An Acad Bras Cienc (2021) 93(2): e20190343 DOI 10.1590/0001-3765202120190343
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CELLULAR AND MOLECULAR BIOLOGY

The essential oils of *Grewia Lasiocarpa* E. Mey. Ex Harv.: chemical composition, *in vitro* biological activity and cytotoxic effect on Hela cells

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Abstract: The chemical composition and biological activity of the essential oil extracted from the fresh leaves and stem bark of *Grewia lasiocarpa* was determined for the first time in this study. The essential oils were extracted by hydrodistillation and identified by GC–MS and FTIR. The antibacterial, antioxidant activity and total phenolic content of essential oils were determined. The major compounds identified were phytol (22.6%); α-farnesene (8.62%); n-hexadecanoic acid (7.24%); farnesol (4.61%) in the leaves, and 2-methylheptadecane (7.24%); heptacosane (7.60%); heptadecane, 2,6,10,14-tetramethyl (7.30%). The presence of aromatic, alkanes and phenolic compounds were revealed by FTIR analysis. The in silico oral prediction shows that some of the components are orally safe. The essential oil from the leaves showed cytotoxic activity at 1mg/mL(IC50 =555.70 µg/mL) against HeLa cells. The oils exhibited no significant antioxidant activity (IC50 >1 000 µg/mL) with <100 mg/g GAE of total phenol. The essential oils showed different degrees of activities against Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853) and Klebsiella pneumoniae (ATCC 314588) at 10 µg/mL, 5 µg/mL and 2.5 µg/mL. These results might provide a future reference basis for further exploration of more of its medicinal application.

Key words: *Grewia lasiocarpa,* essential oil, antibacterial, antioxidant, Cytotoxicity, HeLa cells.

INTRODUCTION

Oil is the carrier of the 'essence' of plants thus, plant volatiles are referred to as 'essential oils' this description was given by Haagen-Smit (1949) (Raut & Karuppayil 2014). Although these essential oils, which are volatile and lipophilic, are commonly obtained from plants, they are also present in bryophytes, *e.g.,* Liverworts (Figueiredo et al. 2008).

The plant family Malvaceae is known to have a significantly high composition of secondary metabolites rich in volatile compounds and essential oils (Baser 1995). The presence of these metabolites supports the economic significance of plants belonging to this family, *e.g.*, food okra- *Abelmoschus esculentus*, fibre cotton -genus *Gossypium*) *etc.* (Zuzarte & Salgueiro 2015, Flachs 2016). *Grewia lasiocarpa* E. Mey. ex Harv. (Malvaceae) is an indigenous South African plant that is not yet well documented in the literature; however, there are recent reports (Akwu et al. 2019a, b, c, 2020a, b, 2021). Globally, numerous plant genera, including the genus *Grewia* (family Malvaceae *sensu lato*) are being exploited for the phytocompounds they possess based on their ethnomedicinal properties (Morton 1987, Robertshawe 2011, Rehman et al.

2013, Ayurveda 2015). Reports on the presence of essential oils/volatile oils in some *Grewia* species have been given *viz*. in the stem bark of *Grewia venusta* FRES (Nep et al. 2013) and the leaf, stem bark and roots of *G. mollis* (Rehman et al. 2013). The essential oil of the seed bark of *G. asiatica* Linn. (*G. subinaequalis* DC) contains β-amyrin, lupeol and betulin, while the essential oil of the flower contains glycosides pelargonidin, cyanidin, and delphinine (Oliver-Bever 1986), 1,2-epoxy [5.6%], 1-(2, cyano2-ethyl butyl)3-isopropyl urea [5.9%] and hexadecanoic acid [6.3%] (Langford et al. 2010). The essential oil present in the flower of *G. bicolor* (syn *G. salicifolia*) constitutes a sedative, farnesol, which is also peculiar to other plants in the Tiliaceae family to which *Grewia* was previously taxonomically grouped (Burt 2004, Kubmarawa et al. 2007, Pathak 2017). Essential oils are good sources of bioactive compounds, and they possess several biological activities (Shagal et al. 2012), *e.g.* antioxidant, antimicrobial, cytotoxic, antiviral *etc*. (Shaaban et al. 2012, Hassan et al. 2016), resulting in an application upsurge in biomedicine (Ullah et al. 2012). Owing to their complexity, characterisation, and identification of essential oils or volatile organic compounds are done using advanced chromatographic and spectroscopic techniques such as gas chromatography and mass spectrometry (GC-MS). Fourier Transform - Infra-Red Spectroscopy (FTIR) is a technique is employed for the characterisation and identification of functional groups. The substance to be analysed could be in any form, namely liquid, solid, solution, fibre, gas, film or with a surface coating (using the attenuated total reflectance (ATR) technique) (Coates 2000, Stuart 2005). As the use of computational technology (*in silico* methods), to predict the biological activity of substances that are found in humans and the environment, is gaining grounds (Maree et al. 2014, Erukainure et al. 2018). Hence, it is crucial to evaluate the pharmacognistic potentials, oral toxicity, and characterization of these substances using *in silico* methods.

To the best of our knowledge, there are no published reports on the chemical composition, cytotoxicity, total phenolic content, antioxidant and antibacterial activity of the essential oils of the *Grewia lasiocarpa*. This present study aimed to extract, identify the chemical composition, evaluate the antioxidant, total phenolic content and biological activity of the essential oils of *G. lasiocarpa* (leaves and stem bark).

MATERIALS AND METHODS

Collection of plant material

Fresh, healthy plant organs (leaves and stem bark) of *G. lasiocarpa* were collected from the Umdoni Trust Park area of KwaZulu-Natal's southern countryside, South Africa. The plant material were taxonomically identified and authenticated by Dr. Syd Ramdhani, curator of the School of Life Sciences, University of KwaZulu-Natal, and a voucher specimen was deposited at the Herbarium with herbarium number (Nneka 002).

Extraction of essential oils

The chopped fresh leaves and stem bark (730 g) each were separately subjected to successive hydrodistillation at normal pressure in a standard Clevenger-type apparatus for 4 h each, in the ratio 1:4 (v/w) of plant material to distilled water. The extracted essential oils were dried over anhydrous sodium sulphate (Merck, Darmstadt, Germany) and measured. The essential oils were stored in screw-capped glass vials in the dark at 4°C until analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of essential oils

The GC-MS analysis was performed on a GCMS-QP2010 Plus Shimadzu instrument and fitted with capillary chromatographic column of 30 m x 0.25 mm ID x 0.25/0.24 µm film thickness of diphenyl dimethyl polysiloxane (5% diphenyl and 95% dimethyl polysiloxane) model Rtx® –5 ms (RESTREK). For the analytical conditions, the oven temperature was programmed as follows: the initial temperature at 50°C, for 1.5 min, then increased to 200°C at a rate of 4°C min^{-1}, and then increased up to 300°C at the rate of 10°C min^{-1} held for 7 min. The injector and interface temperatures were 240 and 220°C, respectively. The carrier gas was helium, adjusted to a column velocity flow of 1.2 mL/min and 2 µL of essential oil diluted (1/100) in *n*-hexane (≥99%, GC grade, Sigma-Aldrich) and filtered using a 0.22 µm filter, which was injected into the "splitless" mode system. The mass detector scan mode range was 40 to 500 atomic mass units (amu) or 40-500 *m/z*., with a total running time of 59 min.

Identification of compounds

The identification of individual compounds was based on the comparison of the retention indices (Kovats indices-IK) and the obtained mass spectral fragmentation patterns with those of known compounds reported and stored in the database of the National Institute of Standards and Technology, Washington, DC, USA (NIST) and literature (Davies 1993, Adams 2007, Babushok et al. 2011).

Fourier transform infra-red analysis

The essential oils were dissolved in 10% DMSO, and the diluents were subjected to Fourier transform infrared (FTIR) spectroscopy (mid-IR spectra) on a Perkin-Elmer FTIR using spectrum software version 6.1. The measurements were carried out at 25 - 27°C, and the spectrum recorded from 4000-400 cm^{-1} with a spectral resolution of 4 cm^{-1}. The peak frequencies were compared to the reference literature (Coates 2000, Stuart 2005) to determine the functional groups.

Biological activities of the essential oil

In vitro cytotoxicity/MTT assay

HeLa cells were maintained in EMEM supplemented with 10% (v/v) gamma-irradiated FBS and 1% antibiotics (100 units/mL penicillin, 100 µg/mL streptomycin) at 37 °C and 5% CO_2, in a HEPA Class 100 Steri-Cult CO_2 incubator (Thermo-Electron Corporation, Waltham, Massachusetts, USA).

MTT assay

The cells were trypsinized and seeded into 48-well plates at a seeding density of approximately 3 x 10^4 cells/well and incubated at 37°C for 24 h to allow for attachment. The medium was then replaced with fresh medium, and the essential oils of the leaves and stem bark (1 mg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL) was added to the wells and allowed to incubate for 48 h at 37°C. A negative control containing only cells was set as 100% cell survival/viability. After the 48-h incubation period, the spent medium was removed, and 200 µL fresh medium and 20 µL MTT reagent (5 mg/mL in PBS) was added to each well.

The cells were incubated at 37°C for an additional 4 h, after which the MTT-medium solution was removed, and 200 µL DMSO was added to solubilize the formazan crystals. Absorbance was read at 540 nm using a Mindray MR-96A microplate reader (Vacutec, Hamburg, Germany). All assays were performed in triplicate.

Antibacterial assay (Disc diffusion method)

Test-bacterial strains

Six clinical isolated strains *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC BAA-1683), *Klebsiella pneumoniae* (ATCC 314588) and *Salmonella typhimurium* (ATCC 14026), provided by Dr. Chunderika Mocktar, School of Pharmacy and Pharmacology, University of KwaZulu-Natal, maintained in 75% glycerol at -80°C, were used to assess the antibacterial activity of the essential oils. 100 µL of each bacterial strain stock were added to 5 mL of nutrient broth (Merck), and the cultures were grown overnight in a test-tube shaker for 24 h at 36 ± 1°C; after which the bacterial cultures (inoculum) were further diluted with sterile nutrient broth to an OD of 0.08-0.1 at 625 nm using a UV–vis spectrophotometer (Agilent Cary 60 Spectrophotometer, USA) to yield a final concentration of approximately $1 \times 10^8 - 1 \times 10^9$ bacteria cells /mL and then swabbed onto the Müller-Hinton agar (MHA) plates.

Sample preparation

The essential oils of the leaves and stem bark were dissolved in 10% DMSO to a concentration of 1 mg/mL, from which final concentrations of 10, 5 and 2.5 µg/mL were prepared. 10 µL of each of the different sample concentrations was impregnated on a sterile Whatmann 1 filter paper disc (6 mm in diameter) and kept dry in sterile Petri dishes. For the positive control, the filter papers were soaked in 10 µg/mL of Gentamicin (Gram-negative) and Streptomycin (Gram-positive) to get a concentration of 10 µg/disc. For the negative control, the discs were soaked in 10% DMSO.

Disc diffusion assay

MHA (Biolab, South Africa) was prepared, poured into sterile Petri dishes and allowed to set and dry at room temperature. The standardized bacteria broth solutions were then swabbed onto the prepared MHA plates and allowed to air dry for 15 min. The impregnated discs were then placed on the prepared agar's surface and incubated at 36°C for 18 h. The diameter of the zone of inhibition was measured in mm inclusive of the disk diameter. The assay was done in triplicate.

Total phenolic content and Antioxidant assay

Preparation of stock solution

Stock solutions (1 mg/mL in methanol) of the leaves and stem bark essential oil was prepared, from which 15, 30, 60, 120 and 240 µg/mL were prepared for the *in vitro* studies.

Estimation of total phenolic contents

The Folin–Ciocalteu reagent assay was used in determining the total phenolic content of each essential oil (Liu & Yao 2007). To 30 µL of each oil (240 µg/mL), 150 µL of 10% diluted Folin-ciocalteau reagent, and 120 µL of 0.7 M Na_2CO_3 were added, and the mixtures were incubated for 30 min at room temperature. Absorbance was measured at 765 nm, and results expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight using the formula (equation 2.1).

$$C_{tp} = C * V/m$$

where C_{tp} = Total phenolic content (mg/g) in GAE (gallic acid) equivalent
C=Concentration of gallic acid obtained from calibration curve in mg/mL
V=volume of extract in mL
m=mass of extract in gram

In vitro antioxidant assays

DPPH scavenging activity

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined using the method of Braca et al. (2002). To 500 µL of each oil, 50 µL of 0.3 Mm of DPPH in methanol was added and the microplate was incubated in the dark at room temperature for 30 min. Ascorbic acid was used as the standard. The absorbance was then read at 517 nm against blank without sample or standard. The IC_{50} was derived from the inhibition curves by plotting the percentage inhibition against the concentration logarithmic scale. The compound's scavenging ability was calculated using equation 2.2:

$$\text{DPPH Scavenging activity}(\%) = \left[\frac{\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)}{\text{Abs}_{\text{control}}}\right] * 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of DPPH and methanol

$\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical + sample (compound or standard)

In Silico oral toxicity prediction of the identified compounds

The oral toxicity classes of the major compounds were reported as LD_{50} values (mg/kg) and categorized in accordance with the globally harmonized system of classification of labelling of chemicals (GHS) on ProTox (http://tox.charite.de/protox_II/) (Drwal et al. 2014).

Statistical analysis

All experiments were carried out in triplicate, and the data derived were subjected to statistical analysis using SPSS 25 for Windows, IBM Corporation, New York, USA. Tukey's-honest significant difference multiple range *post hoc* test. The values expressed as means ± standard deviation and significant difference established at $p<0.05$.

RESULTS AND DISCUSSION

Extraction of essential oils

The essential oil of the leaves and stem bark had a pungent aromatic smell. The leaves' essential oil was liquid at room temperature with a yield of 0.008% (w/w) greenish liquid, and that of the stem bark was 0.003% (w/w) viscous colourless liquid.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: Chemical composition of the identified constituents of *G. lasiocarpa* essential oils

The GC-MS analysis revealed nineteen compounds were present in the leaves, representing (90.20%), out of which twelve were identified (Table I), and in the stem bark, twenty compounds were eluted representing (92.34%), out of which ten were identified (Table II). The major compounds in the essential oil of the leaves were phytol (22.6%); α-farnesene (8.62%); *n*-hexadecanoic acid (7.62%); farnesol (4.61%), and 2-methylheptadecane (7.24%); heptacosane (7.60%); heptadecane, 2,6,10,14-tetramethyl (7.30%) in the stem bark. The compound farnesol was present in the leaves and stem bark but with variations in area percentage. In a relatively large number of plant essential oils that have been investigated, the low-molecular-weight aliphatic compounds, terpenes/terpenoids and aromatic are often present in a large percentage (Pichersky et al. 2006). This general observation agrees with the essential oil composition of the leaves (Table I) but not of the stem bark (Table II). The compounds hexadecanoic acid, tetratricontane, farnesol and their derivatives might be part of the chemotaxonomic essential oil tool for this genus since there are reports of

Table I. Chemical composition of *Grewia lasiocarpa* leaves essential oil.

S/N	RT	Compounds	$RI_{calc.}$	RI_{Lit}	Identification Method	Peak area (%)
1	12.92	trans-2,7-dimethyl-3,6-octadien-2-ol	1100	-	MS	2.16
2	23.87	α-Farnesene	1500	1501[19]	MS, RI	8.62
3	26.48	Farnesol	1600	-	MS	4.61
4	40.35	n-Hexadecanoic acid	1800	-	MS	7.24
5	41.93	Phytol	2100	2116.4[19]	MS, RI	22.60
6	42.52	Heptadecane,2,6,10,15-tetramethyl	2100	-	MS	1.71
7	42.61	Hexacosane	2600	2600[10]	MS	2.17
8	42.98	Tetracosane	ND	-	MS	1.56
9	52.37	Octacosane	2800	2800[10]	MS, RI	2.12
10	53.95	Squalene	3000	2914[1]	MS	0.91
11	55.05	Hexatriacontane	3600	3600[1]	MS; RI	2.41
12	57.07	Tetratetracontane	4400	-	MS	0.92

$RI^{calc.}$ =Calculated retention indices, $RI_{Lit.}$=Retention indices from literature.

Table II. Chemical composition of *Grewia lasiocarpa* stem bark essential oil.

S/N	RT	Compounds	$RI^{calc.}$	RI_{Lit}	Identification Method	Peak area (%)
1	29.27	Tetradecane, 4-methyl	1500	-	MS	4.36
2	33.99	Tetradecane, 4-ethyl	1600	-	MS	5.89
3	34.78	Pentadecane, 2-methyl	1600	-	MS	3.93
4	39.09	2-Methylheptadecane	1800	-	MS	7.24
5	39.71	Hexadecane, 5-butyl	2000	-	MS	3.18
6	53.92	Farnesol	-	-	MS	1.72
7	55.30	Heneicosane	2100	-	MS	4.58
8	55.89	Heptadecane,2,6,10,14-tetramethyl	2100	-	MS	7.30
9	56.02	Tricosane, 2-methyl	2300	-	MS	3.62
10	57.05	Heptacosane	2700	-	MS	7.60

$RI^{calc.}$ =Calculated retention indices, $RI_{Lit.}$ =Retention indices from literature.

these compounds in other *Grewia* spp. (Chen et al. 2008, Langford et al. 2010).

In vitro Cytotoxicity/MTT assay

The major constituents of essential oils determine the biological properties (Bakkali et al. 2008). The significant cytotoxic activity observed at 1 mg/mL of the essential oils from the leaves (Figure 1) may be attributed to the high percentage of phytol present in the leaves (Table I) (Kumar et al. 2010, Silva et al. 2014), which is in agreement with the report of Tisserand & Balacs (1999), which states that phytol is grouped among the uncommon constituents of essential oils and most often when they occur they are in high percentage. The other compounds present in the essential oil of the leaves are tetratricontane (Dandekar et al. 2015), squalene (Murakoshi et al. 1992, Rao et al. 1998, Smith et al. 1998, Reddy & Jose 2013), *n*- hexadecanoic acid (Ji et al. 2005), tetradecane, 4-methyl (Begum et al. 2016) in the stem bark and farnesol (Asakawa et al. 2013) present in both, have cytotoxic properties.

Antibacterial assay

Quite a number of essential oils exhibit remarkable antibacterial activity due to their ability to bind to and fragment the lipids in the cell membranes and mitochondria, resulting in the cytosol's release and its contents (Burt 2004). In addition, essential oils exhibit more inhibitory activity against Gram-positive bacteria than Gram-negative bacteria owing to the cellular morphology of the cell membrane of the former (Burt 2004). On the contrary, the oils from *G. lasiocarpa* leaves and stem bark showed no activity against the Gram-positive bacteria

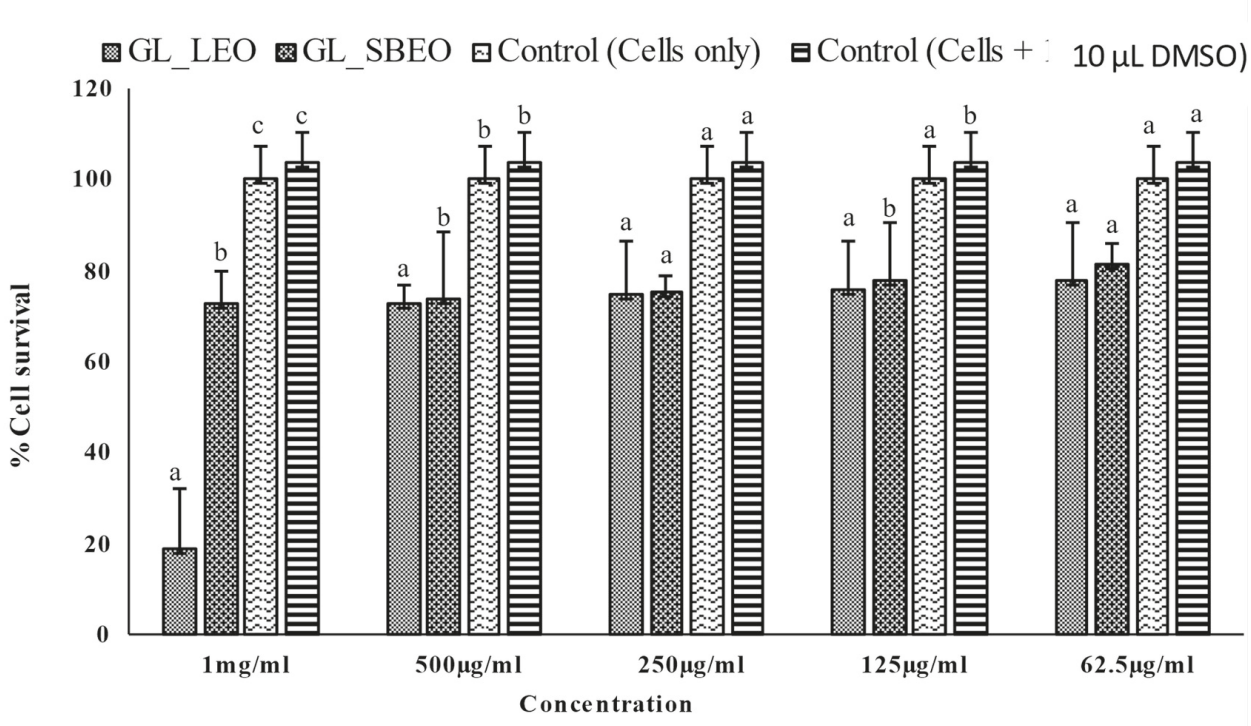

Figure 1. ***In vitro*** **cytotoxicity activity of the leaves and stem bark of Grewia lasiocarpa against HeLa cells. a -c letters above the bars for a given concentration are significantly different from each other (Tukey's honest significant difference multiple range post hoc test p<0.05 IBM SPSS version 25). GL_LEO=Grewia lasiocarpa leaves essential oil, GL_SBEO=Grewia lasiocarpa stem bark essential oil.**

Staphylococcus aureus (ATCC 25923), methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC BAA-1683) and *Salmonella typhimurium* (ATCC 14026), but against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 314588) with a range of dose-dependent antibacterial activity (Table III). The antibacterial activity of the oils from the leaves and stem bark might be due to the presence of phytol (Kumar et al. 2010, Ghaneian et al. 2015), *n*-hexadecanoic acid (Kumar et al. 2010), hexacosane (Rukaiyat et al. 2015) in the leaves and heneicosane (Uma & Parvathavarthini 2010), farnesol (Asakawa et al. 2013) present in the leaves and stem bark. However, the antibacterial activity of the essential oils from the leaves and stem bark may be a synergistic effect of these reported active compounds or some other compounds in the oil.

Fourier transform infrared spectroscopy (FTIR) analysis

To further confirm that the inhibitory effect was due to the essential oil present in the diluent (10% DMSO), FTIR analysis was done. Hence, the functional groups present in the EOs were identified. As typical of aromatic compounds, two sets of bands around 1600 cm^{-1} and 1500 cm^{-1} were observed (Figure 2) (Coates 2000, Stuart 2005). According to Morar et al. (2017), the relatively low number of peaks in the FTIR spectra may be attributed to the low concentrations of most of the essential oil components, and the spectra regions between 640-1840 cm^{-1} and 2770-3070 cm^{-1} ranges are considered as regions with molecular structural information. However, the broad bands 3400 and 2500 cm^{-1} were observed because of moisture or O-H stretching (Figure 2). The presence of characteristic functional groups such as phenols (1646.74 cm^{-1} and 1320.38 cm^{-1}), alkanes (1407.26 cm^{-1} and 1437.85 cm^{-1}) and aromatic compounds (1646.74 cm^{-1} and 1635.30 cm^{-1}) was revealed by the FTIR analysis in the essential oils of the leaves and stem bark respectively (Figure 2). The leaves have more cytotoxic compounds than the stem bark, as portrayed by their IC_{50} values (555.70 and >1000 µg/mL), respectively (Table IV).

Total phenolic contents

There are several assays for evaluating the antioxidant activity of lipophilic substances like essential oils; however, scientists prefer to use the DPPH technique because of its simplicity and sensitivity (Miguel 2010). The % inhibition

Table III. Antibacterial activity of the essential oils from the leaves and stem bark of *Grewia lasiocarpa*.

Bacteria Strain	Zone of inhibition [mm] (Mean ± SD)							
	GL_LEO				GL_SBEO			
	10 µg/mL	5 µg/mL	2.5 µg/mL	Positive control	10 µg/mL	5 µg/mL	2.5 µg/mL	Positive control
Pa	7.25±1.06	6.50±0.00	R	9.30±0.56	7.80±0.29	8.30±1.53	6.50±0.00	10.00±1.73
Ec	8.67±.58	7.50±0.71	6.25±0.00	11.00±3.00	8.50±0.71	6.75±0.35	6.50±0.00	10.33±2.08
Kp	6.50±0.00	7.23±1.54	6.20±0.00	10.00±2.00	7.50±1.41	6.75±0.35	6.50±0.00	9.83±1.04

R= Resistance, Pa= *Pseudomonas aeruginosa* (ATCC 27853), Ec=*Escherichia coli* (ATCC 25922), Kp=*Klebsiella pneumoniae* (ATCC 314588), (*n* = 3), Positive control (Gentamicin [10 µg/disc]). Methicillin-resistant *Staphylococcus aureus* [MRSA] (ATCC BAA-1683), *Staphylococcus aureus* (ATCC 25923) and *Salmonella typhimurium* (ATCC 14026) were observed to be resistant to both essential oils.

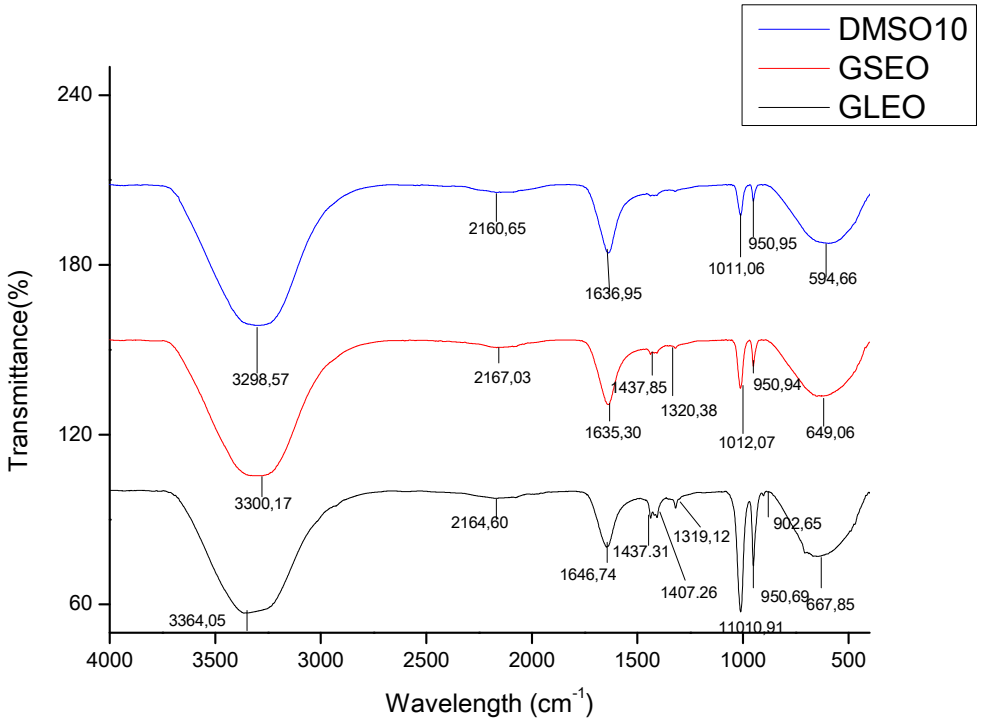

Figure 2. Overlay of the FTIR spectra of 10%DMSO, essential oils of Grewia lasiocarpa leaves and stem bark dissolved in 10%DMSO.

capacity for scavenging DPPH free radicals was observed to be dose-dependent, and a comparison between the leaves and the stem bark % inhibition, the former showed a slightly higher inhibition percentage in all concentration although not significantly different (Figure 3).

Table IV. Biological activities: IC_{50} values of the essential oils from the leaves and stem bark of *Grewia lasiocarpa*.

Sample	Cytotoxicity (µg/mL)	DPPH (mg/mL)
GL_LEO	555.70±25.29	1.04±3.32^{b}
GL_SBEO	>1000	39.87±1.87^{c}
AA	-	0.008±17.85^{a}

The IC_{50} of the cytotoxicity and DPPH assay of the essential oil is the concentration that can result in 50% decrease of cell growth and the concentration needed to scavenge 50% of the DPPH free radicals, respectively. Data are presented as mean ± SD, *n*=3, of triplicate determinations. $^{a-c}$ Different superscript letters within the column for a given parameter are significantly different from each other different Tukey's honest significant difference multiple range *post hoc* test p<0.05 IBM SPSS version 25). GL_LEO=*Grewia lasiocarpa* leaves essential oil, GL_SBEO=*Grewia lasiocarpa* stem bark essential oil, AA=Ascorbic acid.

This observation may be due to the antioxidant properties of terpenoids present in the leaves (Kumar et al. 2010), since the antioxidant activity of essential oils is dependent on the bioactive compounds percentage occurrence (Reddy 2001), most especially owing to the high percentage of the diterpene - phytol (Ruberto & Baratta 2000, Lanfer-Marquez et al. 2005, Santos et al. 2013) and other antioxidants *e.g.* tetratricontane (Dandekar et al. 2015). However, the low IC_{50} value of the essential oils from the leaves (IC_{50}=1.04 mg/mL) and stem bark (IC_{50}=39.87 mg/mL) when compared with ascorbic acid (IC_{50}=0.008 mg/mL) (Table IV) this might be due to the inactive concentrations of the phenolic compounds present in the leaves and stem bark.

The total phenolic content of the leaves and stem bark (Table V) might have also influenced the % inhibition observed (Figure 3). However, the IC_{50} values are significantly different; this implies that any substance's antioxidant capacity is influenced by the quality and quantity of its

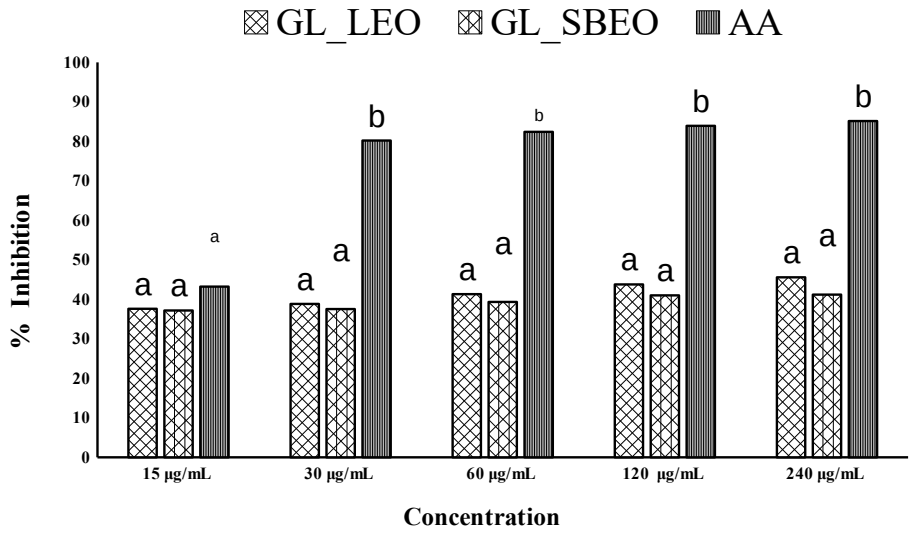

Figure 3. ***In vitro*** **antioxidant activity of the leaves and stem bark of** ***Grewia lasiocarpa.*** **a and b letters above the bars for a given concentration are significantly different from each other (Tukey's honest significant difference multiple range post hoc test p<0.05 IBM SPSS version 25). GL_LEO=Grewia lasiocarpa leaves essential oil, GL_SBEO=Grewia lasiocarpa stem bark essential oil, AA=Ascorbic acid.**

Table V. Total phenolic contents of the essential oils from the leaves and stem bark of *Grewia lasiocarpa.*

Sample	Total phenols (mg/g GAE)
GL_LEO	86.29±0.25[a]
GL_SBEO	78.22±0.25[a]

Data presented as mean ± SD of triplicate [a] Superscript letter within each column for the given parameter is not different from each other Tukey's honest significant difference multiple range *post hoc* test p<0.05 IBM SPSS version 25). GL_LEO=*Grewia lasiocarpa* leaves essential oil, GL_SBEO=*Grewia lasiocarpa* stem bark essential oil.

Table VI. *In Silico* oral toxicity prediction of the Identified compounds in the leaf of *Grewia lasiocarpa*.

Compounds	Predicted LD_{50} (mg/Kg)	Toxicity class	Prediction accuracy (%)
trans-2,7-dimethyl-3,6-octadien-2-ol	3500	5	67.38
α- Farnesene	5000	5	68.07
Farnesol	5000	5	100
Hexadecanoic acid	130	3	100
Phytol	5000	5	100
heptadecane,2,6,10,15-tetramethyl	ND	ND	ND
Hexacosane	154	3	100
Tetracosane	154	3	100
Octacosane	154	3	100
Squalene	5000	5	100
Hexatriacontane	154	3	100
Tetratetracontane	154	3	100

ND=Not determined.

Table VII. ***In Silico*** **oral toxicity prediction of the identified compounds in the stem bark of** ***Grewia lasiocarpa.***

Compounds	Predicted LD_{50} (mg/Kg)	Toxicity class	Prediction accuracy (%)
Tetradecane, 4-methyl	154	3	100
Tetradecane, 4-ethyl	154	3	100
Pentadecane, 2-methyl	154	3	100
2-Methylheptadecane	ND	ND	ND
Hexadecane, 5-butyl	154	3	100
Heptadecane,2,6,10,15-tetramethyl	ND	ND	ND
Hexadecane, 5-butyl	154	3	100
Heneicosane	154	3	100
Heptadecane,2,6,10,14-tetramethyl	ND	ND	ND
Tricosane, 2-methyl	154	3	100

ND=Not determined.

constituents, *i.e.* the chemical composition and amount of individual compounds.

In Silico prediction of oral lethal dose toxicity class of the identified compounds

The advantages of applying the *in silico* method are virtual (no live animal required), quick and reliable. The oral lethal dose toxicity classes are Class I: fatal if swallowed ($LD_{50} \leq 5$), Class II: fatal if swallowed ($5 < LD_{50} \leq 50$), Class III: toxic if swallowed ($50 < LD_{50} \leq 300$), Class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$), Class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$), Class VI: non-toxic ($LD_{50} > 5000$) (Drwal et al. 2014), based on this it is unsafe to orally administer some of the compounds (Table VI and VII); thus the internal use of EOs without a thorough analysis of its chemical composition is not advised. Some essential oils are not safe for consumption (Baser 1995). However, they are generally regarded as safe (GRAS), but it is claimed that they do not cause an adverse detrimental effect when administered orally in considerable low concentrations (hydrosols) (Baser 1995). Phytol is used in aromatherapy; the *in silico* oral prediction of its non-toxicity (Table VI) further supports its safe use in aromatherapy.

CONCLUSIONS

This study presented the first data for the leaves' essential oil constituents and the stem bark of *Grewia lasiocarpa* through hydrodistillation, GC-MS, and FTIR analyses. *Grewia lasiocarpa* has significant antibacterial and cytotoxic potential based on the results obtained; isolation of the individual compounds for further bio-assays might be promising for safe drug development. Therefore, more studies should be conducted on the essential oils from the genus *Grewia* to establish more bioactive components.

Acknowledgments

The authors would like to acknowledge the Organisation for Women in Science for the Developing World (OWSD), and Swedish International Development Cooperation Agency (SIDA) for their intellectual and material contribution (financial assistance). We acknowledge the consumables supply by the National Research

Foundation (NRF). We are grateful to Dr. Chunderika Mocktar for the provision of the clinical bacteria strains.

REFERENCES

ADAMS RP. 2007. Identification of essential oil components by gas chromatography/mass spectrometry. 4th Ed., Carol Stream, IL: Allured publishing corporation, 456 p.

AKWU NA, NAIDOO Y & SINGH M. 2019a. Cytogenotoxic and biological evaluation of the aqueous extracts of *Grewia lasiocarpa*: An *Allium cepa* assay. S Afr J Bot 125: 371-380.

AKWU NA, NAIDOO Y & SINGH M. 2019c. A comparative study of the proximate, FTIR analysis and mineral elements of the leaves and stem bark of *Grewia lasiocarpa* E. Mey. ex Harv.: An indigenous Southern African plant. S Afr J Bot 123: 9-19.

AKWU NA, NAIDOO Y & SINGH M. 2020b. The anatomy and histochemistry of *Grewia lasiocarpa* E. Mey. ex Harv. (Malvaceae). S Afr J Sci Technol 39(1): 91-107.

AKWU NA, NAIDOO Y, SINGH M, CHANNANGIHALLI ST, NUNDKUMAR N & LIN J. 2020a. Isolation of lupeol from Grewia lasiocarpa stem bark: Antibacterial, antioxidant, and cytotoxicity activities. Biodiversitas J Biol Diversity 21(12): 5684-5690.

AKWU NA, NAIDOO Y, SINGH M & LIN J. 2019b. Phytochemical screening, *in vitro* evaluation of the antibacterial, cytotoxicity and antioxidant potentials of *Grewia lasiocarpa* E. Mey. ex Harv. S Afr J Bot 123: 180-192.

AKWU NA, NAIDOO Y, SINGH M, NUNDKUMAR N, DANIELS A & LIN J. 2021. Two temperatures biogenic synthesis of silver nanoparticles from *Grewia lasiocarpa* E. Mey. ex Harv. leaf and stem bark extracts: Characterization and applications. Bio Nano Sci 11: 142-158.

ASAKAWA Y, LUDWICZUK A & NAGASHIMA F. 2013. Chemical constituents of bryophytes: bio-and chemical diversity, biological activity, and chemosystematics. In: Kinghorn AD, Falk H & Kobayashi J (Eds). Progress in the chemistry of organic natural products, Vienna, Austria: Springer 95: 1-796.

AYURVEDA E. 2015. Falsa Fruit-*Grewia asiatica* uses, dose, research. [accessed]. https://easyayurveda.com/2015/06/04/falsa-fruit-grewia-asiatica/.

BABUSHOK V, LINSTROM P & ZENKEVICH I. 2011. Retention indices for frequently reported compounds of plant essential oils. J Phys Chem Ref Data 40(4): 043101.

BAKKALI F, AVERBECK S, AVERBECK D & IDAOMAR M. 2008. Biological effects of essential oils–a review. Food Chem Toxicol 46(2): 446-475.

BASER KHC. 1995. Analysis and assessment of assessment of essential oils: In A manual on the essential oil industry. UNIDO, Vienna Austria. Edited by KT De Silva.

BEGUM IF, MOHANKUMAR R, JEEVAN M & RAMANI K. 2016. GC–MS Analysis of Bio-active Molecules Derived from *Paracoccus pantotrophus* FMR19 and the Antimicrobial activity against bacterial pathogens and MDROs. Indian J Microbiol 56(4): 426-432.

BRACA A, SORTINO C, POLITI M, MORELLI I & MENDEZ J. 2002. Antioxidant activity of flavonoids from *Licania licaniaeflora*. J Ethnopharmacol 79(3): 379-381.

BURT S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. Int J Food Microbiol 94(3): 223-253.

CHEN H, XIAO X, WANG J, WU L, ZHENG Z & YU Z. 2008. Antagonistic effects of volatiles generated by *Bacillus subtilis* on spore germination and hyphal growth of the plant pathogen, Botrytis cinerea. Biotechnol Lett 30(5): 919-923.

COATES J. 2000. Interpretation of infrared spectra, a practical approach. Encyclopedia of analytical chemistry. J Wiley & Sons Ltd., Chichester, p. 10881-10882.

DANDEKAR R, FEGADE B & BHASKAR V. 2015. GC-MS analysis of phytoconstituents in alcohol extract of *Epiphyllum oxypetalum* leaves. Int J Pharmacogn Phytochem Res 4(1).

DAVIES NW. 1993. Mass spectrometry and gas chromatography in chemical analysis and identification: experimental factor and case studies (Doctoral dissertation), University of Tasmania, Hobart, Australia. oai:eprints.utas.edu.au:1915.

DRWAL MN, BANERJEE P, DUNKEL M, WETTIG MR & PREISSNER R. 2014. ProTox: a web server for the in silico prediction of rodent oral toxicity. Nucleic Acids Research 42(W1): W53-W58.

ERUKAINURE OL ET AL. 2018. Suppressive Effects of *Clerodendrum volubile* P Beauv. [Labiatae] Methanolic extract and its fractions on Type 2 Diabetes and its complications. Front Pharmacol 9: 8.

FIGUEIREDO AC, BARROSO JG, PEDRO LG & SCHEFFER JJ. 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour Fragr J 23(4): 213-226.

FLACHS A. 2016. The economic botany of organic cotton farms in Telangana, India. J Ethnobiol 36(3): 683-713.

GHANEIAN MT, EHRAMPOUSH MH, JEBALI A, HEKMATIMOGHADDAM S & MAHMOUDI M. 2015. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environ Eng Manag J 2(1): 13-16.

HAAGEN-SMIT AJ. 1949. Essential oils-a brief survey of their chemistry and production in the united states. Economic Botany 3(1): 71-83.

HASSAN W, REHMAN S, NOREEN H, GUL S & NEELOFAR AN. 2016. Gas chromatography mass spectrometric (GC-MS) analysis of essential oils of medicinal plants. Adv Anim Vet Sci 4(8): 420-437.

JI J, ZHANG L, WANG P, MU Y-M, ZHU X-Y, WU Y-Y, YU H, ZHANG B, CHEN S-M & SUN X-Z. 2005. Saturated free fatty acid, palmitic acid, induces apoptosis in fetal hepatocytes in culture. Exp Toxicol Pathol 56(6): 369-376.

KUBMARAWA D, AJOKU G, ENWEREM N & OKORIE D. 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr J Biotechnol 6(14).

KUMAR PP, KUMARAVEL S & LALITHA C. 2010. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr J Biochem Research 4(7): 191-195.

LANFER-MARQUEZ UM, BARROS RM & SINNECKER P. 2005. Antioxidant activity of chlorophylls and their derivatives. Food Res Int 38(8-9): 885-891.

LANGFORD ML, HASIM S, NICKERSON KW & ATKIN AL. 2010. Activity and toxicity of farnesol towards *Candida albicans* are dependent on growth conditions. Antimicrob Agents Chemother 54(2): 940-942.

LIU Q & YAO H. 2007. Antioxidant activities of barley seeds extracts. Food chemistry 102(3): 732-737.

MAREE J, KAMATOU G, GIBBONS S, VILJOEN A & VAN VUUREN S. 2014. The application of GC–MS combined with chemometrics for the identification of antimicrobial compounds from selected commercial essential oils. Chemometrics Intellig Lab Syst 130: 172-181.

MIGUEL MG. 2010. Antioxidant and anti-inflammatory activities of essential oils: a short review. Molecules 15(12): 9252-9287.

MORAR M-I, FETEA F, ROTAR AM, NAGY M & ANAMARIA C. 2017. Characterization of essential oils extracted from different aromatic plants by FTIR spectroscopy. Bulletin UASVM Food Science and Technology 74: 1.

MORTON JF. 1987. Fruits of warm climates. Folrida Flair Books, Creative resoureces systems, Miami, 517 p.

MURAKOSHI M, NISHINO H, TOKUDA H, IWASHIMA A, OKUZUMI J, KITANO H & IWASAKI R. 1992. Inhibition by squalene of the tumor-promoting activity of 12-O-Tetradecanoylphorbol-13-acetate in mouse-skin carcinogenesis. Int J Cancer 52(6): 950-952.

NEP EI, ODUMOSU PO, NGWULUKA NC, OLORUNFEMI PO & OCHEKPE NA. 2013. Pharmaceutical properties and applications of a natural polymer from *Grewia mollis*. J Polym 2013: 1-8.

OLIVER-BEVER B. 1986. Medicinal plants in tropical West Africa. Cambridge university press.

PATHAK A. 2017. *Grewia* (Malvaceae sensu lato): ethnomedicinal uses and future potential. IJETST 4(9): 5945-5960.

PICHERSKY E, NOEL JP & DUDAREVA N. 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. Sci 311(5762): 808-811.

RAO CV, NEWMARK HL & REDDY BS. 1998. Chemopreventive effect of squalene on colon cancer. Carcinog 19(2): 287-290.

RAUT JS & KARUPPAYIL SM. 2014. A status review on the medicinal properties of essential oils. Ind Crops Prod 62: 250-264.

REDDY LJ & JOSE B. 2013. Evaluation of antibacterial and DPPH radical scavenging activities of the leaf extracts and leaf essential oil of *Syzygium cumini* Linn. from South India. Int J Pharm Pharm Sci 5(3): 1-5.

REDDY S. 2001. University botany I:(algae, fungi, bryophyta and pteridophyta). Vol. 1. New Age International.

REHMAN J, KHAN IU & ASGHAR MN. 2013. Antioxidant activity and GC-MS analysis of *Grewia optiva*. E3 JJ Biotechnol Pharm Res 4(1): 14-21.

ROBERTSHAWE P. 2011. Medicinal Plants in Australia Volume 1: Bush Pharmacy. J Aust Tradit-Med So 17(3): 173-174.

RUBERTO G & BARATTA MT. 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem 69(2): 167-174.

RUKAIYAT M, GARBA S & LABARAN S. 2015. Antimicrobial activities of hexacosane isolated from *Sanseveria liberica* (Gerome and Labroy) plant. Adv Med Plant Res 3(3): 120-125.

SANTOS CCDMP, SALVADORI MS, MOTA VG, COSTA LM, DE ALMEIDA AAC, DE OLIVEIRA GAL, COSTA JP, DE SOUSA DP, DE FREITAS RM & DE ALMEIDA RN. 2013. Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. Neurosci J, p. 1-9.

SHAABAN HA, EL-GHORAB AH & SHIBAMOTO T. 2012. Bioactivity of essential oils and their volatile aroma components. J Essent Oil Res 24(2): 203-212.

SHAGAL M, KUBMARAWA D & IDI Z. 2012. Phytochemical screening and antimicrobial activity of roots, stem-bark and leave extracts of *Grewia mollis*. Afr J Biotechnol 11(51): 11350-11353.

SILVA RO, SOUSA FBM, DAMASCENO SR, CARVALHO NS, SILVA VG, OLIVEIRA FR, SOUSA DP, ARAGÃO KS, BARBOSA A & FREITAS RM. 2014. Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. Fundam Clin Pharmacol 28(4): 455-464.

SMITH TJ, YANG GY, SERIL DN, LIAO J & KIM S. 1998. Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis by dietary olive oil and squalene. Carcinogenesis 19(4): 703-706.

STUART B. 2005. Infrared spectroscopy. Wiley Online Library.

TISSERAND R & BALACS T. 1999. Essential oil safety. A guide for health care professionals. London: Churchill Livingstone. Hartcourt Publishers Limited.

ULLAH W, UDDIN G & SIDDIQUI BS. 2012. Ethnic uses, pharmacological and phytochemical profile of genus *Grewia*. J Asian Nat Prod Res 14(2): 186-195.

UMA B & PARVATHAVARTHINI R. 2010. Antibacterial effect of hexane extract of sea urchin, *Temnopleurus alexandri* (Bell, 1884). Int J Pharmtech Res 2(3): 1677-1680.

ZUZARTE M & SALGUEIRO L. 2015. Essential oils chemistry. Bioactive essential oils and cancer. Springer, p. 19-61.

How to cite

AKWU NA, NAIDOO Y, CHANNANGIHALLI ST, SINGH M, NUNDKUMAR N & LIN J. 2021. The essential oils of *Grewia Lasiocarpa* E. Mey. Ex Harv.: chemical composition, *in vitro* biological activity and cytotoxic effect on Hela cells. An Acad Bras Cienc 93: e20190343. DOI 10.1590/0001-3765202120190343.

Manuscript received on April 24, 2019;
accepted for publication on July 2, 2019

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