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SHORT COMMUNICATION

Phytochemical profiling of aqeous methanolic leaf extract of *Triclisia gilletii* by gas chromatography (GC/MS) and liquid chromatography (HPLC-PDA–ESI/MS^n) tandem mass spectroscopy (MS): a pointer to its nephroprotection

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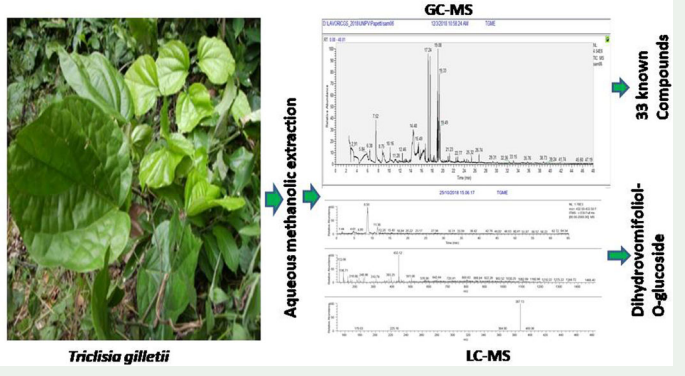
ABSTRACT

The phytochemical constituents in the aqueous methanolic leaf extract of *Triclisia gilletii* responsible for its nephroprotective potentials against ethane-1,2-diol induced nephrolithiasis as previously investigated in our laboratory were elucidated. The extract was prepared using 80% aqueous methanol in 72 h, Phytochemical contents of aqueous methanolic extract of *Triclisia gilletii* (TGME) was identified using both a Thermo Scientific DSQII single quadrupole gas chromatography (GC) and a Thermo Scientific liquid chromatography (LCQ Fleet system) tandem mass spectroscopy. The chromatogram acquisition, detection of mass spectral peaks and their waveform processing were performed using Xcalibur MS Software (Thermo Scientific Inc.). GC-MS analysis revealed the presence of phenols, fatty acids, vitamins and steroids. Likewise, for LC-MS analysis kaempferol and dihydrovomifoliol-O-glucoside were detected. The identified constituents have possible contributively effect on the acclaimed pharmacological potential of *Triclisia gilletii* against ethane-1,2-diol induced nephrolithiasis.



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1. Introduction

There are about 20 species of the genus *Triclisia* known throughout globe with 12 in tropical Africa Region (Troupin 1956). Plants belonging to this genus possess potential therapeutic values which have been used since very ancient times to cure various ailments and infectious diseases (Tiam et al. 2019). Although, in the past, identifying the various species of this genus were difficult as they possess closely related features. *T. angolensis* Exell, *T. gilletii* (De Wild.) Staner, and *T. hypochrysea* had been identified in the past as synonymous of the specie *T. Dictyophylla* (Troupin 1956). The *Triclisia* genus had been a world of puzzle where the same name were given to different species collected.

In recent research, specimens of *Triclisia dictyophylla* from all over its distribution area were compared; conspicuous differences in the shape and size of flowers and fruit carpels were observed (Jongkind 2017). The exceptionally large, fruit carpels were described by Keay and Troupin (1954) as *T. gilletii*. In publications on Central Africa, the fruit carpels of the same species were described as being about half that size and ending in a conspicuous beak and both descriptions are correct for the species (Jongkind 2017). Troupin included two species in *T. dictyophylla* with conspicuously different fruits and flowers. The species involved are *T. dictyophylla* with small flowers and large fruit carpels, and *T. gilletii*, with large flowers and relatively small fruit carpels. The fruits of *T. dictyophylla* are usually found on the bigger stems closer to the forest floor while the fruits of *T. gilletii* are often found on the smaller branches (Jongkind 2017).

Triclisia gilletii Staner is a robust creeper of up to 10 cm diameter, of the lowland dense rain-forest belonging to the Menispermaceae family. Its common names include Moonseed vine (English), Peshe (Ondo town) or Ogbogan (Okeluse town) in Ondo State, Nigeria, and AKAN-AKYEM sanhoma (Ghana). *T. gilletii*, have relatively large flowers and small fruit carpels compared with other species, fruits are conspicuously beaked and often found on the smaller branches away from the forest floor as reported and described by Jongkind (2017).

The plant parts are used in the treatment of several ailments such as malaria, venereal diseases, epileptic attacks, oedema, anaemia, diarrhoea, stomach problems, leprosy, mental health problems, dysentery, respiratory diseases and convulsive coughing (Tiam et al. 2019).

Literature data reveals that *Triclisia* species contains mainly bisbenzylisoquinoline (BBIQ) alkaloids (Schiff 1983; Uche et al. 2017), morphinan alkaloids (Spiff et al. 1981) and other amide alkaloids (Murebwayire et al. 2006; Samita et al. 2017).

Previous reports from *T. gilletii* also revealed the presence of BBIQ (Tackie et al. 1973; Dwuma-Badu et al. 1975; Owusu et al. 1981). Recently, Tiam et al. (2019) reported the isolation of some compounds from the methanol extract of the leaves of *Triclisia gilletii*. These include; (+)-nonacosan-10-ol, stigmasterol, 3-O-β-D-glucopyranosylsitosterol, 3-O-β-D-glucopyranosylstigmasterol, oleanic acid, myricetin, quercetin and 3-methoxyquercetin.

Triclisia species have been known for its diverse biological activities such as *in vivo* anticoagulation and antimicrobial activity which probably affirm its usage as a remedy for oedema and renal related ailments and complications like swellings of lower extremities (Ajugwo and Ezimah 2013). Its root, stem bark and leaves having some

medicinal values and have been reported to possess antiprotozoal activity (Muganza et al. 2012), and antimycobacterial activity (Tiam et al. 2019).

Also, the ameliorative potential of the aqueous-methanolic extract of *Triclisia gilletii* leaves against ethane-1,2-diol induced model of nephrolithiasis has been investigated (Olayeriju et al. 2020). This formed the basis of our search for the possible compound or groups of compounds present in the leaves responsible for the nephroprotective activity.

Until date, there is no holistic phytochemical characterisation of the species of Triclisia especially with the use of GC-MS or LC-MS. In the present study, we attempted to identify and characterise the phytochemical constituents in the aqueous-methanolic leaf extract of *Triclisia gilletii* using GC-MS and LC-MS.

2. Results and discussion

2.1. Gc-MS analysis

The phyto-components present in TGME with their corresponding retention time, molecular formula and molecular weight, as well as their relative abundance, which was expressed in terms of percent peak area detected by GC-MS are presented in Table S1 and depicted in Figure S1 (supplementary material). The results revealed that fifty-four (54) compounds were identified from the chromatogram when compared with the NIST library. The different class of compounds range from fatty acids, phenols, terpenes, steroids, carotenes, and heterocyclic compounds with oleic acid having the highest per cent peak area of 11.1%.

2.2. LC-Ms analysis

Dihydrovomifoliol-O-glucoside (Figure S2) (Supplementary material) was assigned to compound with $[M + HCOOH - H]^-$ ion at m/z 433 (Spínola and Castilho 2016). Dissociation of fragment m/z 593 showed a loss of 308 units (corresponding to a rhamnose plus glucose group) and yielded directly a fragment ion at m/z 285 (assigned as kaempferol). Compared to flavonoid glycosides found in *Gingko biloba* (Zhou et al. 2014), characterised as kaempferol-3-O- glucose- rhamnoside (Figure S3) (Supplementary material). It was not possible to identify the other compounds detected in the chromatographic profile due to their presence in trace amounts (Table S2) (Supplementary material).

2.3. Discussion

Medicinal plant extracts are complex mixture of different classes of chemical substances (i.e., flavonoids, saponins, tannins, steroids, etc.) and many efforts are being made continuously to isolate these molecules that are potentially active in the treatment of various human diseases (Parjapati et al. 2003). Different phytochemicals have been found to possess a wide range of activities; which may help in protection against chronic diseases.

Most of the detected compounds by GC-MS analysis belongs to the following groups of compounds; steroids (sitosterol and stigmasterol), terpenes (oleanic-1,2-en-

3-one and b-amyrin), phenols (2,4-ditertbutylphenol), fatty acids and vitamins, which is similar to the phytoconstituents reported by Tiam et al. (2019) on methanolic extract of moonseed vine. Preliminary phytochemical screening of TGME revealed these classes of compounds and posses pharmacological activities against renal toxicity (Nephrolithiasis) as reported by Olayeriju et al. (2020).

Characterisation of TGME with LC-MS revealed the presence of kaempferol-3-O-glucose-rhamnoside and dihydrovomifoliol-O-glucoside belonging to the flavonoid family. The compounds were detected on the basis of their accurate molecular weight, MS^n fragment data and by comparison with reference.

Dissociation of mass m/z 593 showed a loss of 308 units, corresponding to the masses of rhamnose (146 Da) and glucose (162 Da) moieties, yielding directly a fragment ion m/z 285 assigned kaempferol as previously characterised and reported as kaempferol-O-3-glucose-rhamnoside from Gingko biloba (Zhou et al. 2014).

Fragmentation of m/z 433 gave fragment ions at 387, 225, 179, 161, and 143 due to losses of formate ion, glucose ion, formate ion, water ion and water ion respectively. This has been previously characterised by Li et al. (2011) from *Sarcandra glabra* and Chen et al. (2015) from a typical Chinese Psoriaris medication (PSOR1-CM01) and assigned dihydrovomifoliol-O-glucoside.

The active principles extracted from medicinal plants is believed to produce better, and specific drugs (Hasrat et al. 2004), with low risk of toxicity and high therapeutic efficacy when used in clinical application (Dovi 2013). The individual constituents in TGME as revealed by both GC-MS and LC-MS, validates the acclaimed bioactivity as reported in our laboratory on the effect of moonseed vine on ethane-1,2-diol induced nephrolithiasis and its nephrotoxicity in Wistsr rats (Olayeriju et al. 2020).

Triterpenes generally have been found to protect against calcium oxalate crystal-induced peroxidative changes in experimental urolithiasis by preventing kidney tissue damage, inhibiting aggregation of crystals in urine and enhancement of body defence system (Malini et al. 2000). Also, vitamin E has been known to prevent calcium oxalate deposition (Tugcu et al. 2008). Squalene a precursor of the synthesis of all plant and animal sterols has also been investigated for its capacity to enhance the expression of anti-inflammatory enzymes by targeting pro and anti-inflammatory mediators and pathways to modulate over-activation of neutrophils, monocytes and macrophages (Cárdeno et al. 2015). Electrophilic fatty acid derivatives including nitrolinoleic acid and nitro-oleic acid, can mediate anti-inflammatory signalling reactions (Kansanen et al. 2009). 3-hydroxy-β-damascone and related carotenoid-derived compounds are potent inducers of Nrf2-mediated phase-2 response with concomitant anti-inflammatory activity (Gerhäuser et al. 2009).

3. Experimental section

See supplementary materials.

4. Conclusion

In this study, the phytochemical profile of *Triclisia gilletii* aqueous methanolic extract (TGME) was established for the first time using both GC-MS and LC-MS analysis.

Steroids, terpenes, phenols, fatty acids and vitamins were the major groups of constituents found in TGME as revealed by GC-MS and the flavonoid compounds; kaempferol-3-O-glucose-rhamnoside and dihydrovomifoliol-O-glucoside was identified with LC-MS. These constituents help validate the earlier report on the nephroprotective effect of *T. gilletii* on ethane-1,2-diol-induced neprolithiasis from our laboratory. Further studies are ongoing in our lab to investigate which of the different compounds of the extract is most effective and the possible mechanism of action via molecular approach.

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Disclosure statement

The authors have no conflict of interest to declare.

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