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Bioprospecting of the underutilized endemic taxon *Cissus woodrowii* (Stapf ex Cooke) Santapau for its antioxidant activity and phenolic profiling

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The present study explored the antioxidant potential of endemic *Cissus woodrowii* (CW) which is an underutilised tree taxon of the Vitaceae family. Maximum per cent yield (13.49%), total phenolic content (24.14 mg TAE/g dry weight), and total flavonoid content (18.45 mg QE/g dry weight) were recorded in the methanolic leaf extract. Whereas the *in vitro* antioxidant activities of different extracts were assessed using 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), 2,2-diphenyl-1-picrylhydrazyl, hydroxyl, nitric oxide, hydrogen peroxide percent radical scavenging activity (% RSA), antioxidant power assay (ferric-reducing antioxidant power), and total antioxidant capacity (phosphomolybdate assay). Antioxidant activity of CW extract may be due to its high level of phenolic compounds, which were screened through liquid chromatography–high-resolution mass spectrometry (LC-HRMS), and the selected three phenolic compounds were quantified using high-performance liquid chromatography (HPLC). The HPLC analysis revealed a higher concentration of gallic acid (119.78 µg/g dry weight), followed by quercetin (22.13 µg/g dry weight) and embelin (21.09 µg/g dry weight). This is the first report on this underexplored taxon which could be employed for the development of several nutraceuticals and pharmaceuticals.

Keywords: Antioxidant activity, *Cissus woodrowii*¸ HPLC, LC-HRMS, Phenolic profiling, Woodrow's grape tree.

IPC code; Int. cl. (2015.01)− A61K 36/00, A61K 36/87, A61K 127/00, A61P 39/00

Introduction

Bioprospecting is the exploration and utilization of nutrients, phytochemicals, and medicines from biological resources for the societal and commercial benefit[1]. Till date, many plants have been over-exploited for nutritional and medicinal properties, which make these plants populations more vulnerable[2]. On the other hand, there are several plants which possess various phytochemicals that can be beneficial to humans but are not explored yet[3]. Such plants are termed as "underutilized" as scientific evidence regarding their potential is lacking[4,5]. Identification and exploration of underutilized plants can aid in reducing the burden of many over-exploited plants.

The genus *Cissus* (family Vitaceae) comprises of 350 species, and many of these are used worldwide in traditional medicines to treat various diseases and disorders[6,7]. In India, 22 species of *Cissus* have been reported, of which *C. quadrangularis* is over-exploited for its chemical contents and medicinal uses[7-10]. According to the National Medicinal Plant Board of India, the annual trade for this plant species is approximately 200-500 metric tons which may cause a depletion in its wild population[11]. Phenolics are ubiquitous to plants and exhibit potent antioxidant properties and the similar bioactivity has been reported for *C. quadrangularis* with associated phytochemicals[7,12-14]. To date, hundreds of plant species have been explored for their phenolic compounds and antioxidant activities, since it is associated with cardiovascular diseases, pulmonary diseases, chronic kidney diseases, neurodegenerative diseases, cancer, etc[15-17]. But still there lies a scope for the screening of antioxidant components from different plant species[18].

Cissus woodrowii (Stapf ex Cooke) Santapau is commonly known as Woodrow's grape tree[19]. Taxonomically, it is a unique plant species of *Cissus* due to its shrub-like habit (Fig. 1), while the remaining taxa of the Vitaceae are woody lianas[20]. Bioprospecting of *Cissus woodrowii* (CW) for its phytochemicals and bioactivities is unnoticed. Thus,

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the present investigation was undertaken to explore the antioxidant potential of CW that can be utilised as a substitute for *C. Quadrangularis* to reduce the burden.

Materials and Methods

Collection of CW and its authentication

Leaves of CW were collected from Pasarni ghat area (17°56'14" N and 73°48'54"), Satara, India. The collected plant material was identified using the flora of the presidency of Bombay[20] and further authenticated by Dr M. D. Nandikar (Head and Scientist), Naoroji Godrej Centre for Plant Research (NGCPR). The herbarium specimen (NGCPR-003000) was deposited to NGCPR, Shirwal.

Chemicals and instruments

Organic solvents (high-performance liquid chromatography [HPLC] grade), and different standards were obtained from Sigma, United States of America, and Himedia, India. The Soxhlet extractor assembly used for extraction was obtained from Borosil, India. The rotary evaporator (PBU-6D) for concentrating extracts was obtained from Superfit, India. Spectrophotometric analysis for determination of TPC, TFC, and antioxidant assay was performed using Shimadzu spectrophotometer (UV-1900 UV-VIS Shimadzu, Japan) and Thermo Scientific Multiskan plate reader respectively.

Fig. 1 — *Cissus woodrowii* (Stapf ex Cooke) Santapau indicating shrub like habit

Sequential leaf extraction

By using a Soxhlet extractor for 12 h, the shade-dried, pulverised leaves were sequentially extracted (1:25 v/v) with various organic solvents having increasing polarity. The resulting extracts were filtered through a Whatman filter paper no. 1 and concentrated on a rotary evaporator, whereas the dried residue (40°C for 24 hours) was re-extracted similarly using another solvent as described in Fig. 2. The resulting viscous extracts were stored in an airtight container at 4 °C until further use.

Determination of total phenolic content and total flavonoid content

The TPC of CW leaf extracts was determined using the Folin–Ciocalteu method[21]. The extracts (100 µL, equivalent to 100 µg) were added to the Folin–Ciocalteu reagent and the TPC was determined spectrophotometrically at 765 nm. The TPC of the leaf extract was expressed as milligrams of tannic acid equivalent (TAE) per gram of extract. The TFC of the methanolic leaf extract of CW was determined spectrophotometrically at 420 nm using ethanolic $AlCl_3$ and the content was expressed as milligrams of quercetin equivalent (QE) per gram of extract[22].

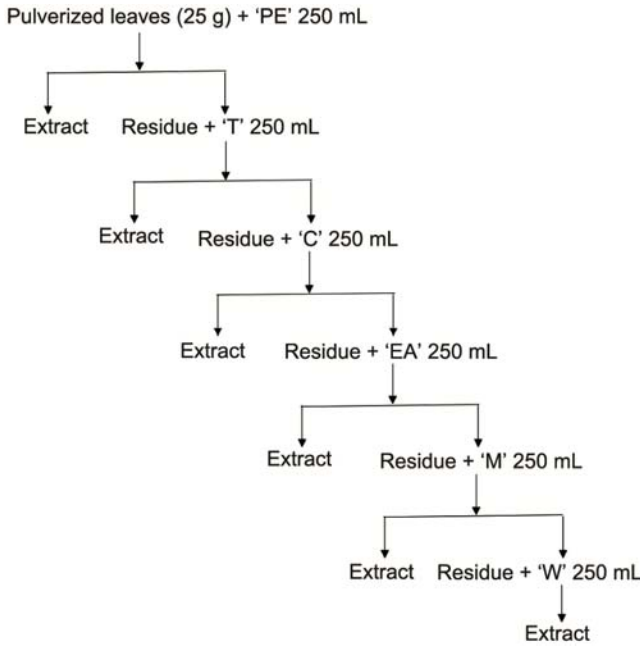

Fig. 2 — Schematic representation of sequential extraction of *C. woodrowii* leaves using different nonpolar, mid polar, and polar solvents

***In vitro* antioxidant activity**

The viscous leaf extracts (10 mg) were dissolved in 1 mL dimethyl sulphoxide (DMSO) to obtain a stock solution. Whereas for determining antioxidant activity various concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL) of the extract were prepared by resuspending the stock in DMSO/ddH_2O.

ABTS assay

ABTS % RSA of CW was evaluated according to Re *et al.*[23] and the absorbance of test solution was measured at 734 nm.

DPPH assay

DPPH % RSA was estimated using the method of Brand-Williams *et al.*[24]. The absorbance of test solution was measured at 518 nm.

Hydroxyl assay

Hydroxyl % RSA was determined using the assay described by Rahman *et al.*[25] and for this assay, the reaction mixture was prepared as described by Omoba *et al.*[26]. Finally, the absorbance was measured at 532 nm.

Nitric oxide assay

Radical scavenging activity (%) of the leaf extract was determined using nitric oxide described by Balakrishnan *et al.*[27]. For this assay, the Griess reagent and buffer were prepared as per Omoba *et al.*[26]. A 150 µL of the reaction mixture (extract + Griess reagent) was transferred to the 96-well plate and absorbance was measured at 546 nm by using a plate reader.

Hydrogen peroxide (H_2O_2) % RSA

H_2O_2 % RSA was determined by the method of Ruch *et al.*[28] where the absorbance of test solution was measured using the spectrophotometer at 230 nm.

Phosphomolybdate assay (Total antioxidant capacity)

Total antioxidant capacity (TAC) was calculated using the phosphomolybdenum method described by Umamaheswari and Chatterjee[29].

Antioxidant power assay (Ferric-reducing antioxidant power)

Antioxidant power assay was performed as described by Benzie and Strain[30]. Various concentrations of the CW extract were mixed with the FRAP reagent and incubated at 37°C for 30 minutes. The absorbance of this solution was measured at 593 nm.

EC_{50} values of various antioxidant assays

Effective concentrations (EC_{50}) were calculated to determine the 50% inhibition of ABTS, DPPH, hydroxyl, nitric oxide, H_2O_2, and phosphomolybdate radicals. The EC_{50} values of extracts were compared with ascorbic acid (AA) and quercetin.

Screening of phenolic compounds in the methanolic leaf extract through LC-HRMS

For screening of phenolic compounds, an Agilent Binary (LC 1260) Triple Quad LC-MS with an Agilent Zorbax Eclipse Plus (C18, 2.1×50 mm 1.8 µM) column was used. A mobile phase of (A) water containing 0.1% formic acid and (B) acetonitrile was used in different ratios with a flow rate of 0.3 mL/min, and the samples were acquired for 30 minutes. Electrospray ionisation (ESI) was used in both positive mode (ESI^+) and negative (ESI^-) mode. About 10 µL of the leaf extract was used as an injection volume and column temperature was maintained at 40 °C. The Agilent 6540 Q-TOF MS system was equipped with a degasser, binary pump, cooled auto-sampler, column oven, and 6540 mass spectrometer. The gas temperature was maintained at 325 °C with 8 L/min flow rate, sheath gas temperature was maintained at 295 °C with 10 L/min flow rate, and nebuliser pressure was maintained at 25 psi for both ESI modes. The capillary voltage was maintained at 2500 and 2000 °C for positive and negative polarities, respectively. Fragment 150 and skimmer were set at 45 in both the ionic modes. Mass range (m/z) used was 80-2000 and the MS scan speed was maintained at 2 spectra/S. The centroid data type was acquired using Mass Hunter Workstation software v.B.05.01 and they were identified through comparison with databases.

Quantification of phenolic compounds through HPLC

The selected phenolic compounds (embelin, gallic acid, and quercitrin) were analysed using the Agilent 1100 HPLC system. Reverse phase chromatographic analyses were performed under gradient conditions using a LiChro CART Purospher STAR column (4.6 mm × 250 mm, 5 µm diameter particles). The mobile phase was similar to that of LC-HRMS. The extract was analysed at a concentration of 10 mg/mL. The flow rate was maintained at 1.0 mL/min, where the injection volume was 10 µL, and the analysis wavelength used was 280 nm. before using, the buffers and extract were filtered through a 0.45 µm membrane filter and degassed using an ultrasonic bath

at RT for 10 minutes. Stock solutions of standard compounds were prepared in the HPLC mobile phase to obtain a calibration curve. Phenolic compounds in the methanolic extract were identified by comparing their retention time and UV absorption spectra with those of commercial standards.

Results and Discussion

Extraction yield, TPC, and TFC

Extraction yield (%), TPC, and TFC of the nonpolar, mid polar, and polar leaf extracts of CW was mentioned in Table 1. All the studied parameters increased with an increase in extraction solvent polarity and these results were in agreement with those of Sowndhararajan and Kang[31] and Vianney *et al.*[32]. A significantly higher extraction yield (13.49%), TPC (24.14 mg TAE/g DW), and TFC (18.45 mg QE/g DW) were observed in the methanolic leaf extract than in the other studied extracts. Among all the leaf extracts tested, the lowest extraction yield, TPC, and TFC were observed in petroleum ether. Variations among TPC and TFC in polar, mid polar, and nonpolar extracts indicate that the extractability of phenolic compounds was governed by the polarity of extracting solvents[17,33]. Phenolic compounds are potent antioxidants[34,35] and the higher TPC and TFC in the methanolic leaf extract of CW are significant indicators of antioxidant properties.

Table 1 — Effect of solvent polarity on extraction yield, total phenolic, and flavonoid content in leaves of *C. woodrowii*

Extracts	EY (%)	TPC mg TAE/ g DW	TFC mg QE/ g DW
PE	5.30±1.02^{d}	11.81±0.62^{f}	7.37±0.46^{e}
T	6.32±1.10^{cd}	14.43±0.78^{e}	8.29±0.34^{d}
C	7.94±1.23^{c}	17.72±0.72^{d}	12.45±0.17^{c}
EA	10.06±1.13^{b}	22.08±0.67^{b}	12.80±0.42^{c}
M	13.49±1.10^{a}	24.14±0.45^{a}	18.45±0.40^{a}
W	12.92±1.41^{a}	19.84±0.66^{c}	16.58±0.44^{b}

PE- Petroleum ether, T- Toluene, C- Chloroform, EA- Ethyl acetate, M- Methanol, and W- Water. *indicates values are the mean of three replicates ± standard deviation. Mean values followed by a different letter in a column are significantly different (P <0.05).

In vitro antioxidant assays

ABTS assay

All the studied extracts exhibited higher ABTS % RSA (Fig. 3) where the methanolic leaf extract (85.77%) exhibited significantly higher (P <0.05) % RSA than AA. The leaf extracts prepared in methanol and ethyl acetate with EC_{50} values of 49.32 and 59.16 µg/mL, respectively, exhibited higher antioxidant activity (Table 2). The higher antioxidant activity for methanolic *extract was probably due to* higher TPC and TFC. Phenolic compounds possess hydroxyl groups[34-36] which significantly contribute to antioxidant activity in living organisms.

DPPH assay

This method is based on the reduction of methanolic DPPH solution in the presence of a hydrogen-donating antioxidant which changes its colour from purple to yellow at 517 nm[37]. The DPPH % RSA of methanolic leaf extract of CW was higher as compared to all the studied extracts (Fig. 4). The EC_{50} values (Table 2) for the DPPH assay were higher for methanolic (22.46 µg/mL) leaf extracts, which were similar to those for AA (21.25 µg/mL) and quercetin (19.71 µg/mL). Therefore, a lower EC_{50} value indicated higher antioxidant activity of the plant material. The EC_{50} value for nonpolar extract (petroleum ether) was 237.88 µg/mL. Thus, polarity dependent antioxidant activities were seen in CW.

Table 2 — Antioxidant effect (EC_{50}) on DPPH radicals, superoxide radicals, total antioxidant capacity and hydroxyl radicals of leaf extract of *C. woodrowii*

Extracts	ABTSL	DPPHL	HYDL	NOL	H_2O_2L	PML
PE	194.18±3.16	237.88±6.72	271.40±3.66	257.34±6.50	240.74±4.53	174.65±3.30
T	176.14±2.03	181.25±3.31	227.31±5.99	151.33±3.61	196.82±4.18	154.86±3.91
C	147.63±2.45	114.04±4.82	160.39±4.26	134.30±3.80	118.82±2.87	123.47±2.97
EA	59.16±1.78	60.06±3.14	73.88±2.17	71.64±3.97	84.79±3.72	84.54±2.06
M	49.32±1.89	22.46±2.07	55.52±3.01	30.26±3.73	47.84±2.35	54.31±3.58
W	69.89±2.50	92.19±4.65	96.13±3.19	49.22±2.99	56.07±1.22	93.29±3.81
AA	21.00±1.30	21.25±1.08	26.25±1.53	22.59±1.13	28.79±1.16	24.19±1.68
Qu	17.36±1.03	19.71±1.62	22.74±1.71	19.08±1.72	24.22±1.78	19.16±1.80

PE- Petroleum ether, T- Toluene, C- Chloroform, EA- Ethyl acetate, M- Methanol, and W- Water, AA- Ascorbic acid, and Qu- Quercetin. ABTSL-ABTS assay of leaves, DPPHL-DPPH assay of leaves, HYDL-Hydroxyl radicle assay of leaves, NOL- Nitric oxide assay leaves, H_2O_2L- Hydrogen peroxide assay leaves, PML-Phosphomolybdate assay leaves

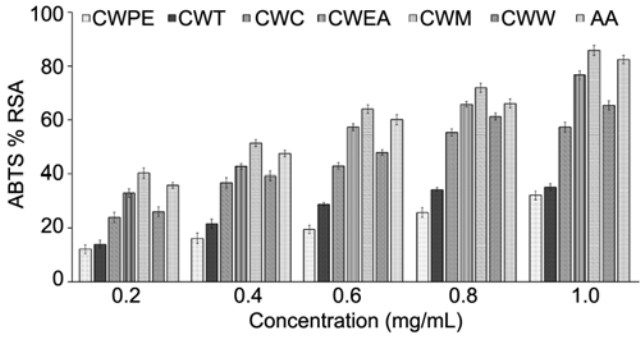

Fig. 3 — ABTS per cent radical scavenging activity of different nonpolar, mid polar, and polar leaves extracts of *C. woodrowii*
CWPE-*C. woodrowii* petroleum ether extract, CWT- *C. woodrowii* toluene extract, CWC-*C. woodrowii* chloroform extract, CWEA-*C. woodrowii* ethyl acetate extract, CWM-*C. woodrowii* methanol extract, CWW-*C.* woodrowii water extract, AA- Ascorbic acid. *indicates values are mean of three replicate determinations (n = 3) ± standard deviation

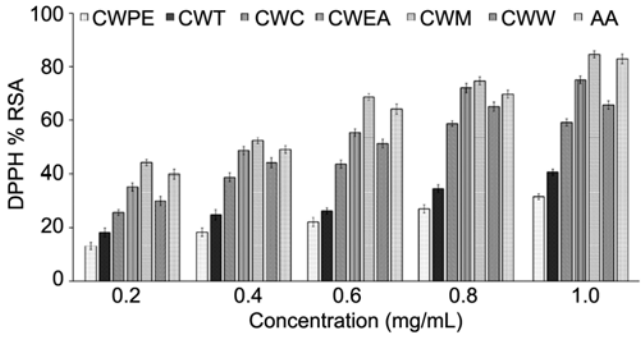

Fig. 4 — DPPH per cent radical scavenging activity of different nonpolar, mid polar, and polar leaves extracts of *C. woodrowii*
CWPE-*C. woodrowii* petroleum ether extract, CWT-*C. woodrowii* toluene extract, CWC- *C. woodrowii* chloroform extract, CWEA-*C. woodrowii* ethyl acetate extract, CWM-*C. woodrowii* methanol extract, CWW-*C.* woodrowii water extract, AA- Ascorbic acid. *indicates values are mean of three replicate determinations (n = 3) ± standard deviation

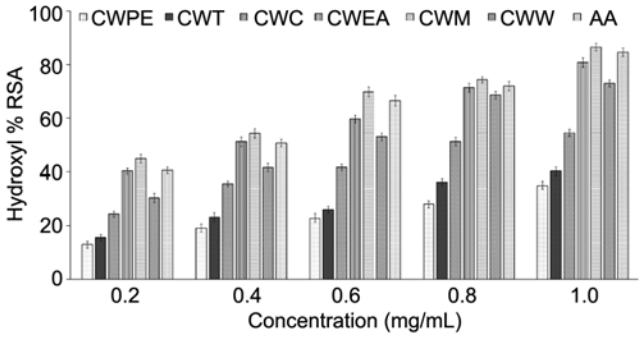

Fig. 5 — Hydroxyl per cent radical scavenging activityof different nonpolar, mid polar, and polar leaves extracts of *C. woodrowii*
CWPE-*C. woodrowii* petroleum ether extract, CWT-*C. woodrowii* toluene extract, CWC-*C. woodrowii* chloroform extract, CWEA-*C. woodrowii* ethyl acetate extract, CWM-*C. woodrowii* methanol extract, CWW-*C.* woodrowii water extract, AA- Ascorbic acid. *indicates values are mean of three replicate determinations (n = 3) ± standard deviation

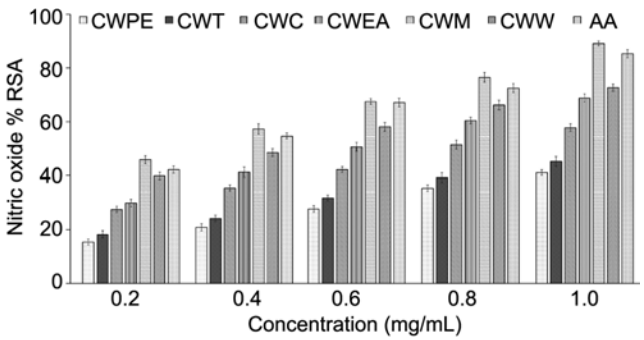

Fig. 6 — Nitric oxide per cent radical scavenging activity of different nonpolar, mid polar, and polar leaves extracts of *C. woodrowii*
CWPE-*C. woodrowii* petroleum ether extract, CWT-*C. woodrowii* toluene extract, CWC- *C. woodrowii* chloroform extract, CWEA-*C. woodrowii* ethyl acetate extract, CWM-*C. woodrowii* methanol extract, CWW-*C.* woodrowii water extract, AA- Ascorbic acid. *indicates values are mean of three replicate determinations (n = 3) ± standard deviation

Hydroxyl assay

In the present study, all the extracts effectively scavenged hydroxyl radicals in a concentration-dependent manner (Fig. 5). Among all the studied extracts, the methanolic leaf extracts showed significantly higher hydroxyl radical scavenging activity and EC_{50} value (Table 2) which could be attributed to the inhibition of lipid peroxidation.

Nitric oxide assay

Various extracts of CW leaf effectively scavenged nitric oxide radicals in a dose-dependent manner (Fig. 6). Methanolic extracts scavenged 89.06% nitric oxide radicals, which was significantly higher than those scavenged by AA (85.28%) and the other studied extracts. Additionally, the EC_{50} value of methanolic extract was 30.26 µg/mL, which was comparable to those of AA (22.59 µg/mL) and quercetin (19.08 µg/mL), thereby proving the antioxidant potential of CW.

H_2O_2 assay

H_2O_2% RSA of methanolic leaf (92.82%) extract of CW was significantly higher (P <0.05) than that of the other studied extracts and AA (Fig. 7). The EC_{50} values of all studied extracts revealed that their H_2O_2 scavenging activity was moderate. The methanolic leaf extract presented a greater EC_{50} value (47.84 µg/mL) which was insignificant to those of AA and quercetin (Table 2).

Phosphomolybdate assay (TAC)

The total antioxidant capacity of CW leaf extracts was estimated using phosphomolybdate assay which showed that methanolic extract exhibited higher TAC and EC_{50} value (54.31 µg/mL) (Fig. 8). However, the antioxidant activity of the positive controls i.e., AA (24.19 µg/mL) and quercetin (19.16 µg/mL) were significant than that of *all the studied extracts* (Table 2).

Antioxidant power assay (FRAP)

In the present study, antioxidant power assay (FRAP) was performed for the various extracts, and the conversion of Fe^{3+} to Fe^{2+} was measured (Fig. 9). Reducing power of plant extracts is frequently correlated with the presence of reductants involved in the antioxidant action[38].

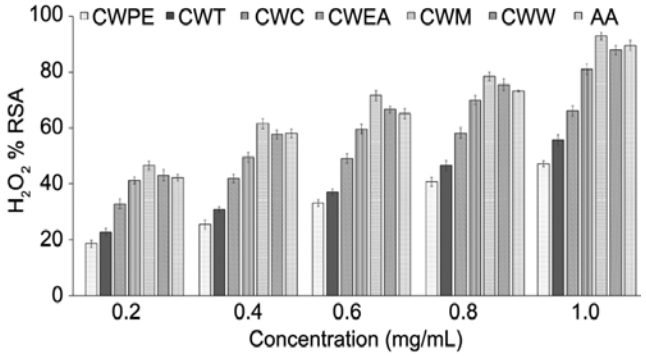

Fig. 7 — H_2O_2 per cent radical scavenging activity of different nonpolar, mid polar, and polar leaves extracts of *C. woodrowii*
CWPE-*C. woodrowii* petroleum ether extract, CWT-*C. woodrowii* toluene extract, CWC-*C. woodrowii* chloroform extract, CWEA-*C. woodrowii* ethyl acetate extract, CWM-*C. woodrowii* methanol extract, CWW-*C.* woodrowii water extract, AA- Ascorbic acid. *indicates values are mean of three replicate determinations (n = 3) ± standard deviation

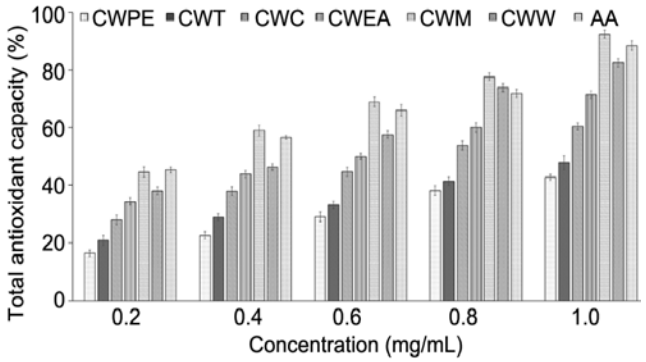

Fig. 8 — Total antioxidant capacity (Phosphomolybdate assay) of different nonpolar, mid polar, and polar leaves extracts of *C. woodrowii*
CWPE-*C. woodrowii* petroleum ether extract, CWT-*C. woodrowii* toluene extract, CWC- *C. woodrowii* chloroform extract, CWEA-*C. woodrowii* ethyl acetate extract, CWM-*C. woodrowii* methanol extract, CWW- C. woodrowii water extract, AA- Ascorbic acid. *indicates values are mean of three replicate determinations (n = 3) ± standard deviation

Correlation between various antioxidant assays and EC_{50} values with TPC and TFC

Significantly positive correlations were observed between antioxidant assays and TPC and TFC of leaf extract (Table 3), indicating that the antioxidant potential of CW depends on the phenolic contents of the extract. The Pearson correlation between EC_{50} and TPC and TFC was evaluated, which was negatively significant (Table 4). The lowest EC_{50} value was associated with the highest antioxidant activity and the correlation was noted by Fidrianny *et al.*[39]. The correlation analysis in the present investigation revealed that TPC and TFC could be the major contributors to the antioxidant activities of CW.

Screening of phenolic compounds and its quantification

The methanolic leaf extract of CW exerted higher antioxidant potential than the other extracts and was further analysed through LC-HRMS to screen for the presence of phenolic compounds. These phenolic compounds were putatively identified on the basis of a database where cosmosiin, dihydrorobinetin, hesperetin, quercitrin, and rutin were observed on both ESI^+ and ESI^-modes (Table 5). This identification revealed the presence of 20 phenolic compounds (Table 5), of which catechin, embelin,

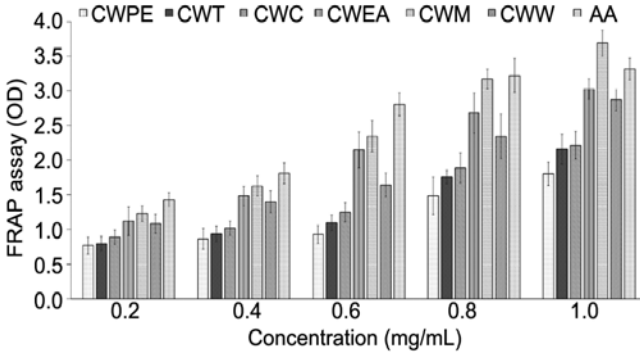

Fig. 9 — Antioxidant power assay (FRAP) of different nonpolar, mid polar, and polar leaves extracts of *C. woodrowii*
CWPE- *C. woodrowii* petroleum ether extract, CWT- *C. woodrowii* toluene extract, CWC-*C. woodrowii* chloroform extract, CWEA-*C. woodrowii* ethyl acetate extract, CWM-*C. woodrowii* methanol extract, CWW- *C. woodrowii* water extract, AA- Ascorbic acid. *indicates values are mean of three replicate determinations (n = 3) ± standard deviation

Table 3 — Correlation between the different antioxidant assays with TPC and TFC of *C. woodrowii* leaves extracts

	ABTSL	DPPHL	HYDL	NOL	H_2O_2L	FRAPL	PML	TPC	TFC
ABTS	1	0.993**	0.981**	0.956**	0.940**	0.918**	0.901**	0.986**	0.892**
DPPH	0.993**	1	0.975**	0.962**	0.945**	0.926**	0.905**	0.992**	0.903**
HYD	0.981**	0.975**	1	0.954**	0.965**	0.930**	0.918**	0.975**	0.898**
NO	0.956**	0.962**	0.954**	1	0.968**	0.940**	0.966**	0.951**	0.967**
H2O2	0.940**	0.945**	0.965**	0.968**	1	0.921**	0.974**	0.950**	0.958**
FRAP	0.918**	0.926**	0.930**	0.940**	0.921**	1	0.888**	0.944**	0.870**
PM	0.901**	0.905**	0.918**	0.966**	0.974**	0.888**	1	0.899**	0.983**
TPC	0.986**	0.992**	0.975**	0.951**	0.950**	0.944**	0.899**	1	0.884**
TFC	0.892**	0.903**	0.898**	0.967**	0.958**	0.870**	0.983**	0.884**	1

ABTSL- ABTS assay of leaves, DPPHL- DPPH assay of leaves, HYDL-Hydroxyl radicle assay of leaves, NOL-Nitric oxide assay leaves, H_2O_2L-Hydrogen peroxide assay leaves, PML- Phosphomolybdate assay leaves. TPC- total phenolic content, TFC- total flavonoid content. **indicates a correlation is significant at the 0.01 level (two-tailed).

Table 4 — Correlation between the EC50 values of antioxidant activities with total phenolic content (TPC) and total flavonoid content (TFC) of *C. woodrowii*

EC_{50} values in different assays	Correlation R^2	
	TPC	TFC
EC_{50} of scavenging ability on DPPH radicals	-.986**	-.900**
EC_{50} of scavenging ability on nitric oxide	-.929**	-.905**
EC_{50} of phosphomolybdate assay	-.986**	-.933**
EC_{50} of scavenging ability on hydroxyl radicals	-.979**	-.908**
EC_{50} of scavenging ability on hydrogen peroxide radicals	-.942**	-.951**
EC_{50} of scavenging ability on ABTS radicals	-.953**	-.892**

**indicates correlation is negatively significant at the 0.01 level (two-tailed),

Table 5 — Profiling of phenolic compounds in methanolic leaves extracts of *C. woodrowii* by LCHR-MS on (a) positive and (b) negative mode

Name	Observed			Database		
	M/z	RT	Mass	Formula	Mass	Mass error*
(a)						
Rutin	611.16	5.57	610.15	$C_{27}H_{30}O_{16}$	610.15	-2.8
Quercitrin	449.10	6.25	448.10	$C_{21}H_{20}O_{11}$	448.10	-1.84
Cosmosiin	415.10	6.72	432.10	$C_{21}H_{20}O_{10}$	432.10	-1.8
Dihydrorobinetin	287.05	7.8	304.05	$C_{15}H_{12}O_7$	304.05	-2.87
Hesperetin	285.07	11.3	302.08	$C_{16}H_{14}O_6$	302.07	-3.53
(b)						
Catechin	289.07	5.47	290.07	$C_{15}H_{14}O_6$	290.07	1.07
Rutin	609.14	5.53	610.15	$C_{27}H_{30}O_{16}$	610.15	-0.12
Gallic acid	169.01	5.76	170.02	$C_7H_6O_5$	170.02	1.32
Diosmetin	327.05	6.33	300.06	$C_{16}H_{12}O_6$	300.06	-0.74
Phloridzin	435.12	6.53	436.13	$C_{21}H_{24}O_{10}$	436.13	0.49
Ellagic acid	300.99	6.57	302.00	$C_{14}H_6O_8$	302.00	3.59
Rhoifolin	605.15	6.66	578.16	$C_{27}H_{30}O_{14}$	578.16	1.84
Cosmosiin	431.09	6.68	432.10	$C_{21}H_{20}O_{10}$	432.10	1.45
Epicatechin	441.08	6.76	442.08	$C_{22}H_{18}O_{10}$	442.09	1.03
Centaurein	539.09	6.94	522.13	$C_{24}H_{26}O_{13}$	522.13	-2.38
Norstictic acid pentaacetate	599.10	7.1	600.11	$C_{28}H_{24}O_{15}$	600.11	0.24
Quercitrin	507.11	7.15	448.10	$C_{21}H_{20}O_{11}$	448.10	0.74
Naringenin-7-o-glucoside	433.11	7.4	434.12	$C_{21}H_{22}O_{10}$	434.12	0.26
Dihydroquercetin	285.04	7.77	304.05	$C_{15}H_{12}$ O7	304.05	0.98

(*Contd.*)

Table 5 — Profiling of phenolic compounds in methanolic leaves extracts of *C. woodrowii* by LCHR-MS on (a) positive and (b) negative mode (*Contd.*)

Name	Observed			Database		
	M/z	RT	Mass	Formula	Mass	Mass error*
(b)						
Lomatin	227.07	7.85	246.08	$C_{14}H_{14}O_4$	246.08	-2.13
Cosmosiin hexaacetate	665.15	8.01	684.16	$C_{33}H_{32}O_{16}$	684.16	1
Dihydrorobinetin	285.04	8.5	304.05	$C_{15}H_{12}O_7$	304.05	-1.16
Hesperetin	283.06	11.27	302.07	$C_{16}H_{14}O_6$	302.07	-2.74
Embelin	293.17	11.67	294.18	$C_{17}H_{26}O_4$	294.18	0.32
Harderoporphyrin	607.25	18.36	608.26	$C_{35}H_{36}N_4O_6$	608.26	-2.44
Rhoifolin	605.15	6.66	578.16	$C_{27}H_{30}O_{14}$	578.16	1.84

*indicates mass error in ppm.

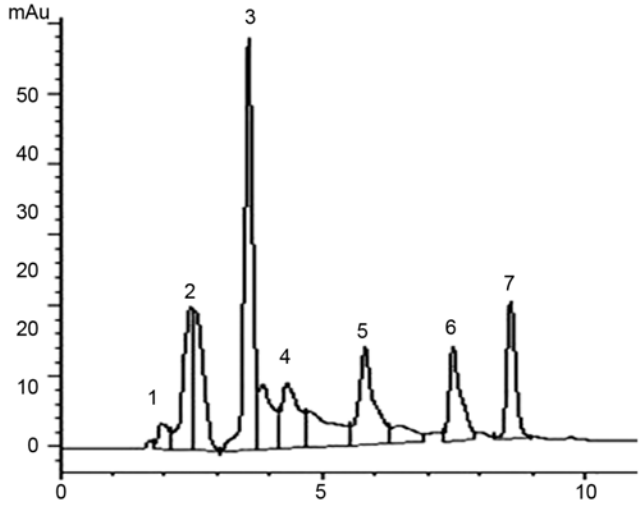

Fig. 10 — HPLC chromatogram indicating the presence of embelin, gallic acid, and quercetinin methanolic leaf extract of *C. woodrowii*
*embelin (RT- 2.601), gallic acid (RT- 3.539), and quercetin (RT

epicatechin, gallic acid, quercitrin, and rutin were recognised as potent antioxidants[40]. The studied plant system belongs to the Vitaceae family and the identified phenolic compounds showed similarities with *Vitis vinifera*[41]. The selected phenolic compounds (embelin, gallic acid, and quercetin) were quantified through HPLC by comparing RT of the commercially available standard compounds. The methanolic extract of CW revealed different peaks, of which three peaks namely 2.601, 3.539, and 8.538 presented RT extremely close to that of the standard compounds (Fig. 10). The concentrations of these phenolic compounds were calculated using calibration curves, where the content of embelin (21.09 µg/g DW) was higher than gallic acid (119.78 µg/g DW) and quercetin (22.13 µg/g DW) (Fig. 9).

Conclusion

The present study revealed that the sequential extraction of *C. woodrowii* leaves significantly influence per cent yield, TPC, and TFC. Higher phenolic and flavonoid contents in the methanolic extract of *C. woodrowii* correlated with significantly higher antioxidant activity. LC-HRMS analysis has revealed 20 phenolic compounds in the leaves of *C. woodrowii* which might play an important role in the antioxidant activity. These phytocompounds can play an important role to overcome oxidative stress-related health complications. Bioprospecting of *C. woodrowii* will help to promote the use of this plant in traditional systems of medicine and can be employed further for identification of lead molecules.

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Conflict of interest

The authors have no conflicts of interest to declare.

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