Phytochemical constituents from the roots and lignotubers of *Rhoicissus tridentata* and their *in vitro* uterotonic activity

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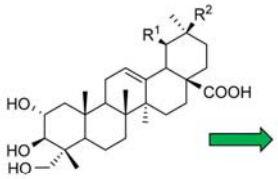
Abstract

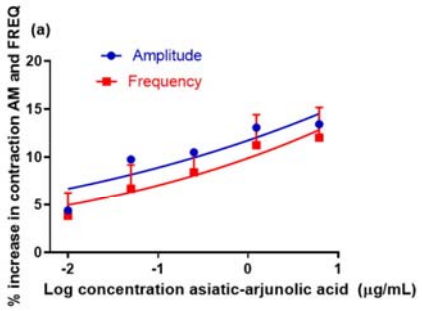
Rhoicissus tridentata is one of the most frequently used plants in preparing *Isihlambezo*, a herbal drink consumed by many South African women to induce labour and tone the uterus in pregnancy. This study aimed to identify the uteroactive compounds in this plant. Chromatographic purification of the methanol and water extracts from the roots yielded eight compounds, i.e. morin 3-*O*-α-L-rhamnopyranoside, *trans*-resveratrol 3-*O*-β-glucopyranoside, a mixture of asiatic and arjunolic acids, quercetin 3-*O*-rhamnopyranoside, catechin, β-sitosterol, and linoleic acid. All compounds were evaluated for their uterotonic effects using uterine smooth muscle isolated from stilboestrol-primed Sprague-Dawley rats. The mixture of asiatic and arjunolic acids showed the highest activity with EC_{50} of 0.02129 µg/mL for amplitude. These results validate the use of *R. tridentata* in ethnomedicine to facilitate labour in childbirth. Morin 3-*O*-α-L-rhamnopyranoside and *trans*-resveratrol 3-*O*-β-glucopyranoside caused a relaxation of the uterine muscle, which suggests that some compounds in *R. tridentata* possess opposing activities.

Rhoicissus tridentata roots and lignotubers



Asiatic acid: $R^1 = CH_3$, $R^2 = H$

Arjunolic acid: $R^1 = H$, $R^2 = CH_3$



Concentration-dependent increase in rat uterine muscle contraction amplitude (AM) and frequency (FREQ) in the presence of asiatic-arjunolic acids.

Keywords: *Rhoicissus*; uterotonic activity; asiatic acid; arjunolic acid; *trans*-resveratrol

1. Introduction

Problems in the onset of the labour process during pregnancy are the main cause of maternal and perinatal deaths in developed countries, and they contribute to childhood developmental problems (Lawn et al. 2009). A significant number of neonatal deaths have been associated with delayed labour caused by post-term pregnancy, lack of progress, oligohydramnios, and suspected intrauterine growth restriction (Gruber and O'Brien 2011). Induction of labour is often required in these cases to prevent neonatal deaths (Gruber and O'Brien 2011). Uterotonic drugs regulate uterine muscle contractions during labour, thereby stimulating the uterine muscle. They are generally used to counteract the abovementioned problems (Gruber and O'Brien 2011). Unfortunately, these drugs lack potency and selectivity, causing harmful side effects for both the mother and the baby (Gruber and O'Brien 2011). For example, oxytocin can induce excessive uterine contractions that may lead to foetal distress due to inadequate oxygen supply, as well as various cardiovascular effects on the mother, such as low blood pressure, irregular heartbeats, and heart muscle oxygen deprivation (Monks and Palanisamy 2021; Vallera et al. 2017). These effects are influenced by the dose and the rate of administration (Vallera et al. 2017).

Plants have been traditionally used for centuries to induce and enhance labour during childbirth (Gruber and O'Brien 2011). *Rhoicissus tridentata* (L.f.) Wild & R.B.Drumm. subsp. *cuneifolia* (Eckl. & Zeyh.) Urton, a shrubby tendril climber in the genus *Rhoicissus* Planch. (Vitaceae), is commonly used by local communities in South Africa to facilitate labour in childbirth (Dube et al. 2021). The water extract of the roots and lignotubers of *R. tridentata* has been reported to enhance the contractions of the isolated uterine smooth muscle and ileum (Brookes and Katsoulis 2006; Dube et al. 2021; Katsoulis et al. 2000). The uterotonic activity of this plant varies with the season and geographic location for collecting. In fact, extracts from plants collected in autumn and summer show higher activity than those harvested during spring and winter (Katsoulis 1999; Katsoulis et al. 2002). Furthermore, the lignotubers displayed higher activity compared to the stems and the roots (Katsoulis et al. 2002). However, the active compounds responsible for the uterotonic activity of this plant have not been isolated (Bayisa 2018; Brookes and Katsoulis 2006; Dube 2014).

In our current study, we document the isolation and structural characterisation of compounds derived from the uteroactive extract obtained from the roots and lignotubers of *R. tridentata*. Additionally, we present the analysis of the uterotonic activity of the isolated compounds, aiming to identify the active principles from this plant.

2. Results and discussion

2.1. Characterisation of compounds

Fractionation of the most active acetone fraction from the roots and lignotubers of *R. tridentata* led to the isolation of eight known natural products (Figure 1). By comparing the NMR (Figure S6–S19), UV-Vis, and MS data with literature, the structures of these compounds were identified as morin 3-*O*-α-L- rhamnopyranoside (**1**) (Lu and Foo 1997; Yen et al. 2009), *trans*-resveratrol 3-*O*-β-glucopyranoside (**2**) (Ha et al. 2009), a mixture of asiatic acid (**3**) and arjunolic acid (**4**) (Aguirre et al. 2006), quercetin 3-*O*-rhamnoside (**5**) (Lu and Foo 1997), catechin (**6**) (Zu et al. 2006), β-sitosterol (**7**) (Ahmad et al. 2010), and linoleic acid (**8**) (Marwah et al. 2007). This is the first report on the isolation of compounds **1–4** and **8** from the genus *Rhoicissus*. Compounds **5–7** were previously isolated from *Rhoicissus* species and several

plants in the family Vitaceae (Brookes and Katsoulis 2006; Lakornwong et al. 2014; Mshengu et al. 2020; Raman et al. 2014; Uwumubyeyi 2019; Zu et al. 2006).

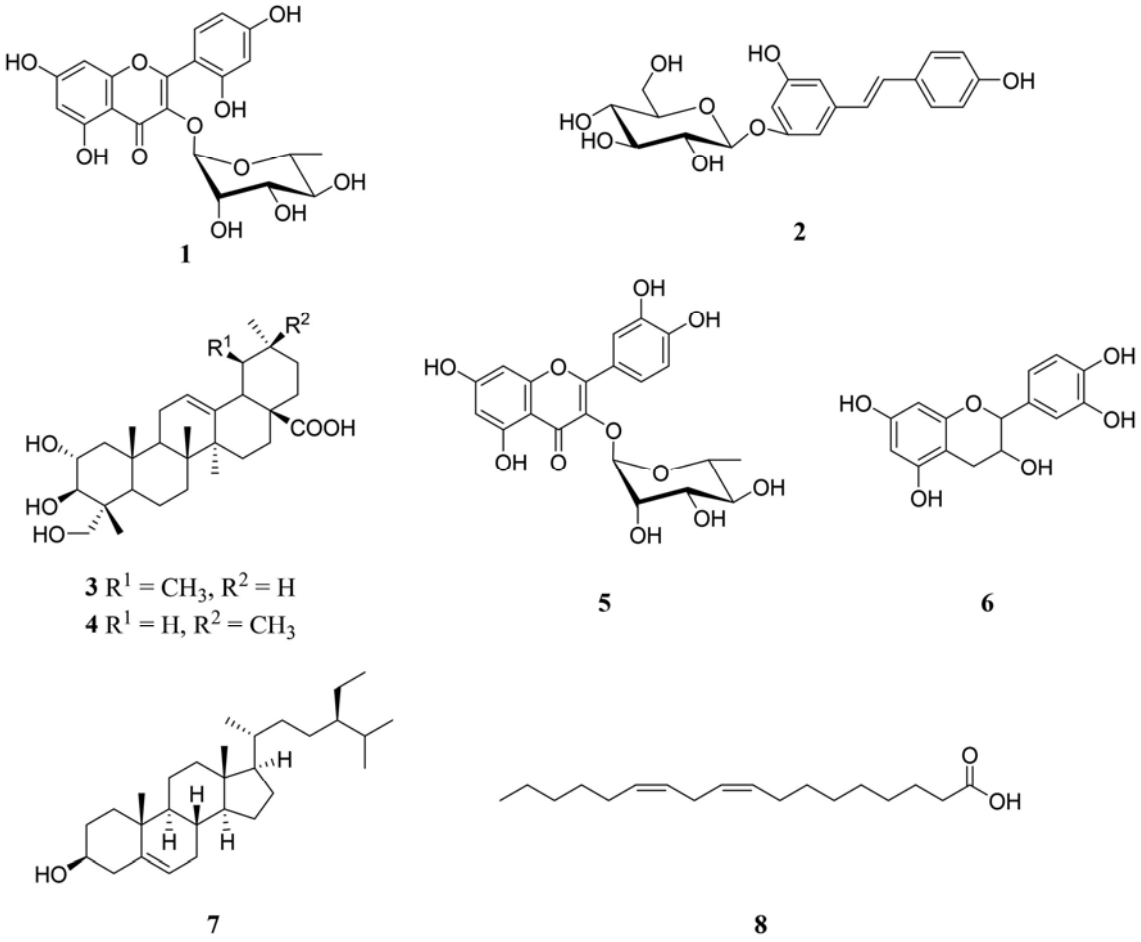


Figure 1. Structures of compounds **1–8** isolated from the roots and lignotubers of *R. tridentata.*

2.2. The uterotonic activity of isolated compounds

The methanol-water extract of the roots and lignotubers of *R. tridentata* stimulated a concentration-dependent increase in contractions of the uterus (Figure S2), consistent with literature findings for plants collected from the same geographic region (Brookes and Katsoulis 2006). This extract exhibited lower activity compared to the control drug (oxytocin) (Table S2), despite being harvested in autumn (Katsoulis et al. 2002), which is typically the season when the plant yields the highest uteroactive aqueous extracts. This may be due to the presence of other compounds with opposing tocolytic effects in the crude extract. The extract was fractionated to give dichloromethane (F1), acetone (F2), and methanol (F3) fractions. F2 exhibited the highest potency, with a notable increase in both the force (74.30% ± 0.535) and frequency (33.2% ± 5.766) of contractions at a lower concentration (2.13 mg/ml) when compared to F1 and F3 (Table S2). A smaller EC_{50} value of 0.2660 mg/mL (amplitude) and 0.2659 mg/mL (frequency) was obtained for the acetone fraction, in comparison to EC_{50} values of 2.299 mg/mL (amplitude) and 2.539 mg/mL (frequency) for F3; and an EC50 of more than

10 mg/mL for F1 (Figure S3). F2 was purified further to obtain active compounds, which were assessed for their uteroactive effects on isolated uterine muscle strips.

The mixture of asiatic-arjunolic acid (**3** and **4**) and β-sitosterol (**7**) stimulated the direct contractile response of the uterus. The mixture of **3** and **4** caused an increase of 13.4% (±0.084) at 6.14 µg/mL in amplitude, while β-sitosterol caused a significant increase in the contraction amplitude of 42.0% (±0.145) at 57.1 µg/mL (Table S2 and Figure S4). The rate of contractions increased by 12.0% (±3.128) at 6.14 µg/mL after exposing the tissue to the mixture of **3** and **4**, while β-sitosterol caused an increase of 19.0% (±4.164) at a dose of 57.1 µg/mL (Figure S4). These findings indicated that the mixture of asiatic-arjunolic acids was more potent than β-sitosterol. The highest activity of this mixture may be due to the synergistic activities of the individual acids; however additional research is necessary to substantiate such collaborative effects. This marks the first documentation of the uterotonic activity of asiatic and arjunolic acids. The uterotonic activity of β-sitosterol observed in the present study agrees with the findings reported by Brookes and Katsoulis (2006). Promprom et al. (2010) also reported on the uterotonic effect of **7** and postulated that this effect was due to Ca^{2+} influx and through the activation of myosin light kinase (Dube 2014; Promprom et al. 2010). We suggest that this could be one of the likely mechanisms through which β-sitosterol, isolated from *R. tridentata*, accelerate myometrium contractions.

Interestingly, compounds **1** and **2** caused a relaxation of the uterine muscle, which indicated that these compounds had inhibitory effects on the myometrium contractions (Figure S5). These findings may suggest that some compounds in the *R. tridentata* possess opposing activity (tocolytic effect). This finding was unexpected, and there is still more work to be done in this regard. For instance, the effect of using the standard drugs (e.g. oxytocin) as a baseline on the relaxant effect of compounds (1) and (2) will be investigated. This is the first report on the inhibitory effects of compounds **1** and **2**. However, the inhibitory response of compound **2** agrees with the data reported in the literature for its aglycone, resveratrol (Hsia et al. 2011; Novakovic et al. 2013). The addition of quercetin 3-rhamnoside (**5**) and catechin (**6**) into the organ bath completely stopped uterine muscle contractions. This indicated that quercetin 3-rhamnoside and catechin may be cytotoxic to the uterine tissue. However, this aspect of the work will be explored in future investigations. Linoleic acid (**8**) was not assayed for uterotonic activity in this study as it was previously reported to induce uterine smooth muscle contractility (Sewram et al. 2001).

3. Conclusions

This study has successfully demonstrated the uterotonic effect of the polar extract of the roots and lignotubers of *R. tridentata* on isolated rat uterine muscle strips. The active principles were identified as the mixture of asiatic-arjunolic acids (**3** and **4**) and β-sitosterol, with the mixture of **3** and **4** showing the highest activity. Morin 3-*O*-α-L-rhamnopyranoside (**1**) and *trans*-resveratrol 3-*O*-β-glucopyranoside (**2**) induced relaxation of the uterine smooth muscle contractility. This finding was unexpected, and further research is needed in this area. This study provides scientific data supporting the use of the aqueous extract of *R.tridentata* in traditional medicine as an oxytocic agent. However, further research needs to be conducted to quantitatively compare the tocolytic compounds with the uteroactive compounds in the crude extract, as this might explain how the opposing activities are modulated. Furthermore, studies to determine the mechanism of action of the isolated mixture of asiatic-arjunolic acids (**3** and **4**) are also essential.

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Disclosure statement

The authors declare no conflicts of interest.

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