) and *T. tschonoskii* Maxim. (Wang et al., 2013). Ttraditionally *Trillium. govanianum* have been reported to used as immuno-regulation, to cure sepsis, inflammation, wound healing, dysentry, menstrual, antiaging agent skin boils and verious sexual disorders (Pant and Samant, 2010) . The powder of this plant has also been used to treat anthelmintic and were found to have antitumor property (Khan et al., 2016).

Determination of phenolic and flavonoids contents in plants has vital importance, as its linked with the antioxidant phenomenon of plants. GCMS is a powerful technique in analytical

chemistry to determine molecular mass and structure with high accuracy and sensitivity. It is faster and provides better results than other conventional methods in this field such as chromatographic techniques. Resazurin dye based on colorimetric method, which determined the cellular metabolic reaction with reduced cell toxicity. Due to the bacterial resistance against certain antibiotic groups, there is need to develop consolidated drug testing methods against resistant bacteria to achieve a target of novel scaffolds in the field of medicine (Rates, 2001). Therefore, the present study was undertaken to explore the antimicrobial potential, to determine the minimum inhibitory concentration (MIC) values of the MeOH extract of the roots of *Trillium govanianum* and its SPE fractions by using resazurin microtiter assay (REMA) against Gram positive and negative bacterial registered strains and to carry out GCMS analysis.

2. Results and Discussion

2.1. *Resazurin microtiter assay (REMA)*

The MeOH extract of the roots of *Trillium govanianum* and its SPE fractions (TGMF1, TGMF2, TGMF3 & TGMF4**)** exhibited varying antibacterial activity by using Resazurin microtiter plate assay as shown in (Table 1). The MIC (mg/mL) values of the MeOH extract and its SPE fractions are shown in Table 1S. The results from (Table 1S) indicated that the MeOH extract (TGM) and four SPE fractions showed higher antibacterial activity against Gram-positive bacteria (MIC: 2.5-0.039 mg/mL) than against Gram-negative bacteria (MIC: 2.5-0.165 mg/mL). The SPE fraction TGMF1, which had the most polar components of the parent MeOH extract, showed most significant antibacterial activity against *M. luteus* (MIC: 0.156 mg/mL), and considerable antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli* and *K. oxytoca* (MIC: 2.5, 2.5, 1.25 and 2.5 mg/mL respectively).The SPE fraction TGMF2 showed most prominent antibacterial activity against *B. subtilis* and *M. luteus* (MIC: 0.615 and 1.25 mg/mL respectively), and showed mild activity against *S. aureus*, *E. coli* and *K. oxytoca* (MIC: 2.5, 2.5 and 2.5 mg/mL respectively). The SPE fraction TGMF3 showed most significant antibacterial activity against *B. subtilis* , *M. luteus* ,*S. aureus* and (MIC: 0.0195, 0.039 and 0.31 mg/mL respectively). The SPE fraction TGMF4 which contained the least polar components of the parent MeOH extract, exhibited notable antibacterial activity against *S. aureus, B. subtilis* and *M. luteus* (MIC: 0.615, 0.039 and 0.156 mg/mL) and showed no activity against *E. coli* and *K. oxytoca* (MIC: ≥10) activity against *E. coli* and *K. oxytoca* (MIC: 1.25 and 0.615 mg/mL respectively). Figure 6S shows result after 24 hours incubation. Literature reported that phenolic compounds and saturated and unsaturated

components are more sensitive to Gram positive than Gram negative bacteria (Gulluce et al., 2007), which is extended and confirmed in present studies. As reported earlier regarding the Resazurin microtiter plate assay (REMA), we concluded this method to be very effective, fast and reproducible (Khan et al., 2018).

2.2 Total phenolic contents in *Trillium govanianum*

The total phenolic content of MeOH extract and SPE fractions in terms of gallic acid equivalent per gram dry weight are presented in Figure S1. The MeOH extract showed highest content of gallic acid equivalent phenols (20.27± 3.03 mg GAE/ g DW), followed by the SPE fractions TGMF1, TGMF2, TGMF4 and TGMF3 (19.07±2.53, 16.70± 0.56, 15.768±1.44, and 13.35±3.43 mg GAE/ g DW, respectively).

2.3 Total flavonoid contents in *Trillium govanianum*

The total flavonoid content of MeOH extract and SPE in terms of quercetin equivalent per gram dry weight exhibited different levels of significant flavonoid contents. The total flavonoid content of MeOH extract of roots of *T. govanianum* and its SPE are presented in Figure S3. The TGM (MeOH extract) showed highest flavonoid content (9.250±0.50 mg QE/ g DW), followed by the SPE fractions TGMF1, TGMF2, TGMF4 and TGMF3 (8.882±0.79, 7.038±1.23, 6.684±1.23, and 6.407±1.14 GAE/ g DW respectively). A positive correlation was found to be present between the phenolic and flavonoid contents (correlation coefficient; R^2 = 0.9992 and 0.9877 respectively, Figure S2 & S4) suggesting that the antioxidant potential of phenols might be caused by the presence of flavonoids.

2.4 GC/MS identification of components of *Trillium govanianum*

The GC/MS analysis of *n*-hexane fraction of MeOH extract of the roots of *T. govanianum* revealed the presence of six components (Table S2). The major constituents were n-Hexadecanoic acid methyl ester (t_R: 6.67), n-Hexadecanoic acid (t_R: 7.01), 9,12-Octadecadienoic acid (Z,Z) (t_R: 7.48) and Octadecanoic acid (t_R: 7.53). The above mentioned identified components were reported biological active against bacterial strains. The GC/MS analysis of *n*-hexane revealed the presence of saturated and unsaturated components and reported biologically active (ur Rahman et al., 2015).

3. Experimental

See supplementary materials

4. Conclusion

The findings of current study support the notion that the use of different fractions on the basis of different solvent systems truly retrieves a complete phytochemical and biological profiling of plants. The presence of saturated and unsaturated components by GC/MS analysis was in agreement with that of other *Trillium* species and reported are biologically active. From phytochemical analysis, it is reasonable to assume that the antibacterial activity of the MeOH extract and its SPE fractions of the roots of *Trillium govanianum* might be, at least partly, owing to the presence of phenolic compounds and could be potential source of antimicrobial compounds. To our best knowledge, this is the first report showing significant phytochemical and antibacterial potential of *Trillium govanianum* indigenous to Pakistan.

Disclosure statement

The authors have declared that there is no conflict of interest.

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