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Phytochemical and antioxidant properties of different solvent extracts of *Kirkia wilmsii* tubers

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ABSTRACT

Objective: To determine suitable phytochemical extraction solvents, screen for phytochemicals, determine the total phenol and flavonoid contents and the antioxidant activities of different solvent extracts of *Kirkia wilmsii* (*K. wilmsii*), an ethnomedicine in South Africa.
Methods: Extractions were performed from dried tubers of the *K. wilmsii* plant, using several solvents and varying extraction times. Extract yields were determined and suitable extraction solvents were selected. Total phenol and flavonoid contents of the extracts were determined spectrophotometrically using gallic acid and quercetin as standards. The free radical scavenging activity of the extracts was investigated using 1,1-diphenyl-2-picrylhydrazyl radical.
Results: Phytochemical screening confirmed the presence of phenolics, flavonoids, terpenoids, tannins, cardenolide deoxy sugars and reducing sugars. Of the 12 solvent extracts used, six gave yields higher than 5%, while the other six gave yields less than 1%. The highest extract yield of 52.9% was obtained using 80% methanol while the lowest yield of 7.3% was obtained using ethanol at 60 min. The 80% methanol, methanol/chloroform/water (12:5:3) and 60% methanol extracts were significantly higher than those of ethanol, methanol and water ($P < 0.05$). Total phenolic content recorded extracts ranged from (45.32 ± 0.50) to (122.84 ± 0.31) mg gallic acid equivalent per gram. A maximum total flavonoid content of (917.02 ± 0.10) mg quercetin equivalent per gram and a minimum of (206.26 ± 0.10) mg quercetin equivalent per gram were recorded for methanol and water, respectively. The flavonoid content for methanol was significantly higher than all the other extracts ($P < 0.05$). The scavenging profiles of *K. wilmsii* extracts were significantly lower ($P < 0.05$) than that of ascorbic acid and IC_{50} values ranged from 129.94 μg/mL for methanol to 225.04 μg/mL for water. An IC_{50} value of 56.52 μg/mL was obtained with ascorbic acid.
Conclusions: Ethanol, methanol, methanol/chloroform/water, 80% methanol, 60% methanol and water can be used as suitable phytochemical extraction solvents for *K. wilmsii* tubers. Total phenolic content and total flavonoid content analysis proved the presence of high levels of phenolic compounds as well as flavonoids. The presence of phenols and flavonoid could be responsible for the radical scavenging activities observed.

1. Introduction

Secondary metabolites are produced by plants mainly as products of primary metabolism and as part of the defence mechanisms of plants. Phytochemicals such as, alkaloids, tannins and flavonoids are examples of secondary metabolites produced by plants, from which the plants are thought to get their healing properties [1]. Phenolic compounds have been associated with antioxidant activity due to their free radical scavenging activities [2,3].

Common names of *Kirkia wilmsii* (*K. wilmsii*) are wild pepper or Mountain Seringa (English) and Legaba or Modumela (Northern Sotho) [4]. The *K. wilmsii* belongs to the Sapindales

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family [5]. This family (Kirkiaceae) includes a wide range of small to medium sized trees, mainly found in eastern tropical Africa, Madagascar and South Africa. This tree is one of the plants that have underground storage organs (such as rhizomes, tubers, corms, bulbs and caudex) [6].

The *K. wilmsii* tuber is used by the Bapedi people in Lebowakgomo district located in the Limpopo Province, South Africa for the treatment of various ailments. The local people believe that chewing it regularly helps to maintain general good health.

Studies on the leaves have shown that the leaves of the tree have antiplasmodial properties [7]. Studies conducted on leaf extracts have shown antimicrobial activity against *Staphylococcus aureus* (*S. aureus*), *Enterococcus faecal*, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, *Sporothrix schenckii*, *Microsporum canis*, *Cryptococcus neoformans* and *Candida albicans* [8]. The leaves further showed biological activity against the animal fungal pathogen *Aspergillus fumigatus*.

Extracts of the dried and powdered bark of the *K. wilmsii* were found to contain secondary metabolites, lignans, isocoumarins and flavonoids [9]. Ethnobotanical surveys have recorded that *K. wilmsii* tubers are traditionally used in the treatment of diabetes mellitus [4] and hypertension [10]. Antimicrobial activity against *Shigella dysenteriae*, *Aeromonas hydrophila*, *Salmonella thyphii*, *Proteus mirabilis*, *E. coli* and *S. aureus* was recorded [11].

The presence of an excess of oxygen in the human body has some negative effects as it can trigger radical chain reactions in the presence of reactive species. This can cause health problems, such as aging and cell destruction [12]. Antioxidants have been found to be the solution to this problem as they interrupt these chain reactions to form radicals that can easily be removed from the human body, thereby generally improving health, assisting cell rejuvenation, cancer prevention and cardiovascular diseases prevention [13]. Thus it is important to investigate the antioxidant potential of *K. wilmsii*.

A considerable number of publications have been reported on the phytochemistry of leaves and the bark of *K. wilmsii* [5,7–9,14]. Limited studies on the tuber have been directed at the evaluation of its traditional medicinal applications [4,10]. No reports are available on the phytochemical analysis and antioxidant activities of the tubers of *K. wilmsii*. The study was therefore aimed at determining suitable solvents for extraction of phytochemicals, phytochemical screening and quantitative analysis of total phenols, flavonoids and antioxidant activity of *K. wilmsii* tubers. Our results provide a basis for future studies on isolation, identification and characterization of active compounds with potential applications in drug development.

2. Materials and methods

2.1. Plant materials

The tubers of *K. wilmsii* were collected from the Lebowakgomo Region in Polokwane District between November 2015 and July 2016, which is situated in the Limpopo Province of South Africa. The plant name was identified by the Department of Botany at the University of Venda and the name was further confirmed by the National Herbarium (Voucher number MPT00112) in Pretoria, South Africa. The plant name has been checked with www.theplantlist.org and has been reported as an accepted name (record 29400130), website accessed 28 November 2015.

2.2. Sample preparation and extraction

Only the tuber of *K. wilmsii* tree was used in the study. The tubers are covered with a bark which was removed from the tubers as the indigenous people remove the bark from the tubers before eating them. The peeled tubers were washed with deionised water (Milli-Q Millipore, 0.054 μS/cm) to remove soil and dirt. The tuber was cut into small pieces using an ordinary kitchen knife and dried at ambient temperature (± 30 °C in Thohoyandou) in the laboratory for 5 days. After drying, the plant was ground to a fine powder using a Retsch Muhle grinding mill.

The extraction process was performed by adding 25 mL of each extraction solvent to the weighed portions (2 g) of dried powder in stoppered volumetric flasks and extraction performed by sonicating in an Integral Systems ultrasonic bath for the prescribed extraction times below. Extraction was performed with the required solvent for the required length of time. The extraction times were 15, 30 and 60 min [15]. The solvents used were ethanol, methanol, methanol/chloroform/water (MCW), 80% methanol, 60% methanol, water, dichloromethane, chloroform, acetone, hexane, diethyl ether, ethyl acetate [16].

For the extracts with the yields higher than 5%, *i.e.* ethanol, methanol, MCW, 80% methanol, 60% methanol and water, further extractions were performed in triplicate.

In all cases the filtrate was evaporated to dryness using the Vacutex Flexi-dry μp freeze drier. The freeze dried extracts were kept in a deep freezer at −20 °C for storage.

2.3. Phytochemical screening tests

Phytochemical screening tests were performed according to standardised recent methods as described in literature [1,2,17–20]. Various solvent extracts of *K. wilmsii* were used to screen for phenolics and flavonoids [1,20], steroids and terpenoids [18,20], saponins [20], cardiac glycosides [20], cardenolide deoxy sugars [20], tannins [2], phlobatannins [2], anthraquinone glycosides [17] and reducing sugars [19].

2.4. Total phenolic content

A modified method for the determination of total phenolic content was carried out using modification of the method cited by Sahu and Saxena [21]. Gallic acid standard solutions were prepared in methanol to give the following final concentrations; 20.00, 40.00, 60.00, 80.00, and 100.00 μg/mL. Each plant extract was dissolved in methanol to give a final concentration of 1.0 mg/mL and 0.5 mL of each sample and standards were introduced into different test tubes and mixed with 2.5 mL of a 10-fold dilute Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate were added. The test tubes were covered with parafilm and allowed to stand for 30 min at room temperature before the absorbance was read at 760 nm. The results of the total phenolics were expressed as mg of gallic acid equivalents (GAE) per gram of sample and calculated by the formula [22]:

$$TPC = (C \times V)/M$$

where, TPC is total phenolic content (mg/g plant extract in GAE), C is concentration of gallic acid established from the

calibration curve (μg/mL), V is volume of the extract (mL), M is mass of the extract of the plant (g).

2.5. Total flavonoid content

Total flavonoid content was estimated using standard methods with minor modifications [21,23] using quercetin as a standard. The standard solutions with the following final concentrations were prepared; 50, 100, 150, 200 and 250 μg/mL. One milliliter of each standard solution and extract solutions was taken into 10 mL volumetric flask, containing 4 mL of deionised water. Then 0.3 mL of 10% $AlCl_3$ was added to the mixture. At the 6th min, 2 mL of NaOH (1 mol/L) was added and volume made up to 10 mL with distilled water. The absorbance was read at 510 nm using Beckman Coulter DU 650i UV–vis spectrophotometer. The results of the total flavonoids were expressed as quercetin equivalents (QE).

2.6. Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the different plant extracts were compared by adapting the procedure reported by Iqbal *et al.* and Sahu *et al.* [17,24]. Each extract was dissolved in an appropriate volume of methanol to give final concentrations ranging from 10.00 to 250.00 μg/mL. Standard ascorbic acid solutions were also prepared in methanol to give the same concentration range. Two milliliter of each solution was transferred to a test tube and mixed with 4 mL of 0.3 mmol/L DPPH. A control solution was prepared by adding methanol (2 mL) to 0.3 mmol/L DPPH (4 mL). The experiment was run in triplicate. The solutions were mixed well and left in the dark for 30 min. After 30 min the solutions were analyzed on a UV–vis spectrophotometer at 517 nm. The percentage of antioxidant potential was calculated using the formula:

$$\%\ \text{Inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

where, A_c is absorbance of the control solution, A_s is absorbance of the sample or standard.

2.7. Statistical analysis

Means of triplicate analysis were calculated and data was expressed as mean ± SD. *Post hoc* ANOVA statistical analysis was performed using SPSS 22 software for comparison between two or more treatments. A difference was considered to be statistically significant when $P < 0.05$.

3. Results

3.1. Phytochemical screening

The phytochemical screening tests of the tuber revealed the presence of phenolics, flavonoids, terpenoids, tannins, cardenolide deoxy sugars and reducing sugars (Table 1).

3.2. Solvent extraction

The highest extract yield (52.9%) was obtained from extraction with 80% methanol (Figure 1). This result was not significantly different from 48.2% to 46.9% extract yields for MCW and 60% methanol, respectively ($P > 0.05$). The results for ethanol (7.3%), water (20.9%) and methanol (36.6%) were significantly lower than the above mentioned results. An increase in the extraction time also showed a significant increase in the % yield obtained ($P < 0.05$). We conclude that 80% methanol is the best solvent to use for extraction as the extracts are more soluble in polar solvents and 80% methanol has more polar organic properties.

Table 1
Phytochemical screening test results.

Test	Extract					
	Water	Methanol	Acetone	Ether	Ethyl acetate	Chloroform
Phenolics	+	+	+	–	+	–
Flavonoids (Alkaline test)	+	+	+	–	+	–
Flavonoids (Lead acetate)	+	+	+	–	+	–
Terpenoids	–	–	–	–	–	+
Saponins	–	–	–	–	–	–
Cardiac glycosides	–	–	–	–	–	–
Cardenolide deoxy sugar	–	+	+	–	–	–
Tannins	+	+	+	–	–	–
Phlobatannins	–	–	–	–	–	–
Anthraquinone glycosides	–	–	–	–	–	–
Reducing sugars	+	+	+	–	–	–

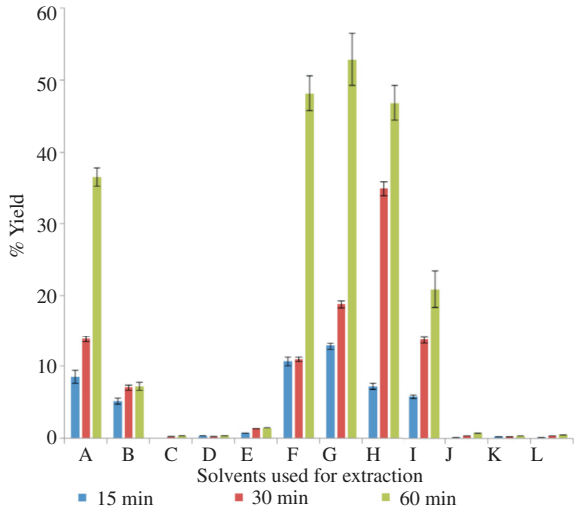

Figure 1. Effect of varying extraction time and extraction solvent on extract yield of *K. wilmsii*.
A: Methanol; B: Ethanol; C: Dichloromethane; D: Chloroform; E: Acetone; F: MCW; G: 80% Methanol; H: 60% Methanol; I: Water; J: Hexane; K: Diethyl ether; L: Ethyl acetate.

3.3. Total phenolic content

The total phenolic contents of *K. wilmsii* extracts ranged from 45.32 to 122.84 mg GAE/g from water and methanol extracts, respectively (Table 2). Pure methanol produced extracts with the highest levels of total phenolics. The content is significantly higher than the phenolic contents of all the other solvents used ($P < 0.05$). No significant differences were recorded in the total phenolic contents of ethanol, 80% methanol, MCW, 60% methanol and water ($P > 0.05$).

3.4. Total flavonoid content

The total flavonoid contents of *K. wilmsii* were recorded ranging from 206.26 mg QE/g to 917.02 mg QE/g from the water extract and the methanol extract, respectively. The methanol extract exhibited a total flavonoid content that is significantly higher than the rest of the extractants ($P < 0.05$). The flavonoid contents of the rest of the solvents are also significantly different ($P < 0.05$) from each other except for ethanol, 60% methanol and water extracts whose flavonoid contents are not significantly different (Table 2).

The total flavonoid content for the methanol extract was very high (917 mg/g) and the other extracts exhibited results ranging from 200 to 450 mg/g.

Table 2
Total phenolic content and total flavonoid content of the different extracts of *K. wilmsii*.

Extract	Total phenolic content GAE (mg/g)	Total flavonoid content QE (mg/g)
Ethanol	58.98 ± 0.45[a]	256.95 ± 0.45[c]
Methanol	122.84 ± 0.31[b]	917.02 ± 0.10[d]
MCW	69.34 ± 0.91[a]	437.64 ± 0.17[e]
80% Methanol	57.16 ± 0.47[a]	351.37 ± 0.06[f]
60% Methanol	62.05 ± 0.30[a]	262.49 ± 0.25[c]
Water	45.32 ± 0.50[a]	206.26 ± 0.10[c]

Results with different superscripts are significantly different from each other ($P < 0.05$).

3.5. DPPH free radical scavenging activity

The scavenging profiles of the extracts and ascorbic acid are shown in Figure 2, from which it can be observed that all the extracts possess radical scavenging potential. Methanol proved to be the most active of the extracts while the water extract was the least.

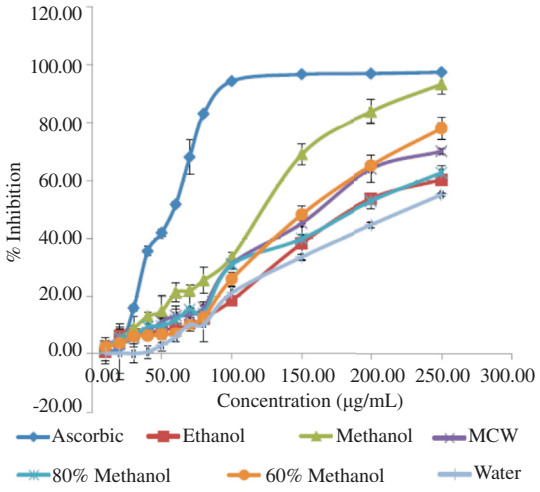

Figure 2. Percentage inhibition of the various extracts on DPPH.

The IC_{50} values of all our extracts are significantly different from each other ($P < 0.05$) except for MCW and 60% methanol which are not significantly different (Table 3). Therefore, the IC_{50} value for the methanol extract is significantly lower than all the other values. The best activity was recorded by the methanol extract with an IC_{50} value of 129.94 µg/mL while the water extract gave the lowest IC_{50} around 225.04 µg/mL.

Table 3
IC_{50} values for the extracts as well as ascorbic acid.

Extract	IC_{50} (µg/g)
Ethanol	204.78 ± 1.51[a]
Methanol	129.94 ± 0.20[b]
MCW	172.44 ± 0.62[c]
80% Methanol	194.62 ± 1.00[d]
60% Methanol	167.27 ± 0.57[c]
Water	225.04 ± 0.72[e]
Ascorbic acid	56.52 ± 0.07[f]

All extracts with different superscripts are statistically different ($P < 0.05$).

4. Discussion

4.1. Phytochemical screening

Compounds such as cardenolides, flavonoids, resins, saponins and tannins have been shown to have healing properties against most disease causing organisms [20,25]. These properties include antioxidant activity, anti-allergic, anti-inflammatory and many others. In our earlier research, water extracts of *K. wilmsii* tuber were found to be active against *Shigella dysenteriae*, *Aeromonas hydrophila*, *Salmonella thyphii*, *Proteus mirabilis*, *E. coli* and *S. aureus* [11].

The methanol, water and acetone extracts yielded positive results for many groups of compounds in the phytochemical screening tests. These results are consistent with other reported results, where methanol extracts tested positive for the highest number of different classes of phytochemicals in *Bauhinia variegata* L. bark [26] as well as in tests on the extracts from *Anamirta cocculus* seeds [27]. Further, the root and bark of *Eurycoma longifolia* also showed that the methanol, ethyl acetate and chloroform extracts were good sources of different classes of compounds [20]. However, the methanol and ethanol extracts of *Strychnos minor* Dennst leaves gave poor results [2]. Further the extracts gave different results with methanol, ethyl acetate and chloroform extracts testing positive for phenolics, flavonoids, terpenoids, alkaloids, proteins and cardiac glycosides in the study done on root and stem extracts of wild *Eurycoma longifolia* Jack by Zakia *et al*. [20]. Our ethyl acetate and chloroform extracts tested negative for most groups of compounds. The ether extract proved negative for all the tests conducted.

We therefore conclude that water, methanol and acetone or their combinations would give good extraction yields that can be used for the phytochemical screening of *K. wilmsii*.

4.2. Solvent extraction

Since biologically active compounds occur naturally in very small concentrations, the choice of an extraction method and the corresponding suitable solvent is an important step in the drug discovery process.

Solvents with a wide range of polarity were used for the extraction. The results suggest that polar solvents gave better extraction yields [28], which is true in our study.

Therefore, the *K. wilmsii* tuber secondary metabolites can be extracted with polar protic solvents with high yields ranging from 7.3% to 52.9%. Non polar solvents, such as, hexane, ether produced very small amounts of extracts, less than 1%. This

observation is also supported by results obtained from the extraction from *Paramignya trimera* root where the polar protic solvents, methanol and water, gave the best extraction yields [29].

Extraction with a MCW combined solvent also produced a significantly high yield (48.2%), consistent with other research work using the same solvent [16]. However, we reported a slightly higher extract yield of 48.2% as compared to 45% and 35% extraction yields for *Anthocleista grandiflora* and *Combretum erythrophyllum* leaves reported by Eloff [16]. However, the MCW combination tended to separate into different phases as chloroform and water are immiscible.

Therefore, addition of water to methanol proved that the extraction efficiency can be increased significantly as the extraction yields obtained with 80% methanol (52.9% yield) and 60% methanol (46.9% yield) were much higher than the yield obtained from the use of pure methanol (36.6% yield). This observation is consistent with other reported results [30,31], as the water tends to increase the polarity of the extractant.

4.3. Total phenolic content

The total phenolic content results obtained in our study are higher than the results reported by Dhanani *et al*. [32], who reported a maximum of 30 mg GAE/g for *Withania somnifera* roots extracted with ethanol, 10% ethanol and water [32]. Some other researchers reported total phenolic content values between 100 and 150 mg GAE/g, with the methanol and acetone extracts of the leaves, stem and flowers of *Thermopsis turcica* [33]. These values are generally higher than our results and only our methanol extract matches these values. Our methanol extract compares well with these phenolic contents.

The other solvents produced extracts whose total phenolic contents is similar to those reported in literature, from the extracts of *Goniothalamus velutinus* (*G. velutinus*) whose bark extract gave 68 GAE mg/g and the leaves 78 GAE mg/g [17]. *G. velutinus* is reported to have antitumor and anticancer properties. The water extracts of *Hedychium spicatum*, *Hedychium coronarium* and *Hedychium rubrum* were found to be 30, 35 and 67 GAE mg/g [21]. However, our results are much lower than total phenolic contents recorded for the resurrection plant *Myrothamnus flabellifolius* water, ethanol and methanol extracts (all 400 mg GAE/g) [34]. Stanojević *et al*. [35] also reported much higher total phenolic contents of 250 mg GAE/g when *Hieracium pilosella* water, ethanol and methanol extracts were tested.

Phenolic contents ranging 90–260 mg GAE/g were recorded with three species of *Curcuma* methanol extracts [21]. All these results are also higher than our reported results except for our methanol extract. *Curcuma* species are used for the treatment of asthma tumors and also as antifungal.

Total phenolic content is an important factor in the consideration of antioxidant activity. Therefore, the higher the value of phenolic content, the more beneficial the extract is to human health as they can quench reactive free radicals or primary oxidants.

4.4. Total flavonoid content

The methanol extract's flavonoid content matches the very high flavonoid contents values obtained with wheat methanol extracts ranging from 791.3 to 987.7 mg QE/g [36]. All our other results are comparable to the results reported with the hexane, chloroform, ethyl acetate, butanol, methanol and water extracts of *Azadirachta indica* with results ranging from 63 mg QE/g to 529.5 mg QE/g [37]. In their work, all the other results are above 350 mg QE/g except for the butanol extract. Research has proven that flavonoids are important in the fight against diseases and can also act as antioxidants depending on their structure [17]. Coupled with phenols, flavonoids have been reported to have high antioxidant activity [2]. Therefore, these high total phenolic content results could mean the abundance of possible compounds of pharmaceutical importance. Other published results are very low, pigeon pea extracts gave flavonoid contents ranging from 0.16 to 1.58 mg QE/g [38], and from 20 to 80 mg QE/g content for *Curcuma extracts* [21], 79.13–82.18 mg QE/g for *Hieracium pilosella* [35]. Further work is necessary to determine the flavonoid types, biological, anti-inflammatory activity, antimicrobial and anticancer activities of *K. wilmsii*.

4.5. DPPH free radical scavenging activity

Antioxidant agents with high scavenging activity should have a low IC_{50} value [39]. This is supported by the lowest value being exhibited by ascorbic acid, a well-known antioxidant. The IC_{50} results obtained are significantly higher than that of ascorbic acid (56 μg/mL). This is to be expected as crude extracts were used before purification. Results for purified extracts are expected to be much more closely related to those of ascorbic acid.

The methanol IC_{50} (130 μg/mL) result is comparable to that obtained from 90% ethanol *Cyclocarya paliurus* extracts (146 μg/mL) [31]. Furthermore, this observation closely relates to results obtained by Alkhawalisy and Hossain [40], where they reported that they recorded the highest antioxidant activity with methanol extracts. However, the concentration of the DDPH concentration used in our study is higher (0.3 mmol/L) as compared to 0.1 mmol/L used by Xie *et al*. [31].

Other IC_{50} results from *Pistacia atlantica* subsp. *mutica* extracts yielded IC_{50} values ranging from 0.6 to 1 105.3 μg/mL [1], with protic polar solvents giving higher activity, which is consistent with our work. The major differences observed can be attributed to the very low concentration of DPPH used by Rezaie *et al*. [3].

Our results are also comparable to IC_{50} values of 155 and 204 μg/mL from the leaves and bark respectively of the extracts of *G. velutinus* [17].

An extract is considered to be active against free radicals if $IC_{50} < 5$ mg/mL [41]. All our extracts have IC_{50} values less than 5 mg/mL, therefore all the extracts for the solvents used are a possible good source of antioxidants. There is a positive correlation between the IC_{50}, total phenolic content and total flavonoid content ($r = 0.853$ for IC_{50} and total flavonoid content and $r = 0.899$ for IC_{50} and total phenolic content). Furthermore, there is also a very high correlation between total flavonoid content and total phenolic content ($r = 0.98$).

The phytochemical screening and solvent extraction analysis give a good guide of the phytochemicals present in the extracts as well as suitable extraction solvents. And 80% methanol is the best solvent to use for extraction as the extracts are more soluble in polar solvents and 80% methanol has more polar organic properties.

Successive extractions proved that yields can be considerably increased by performing short repetitive extractions.

Total phenolic content and total flavonoid content analysis proved the presence of high levels of phenolic compounds as well as flavonoids. In comparison to other ethno-medicines studied, the flavonoid content was particularly high for *K. wilmsii*. The results are consistent with the antioxidant activity produced by the extracts. Positive correlations between IC_{50}, total phenolic content and total flavonoid contents show that the antioxidant activity is caused by the presence of phenolic compounds and flavonoids.

Further studies would involve antimicrobial and anti-inflammatory activity tests, chemical properties tests, separation and identification of individual components.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Bhandary SK, Kumari N, Bhat VS, Sharmila K, Bekal MP. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. *Nitte Univ J Health Sci* 2012; **2**: 34-8.

[2] Maria John KM, Ayyanar M, Arumugam T, Enkhtaivan G, Jin K, Kim DH. Phytochemical screening and antioxidant activity of different solvent extracts from *Strychnos minor* Dennst leaves. *Asian Pac J Trop Dis* 2015; **5**: 204-9.

[3] Rezaie M, Farhoosh R, Iranshahi M, Sharif A, Golmohamadzadeh S. Ultrasonic-assisted extraction of antioxidative compounds from Bene (*Pistacia atlantica* subsp. *mutica*) hull using various solvents of different physicochemical properties. *Food Chem* 2015; **173**: 577-83.

[4] Semenya S, Potgieter M, Erasmus L. Ethnobotanical survey of medicinal plants used by Bapedi healers to treat diabetes mellitus in the Limpopo Province, South Africa. *J Ethnopharmacol* 2012; **141**: 440-5.

[5] Bachelier JB, Endress PK. Floral structure of *Kirkia* (Kirkiaceae) and its position in Sapindales. *Ann Bot* 2008; **102**: 539-50.

[6] Laden G, Wrangham R. The rise of the hominids as an adaptive shift in fallback foods: plant underground storage organs (USOs) and australopith origins. *J Hum Evol* 2005; **49**: 482-98.

[7] Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, et al. *In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *J Ethnopharmacol* 2004; **92**: 177-91.

[8] Suleiman MM, McGaw LJ, Naidoo V, Eloff JN. Evaluation of several tree species for activity against the animal fungal pathogen *Aspergillus fumigatus*. *S Afr J Bot* 2010; **76**: 64-71.

[9] Mulholland DA, Cheplogoi P, Crouch NR. Secondary metabolites from *Kirkia acuminata* and *Kirkia wilmsii* (Kirkiaceae). *Biochem Syst Ecol* 2003; **31**: 793-7.

[10] Semenya SS, Potgieter MJ. *Kirkia wilmsii*: a Bapedi treatment for hypertension. *S Afr J Bot* 2015; **100**: 228-32.

[11] Chigayo K, Mojapelo PE, Bessong P, Gumbo JR. The preliminary assessment of antimicrobial activity of HPLC separated extracts of *Kirkia wilmsii*. *Afr J Tradit Complement Altern Med* 2014; **11**: 275-81.

[12] Ye F, Liang Q, Li H, Zhao G. Solvent effects on phenolic content, composition, and antioxidant activity of extracts from florets of sunflower (*Helianthus annuus* L.). *Ind Crops Prod* 2015; **76**: 574-81.

[13] Li JE, Fan ST, Qiu ZH, Li C, Nie SP. Total flavonoids content, antioxidant and antimicrobial activities of extracts from *Mosla chinensis* Maxim. cv. Jiangxiangru. *LWT-Food Sci Technol* 2015; **64**: 1022-7.

[14] Pillay P, Maharaj VJ, Smith PJ. Investigating South African plants as a source of new antimalarial drugs. *J Ethnopharmacol* 2008; **119**: 438-54.

[15] Razafintsalama V, Sarter S, Mambu L, Randrianarivo R, Petit T, Rajaonarison JF, et al. Antimicrobial activities of *Dilobeia thouarsii* Roemer and Schulte, a traditional medicinal plant from Madagascar. *S Afr J Bot* 2013; **87**: 1-3.

[16] Eloff J. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *J Ethnopharmacol* 1998; **60**: 1-8.

[17] Iqbal E, Salim KA, Lim LBL. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *J King Saud Uni Sci* 2015; **27**: 224-32.

[18] Kumar D, Ghosh R, Pal BC. α-Glucosidase inhibitory terpenoids from *Potentilla fulgens* and their quantitative estimation by validated HPLC method. *J Funct Foods* 2013; **5**: 1135-41.

[19] Singh R, Kumari N. Comparative determination of phytochemicals and antioxidant activity from leaf and fruit of *Sapindus mukorossi* Gaertn. – A valuable medicinal tree. *Ind Crops Prod* 2015; **73**: 1-8.

[20] Khanam Z, Wen CS, Bhat IUIH. Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). *J King Saud Uni Sci* 2015; **27**: 23-30.

[21] Sahu R, Saxena J. Screening of total phenolic and flavonoid content in conventional and nonconventional species of *Curcuma*. *J Pharmacogn Phytochem* 2013; **2**: 176-9.

[22] Singh KL, Bag G. Phytochemical analysis and determination of total phenolics content in water extracts of three species of *Hedychium*. *Int J PharmTech Res* 2013; **5**: 1516-21.

[23] Kapewangolo P, Hussein AA, Meyer D. Inhibition of HIV-1 enzymes, antioxidant and anti-inflammatory activities of *Plectranthus barbatus*. *J Ethnopharmacol* 2013; **149**: 184-90.

[24] Sahu RK, Kar M, Routray R. DPPH free radical scavenging activity of some leafy vegetables used by Tribals of Odisha, India. *J Med Plants Stud* 2013; **1**: 21-7.

[25] Pandey S. Preliminary phytochemical screening and *in vitro* antibacterial activity of *Bauhinia variegata* Linn. against human pathogens. *Asian Pac J Trop Dis* 2015; **5**: 123-9.

[26] Parekh J, Karathia N, Chanda S. Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark. *Afr J Biomed Res* 2006; **9**: 53-6.

[27] Qadir U, Paul V, Ganesh P. Preliminary phytochemical screening and *in vitro* antibacterial activity of *Anamirta cocculus* (Linn.) seeds. *J King Saud Uni Sci* 2015; **27**: 97-104.

[28] Ahmed AS, McGaw LJ, Elgorashi EE, Naidoo V, Eloff JN. Polarity of extracts and fractions of four *Combretum* (Combretaceae) species used to treat infections and gastrointestinal disorders in southern African traditional medicine has a major effect on different relevant *in vitro* activities. *J Ethnopharmacol* 2014; **154**: 339-50.

[29] Nguye VT, Bowyer MC, Van Vuong Q, Van Altena IA, Scarlett CJ. Phytochemicals and antioxidant capacity of Xao tam phan (*Paramignya trimera*) root as affected by various solvents and extraction methods. *Ind Crops Prod* 2015; **67**: 192-200.

[30] Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* 2009; **14**: 2167-80.

[31] Xie JH, Dong CJ, Nie SP, Li F, Wang ZJ, Shen MY, et al. Extraction, chemical composition and antioxidant activity of flavonoids from *Cyclocarya paliurus* (Batal.) Iljinskaja leaves. *Food Chem* 2015; **186**: 97-105.

[32] Dhanani T, Shah S, Gajbhiye N, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arab J Chem* 2013; http://dx.doi.org/10.1016/j.arabjc.2013.02.015. in press.

[33] Aksoy L, Kolay E, Ağılönü Y, Aslan Z, Kargıoğlu M. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic *Thermopsis turcica*. *Saudi J Biol Sci* 2013; **20**: 235-9.

[34] Cheikhyoussef A, Summers RW, Kahaka G. Qualitative and quantitative analysis of phytochemical compounds in Namibian *Myrothamnus flabellifolius*. *Int Sci Technol J Namibia* 2015; **5**: 71-83.

[35] Stanojević L, Stanković M, Nikolić V, Nikolić L, Ristić D, Čanadanovic-Brunet J, et al. Antioxidant activity and total phenolic and flavonoid contents of *Hieracium pilosella* L. extracts. *Sensors* 2009; **9**: 5702-14.

[36] Sumczynski D, Bubelova Z, Sneyd J, Erb-Weber S, Mlcek J. Total phenolics, flavonoids, antioxidant activity, crude fibre and digestibility in non-traditional wheat flakes and muesli. *Food Chem* 2015; **174**: 319-25.

[37] Khamis Al-Jadidi HS, Hossain MA. Studies on total phenolics, total flavonoids and antimicrobial activity from the leaves crude extracts of neem traditionally used for the treatment of cough and nausea. *Beni-Suef Uni J Basic Appl Sci* 2015; **4**: 93-8.

[38] Al-Saeedi AH, Hossain MA. Total phenols, total flavonoids contents and free radical scavenging activity of seeds crude extracts of pigeon pea traditionally used in Oman for the treatment of several chronic diseases. *Asian Pac J Trop Dis* 2015; **5**: 316-21.

[39] Zhang Y, Shen Y, Zhu Y, Xu Z. Assessment of the correlations between reducing power, scavenging DPPH activity and anti-lipid-oxidation capability of phenolic antioxidants. *LWT-Food Sci Technol* 2015; **63**: 569-74.

[40] Alkhawalidy ASR, Hossain MA. Study on total phenolics and antioxidant activity of leaves crude extracts of *Annona squamosa* traditionally used for the treatment of cancerous tumours. *Asian Pac J Trop Dis* 2015; **5**(Suppl 1): S142-4.

[41] Abdillah S, Tambunan RM, Farida Y, Sandhiutami NMD, Dewi RM. Phytochemical screening and antimalarial activity of some plants traditionally used in Indonesia. *Asian Pac J Trop Dis* 2015; **5**: 454-7.