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Research Article
Metabolite Profiling of *Crotalaria verrucosa* Leaf Extract and Evaluation of its Antioxidant and Cytotoxic Potency

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Abstract

Background and Objective: *Crotalaria verrucosa (C. verrucosa)* Fabaceae is a Bangladeshi medicinal plant, native to Sylhet and traditionally this plant is used as medicine for treating impetigo, scabies, salivation, jaundice, cough, biliousness, dyspepsia, fever, cardiac abnormalities and oral diseases. Since no previous study investigated its antioxidant or cytotoxic potential, the present study was aimed to analyze its antioxidant and cytotoxic potential for identification and quantification of those metabolites which contributed to these activities. **Materials and Methods:** Cold maceration using methanol was used for extraction from *C. verrucosa* leaves. Antioxidant activity was determined by total phenol content, total flavonoid content, total antioxidant capacity and DPPH free radical scavenging assays. Metabolite profiling was obtained by HPLC-DAD. Finally, cytotoxicity was evaluated using an MTT assay against HeLa cells. **Results:** The extract confirmed high phenolic content and high flavonoid content yet a moderate total antioxidant capacity and moderate inhibition (%) of DPPH free radical scavengers was found. Further HPLC analysis revealed the presence of 7 polyphenolic compounds from which gallic acid was predominantly present along with small quantities of (+) catechin hydrate, vanillic acid, caffeic acid, syringic acid, (-) Epicatechin and vanillin. Lastly, MTT assay evident the highest cell growth inhibition at a concentration of 2.5 mg mL^{-1} and the IC_{50} value was found to be 0.83mg mL^{-1} which indicated the strong cytotoxic potential of the extract. **Conclusion:** Strong cytotoxic potential and a moderate antioxidant potential were observed which justifies its role in folkloric remedies but extract needs further purification to use it as a folkloric remedy for oxidative stress-mediated diseases.

Key words: Polyphenolic compounds, *Crotalaria verrucosa*, antioxidant, cytotoxic, stress mediated diseases, oral diseases, metabolite profiling

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reactive Oxygen Species (ROS) and sometimes pathologic conditions or overproduction of oxidants in our metabolic system may lead to oxidative stress, potentially leading to damage[1]. It has been recognized as one of the causes of ageing[2] and other diseases such as atherosclerosis[3], cancer[4], cardiovascular diseases[5], mild cognitive impairment[6], Alzheimer's disease[7], Parkinson's disease[8], alcohol induced hepatic disease[9] and ulcerative colitis[10]. If the free radicals and other ROS are not eliminated or neutralized within the body at an early stage, it can then target the various cellular elements such as lipid membrane, proteins, DNA and RNA and this oxidation-induced by ROS can cause cell membrane disintegration, membrane protein damage and DNA mutation which can eventually cause cancer[11]. Cervical cancer is deadly cancer and the second leading cause of deaths in cancer among Bangladeshi women. Recently in 2015, a study among Bangladeshi women was conducted by Ganga and Shabnam[12] and they found out that 13,000 women die every year due to cervical cancer in Bangladesh. A number of risk factors such as early marriage, early starting of sexual activity, low socioeconomic condition and high incidence of sexually transmitted diseases and Human papillomavirus (HPV) infection are associated with the prevalence of cervical cancer in developing countries like Bangladesh[13].

Polyphenolic compounds and flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential benefits to human health in fighting diseases, especially, aging and cancer. The capacity of flavonoids to act as antioxidants depends upon their molecular structure where the positions of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities[14]. As defined by Amic *et al.*[15], the antioxidant potential of phytoconstituents describes the ability of either preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body or reducing/chelating the transition metal composition. Flavonoids are polyphenolic compounds possessing a structural backbone of diphenylpropane. While phenolic compounds may function as terminators of free radical chain reaction, it most actively participates in the protection against "oxidative stress". Due to their favorable reduction potential relative to alkyl peroxyl radicals, flavonoids are ideal scavengers of peroxy radicals and in this manner; they effectively inhibit lipid peroxidation[16]. Another structural feature worth taking notice is the presence of 2,3-unsaturation in conjugation with a 4-oxo group in C-ring. In addition to this, the presence of dihydroxylated ring-B, exposes the molecule to donate hydrogen/electron that subsequently scavenges reactive radical species[17].

Phytoconstituents comprise an important source of medicine for traditional use and thus, are potential in providing direct treatment. It is also used in synthetic and semi-synthetic drug development. Thus, the extraction and quantification of phytoconstituents are essential for the development of new and novel therapeutics to treat oxidative stress-mediated diseases and cancer[18].

Crotalaria verrucosa, available in many areas of Bangladesh, is the only perennial shrub among the *Crotalaria* species that is described as solely possessing medicinal value[19,20]. Ghani[21] categorized *C. verrucosa* in his book as a medicinal plant of Bangladesh. *C. verrucosa* has found its use in traditional medicine and is mainly effective for impetigo, scabies, salivation, jaundice, cough, biliousness, dyspepsia, fever, cardiac abnormalities and oral diseases[22-24]. Chakma and Marma tribes of Bangladesh also use this as a medicinal plant for the treatment of skin allergies.

The previous study on this legume plant included the investigation into its *in vivo* anti-fertility[25], wound-healing[22], hepatoprotective activity[26], anti-diabetic, antipyretic and CNS depressant potential[27] and *in vitro* anticoagulant activity, thrombolytic[27] and antibacterial activity[23,28]. No previous bioactivity study has yet been conducted on determining the antioxidant and cytotoxic potential of *C. verrucosa* methanolic leaf extract (CVMLE).

However, in a study conducted by Kumar *et al.*[29] it was found that *C. verrucosa* L. is composed of flavonoids, steroids and steroidal nucleus. Since plants rich in polyphenolic compounds like flavonoids have demonstrated to possess anti-aging, anti-carcinogenic, anti-inflammatory, antiallergenic and antiviral activities; thus, this study was aimed to evaluate the antioxidant and cytotoxic potency of CVMLE and profile the metabolites responsible for such activity.

MATERIALS AND METHODS

Collection and authentication: The whole plant, *C. verrucosa*, was collected in August, 2015 from Sylhet, Bangladesh and the plant was identified by the National Herbarium of Bangladesh (NHB), Mirpur, Dhaka (ACCESSION NO.: DACB-41865), its voucher specimen was deposited for future reference.

Extract preparation: Fresh leaves were removed from the plant, washed thoroughly and shade-dried. The dried leaves were then grinded using a high capacity grinding machine (Retsch™ RM 200 Mortar Grinder, UK) and the powdered plant material produced was macerated in methanol for a period of 48 h at ambient room temperature (22-25°C) with occasional agitation[30]. Afterward, the contents of the beaker were filtered and concentrated using a vacuum rotary evaporator (Heidolph) at 100 rpm, maintained at 30°C. The extract was then refrigerated for further use.

Preliminary phytochemical screening of CVMLE: A preliminary phytochemical screening was performed on CVMLE in order to assess its qualitative chemical compositions.

Determination of antioxidant potential of CVMLE: The antioxidant potential of CVMLE was determined by performing four tests, namely, (1) DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging, (2) Total Antioxidant Capacity (TAC), (3) Total Phenolic Content (TPC) and (4) Total Flavonoid Content (TFC).

First of all, CVMLE was dissolved in methanol to yield a concentration of 1200 μg mL^{-1}. By means of serial dilution, it yielded concentrations ranging from 200, 400, 800 and 1200 μg mL^{-1} which was then chosen for the following tests.

DPPH free radical scavenging assay: The DPPH free radical scavenging activity of CVMLE was determined according to the method described by Braca *et al.*[31] using L-ascorbic acid as the standard. Here, the absorbance of the sample solutions was measured at 517 nm using U-2910 UV-Vis Spectrophotometer.

The percentage inhibition of free radical scavenging activity (expressed in terms of inhibition (%) was then calculated from the formula below and the IC_{50} value determined (denoting the concentration of the sample required to scavenge 50% of the DPPH free radical scavengers).

$$\text{Inhibition (\%)} = \frac{A_0 - A_s}{A_0} \times 100$$

where, A_0 is the absorbance of the control (DPPH and methanol) and A_S is the absorbance of the sample in the presence of free radical scavengers.

Total Antioxidant Capacity (TAC): The TAC of CVMLE was determined according to the method described by Prieto *et al.*[32] using L-ascorbic acid as the standard. The absorbance of the sample solutions was measured at 695 nm using U-2910 UV-Vis Spectrophotometer and the result was expressed as ascorbic acid equivalent (AAE) per gram extract (mg of AAE g^{-1} dried extract).

Total Phenolic Content (TPC): The TPC of CVMLE was determined by the Folin-Ciocalteu method modified by Wolfe *et al.*[33] using gallic acid as the standard. Here, the absorbance of the sample solutions was measured at 765 nm using U-2910 UV-Vis Spectrophotometer and TPC was expressed as Gallic Acid Equivalent (GAE) per gram extract (mg of GAE g^{-1} dried extract).

Total Flavonoid Content (TFC): According to the method described by Kumaran and Karunakaran[34], the TFC was determined by using Quercetin as the standard. The absorbance of the sample solutions was measured at 415 nm using U-2910 UV-Vis Spectrophotometer and TFC was then expressed as Quercetin Equivalent (QE) per gram extract (mg of QE g^{-1} dried extract).

Phytochemical analysis using HPLC-DAD: Detection and quantification of polyphenolic antioxidants in CVMLE were determined by HPLC-DAD analysis[35] with some modifications. It was carried out on a Dionex UltiMate 3000 system equipped with a quaternary rapid separation pump (LPG-3400RS) and photodiode array detector (DAD-3000RS).

Separation was performed using Acclaim® C_{18} (5 μm) Dionex column (4.6×250 mm) at 30°C with a flow rate of 1 mL min^{-1} and an injection volume of 20 μL. The mobile phase consisted of acetonitrile (solvent A), acetic acid solution pH 3.0 (solvent B) and methanol (solvent C) with the gradient elution program of 5%A/95%B (0-5 min), 10%A/90%B (6-9), 15%A/75%B/10%C (11-15), 20%A/65%B/15%C (16-19 min), 30%A/50%B/20%C (20-29 min), 40%A/30%B/30%C (30-35) and 100%A (36-40 min). The UV detector was set to 280 nm for 22.0 min, changed to 320 nm for 28.0 min, again change to 280 nm for 35 min and finally to 380 nm for 36 min and held for the rest of the analysis period while the diode array detector was set at an acquisition range from 200-700 nm. For the preparation of calibration curve, a standard stock solution was prepared in methanol containing arbutin (AR), (-)-Epicatechin (ECA) (5 μg mL^{-1} each), Gallic Acid (GA), Hydroquinone (HQ), Vanillic Acid (VA), Rosmarinic Acid (RA),

Myricetin (MC) (4 µg mL^{-1} each), Caffeic Acid (CA), Syringic Acid (SA), Vanillin (VL), trans-Ferulic acid (FA) (3 µg mL^{-1} each), p-Coumaric acid (PCA), Quercetin (QU), kaempferol (KF) (2 µg mL^{-1} each), (+)-Catechin hydrate (CH), Ellagic acid (EA) (10 µg mL^{-1} each), trans-Cinnamic acid (TCA) (1 µg mL^{-1}), Rutin Hydrate (RH) (6 µg mL^{-1}) and Benzoic Acid (BA) (8 µg mL^{-1}). A solution of the extract was prepared in methanol with a concentration of 10 mg mL^{-1}.

Sprior to HPLC analysis, all the solutions (mixed standards, sample and spiked solutions) were filtered through 0.20 µm syringe filter (Sartorius, Germany) and then degassed in an ultrasonic bath (Hwashin, Korea) for 15 min. Data acquisition, peak integration and calibrations were calculated with Dionex Chromeleon software (Version 6.80 RS 10).

Cytotoxic screening of CVMLE

Cell culture: The MTT colorimetric assay was performed by using celltiter 96 non-radioactive cell proliferation assay kit (Promega, USA). Cells were seeded onto 96 well plates and incubated at 37°C and 5% of CO_2 atmosphere. This cell line was cultured and maintained in DMEM (Dulbecco's Modified Eagles Medium) supplemented with 1% penicillin-streptomycin, 0.2% Gentamicin and 10% fetal bovine serum (FBS).

MTT colorimetric assay: The cytotoxic activity of CVMLE was performed by MTT assay on HeLa cell line following the method described by Ifere *et al.*[36] in the Centre for Advanced Research in Sciences (CARS), Dhaka. A number of 1×10^4 cells were seeded onto 96 well plates and allowed to adhere for 24 h. After 24 h of incubation, cells were treated with varying concentrations (0.0025-2.5 mg mL^{-1}) of the CVMLE. After 48 h of incubation, the cells were examined for cytotoxicity using celltiter 96® non-radioactive cell proliferation assay kit (Promega, USA). This was followed by measuring absorbance at 570 nm using a 96 well plate reader. Cycloheximide, a standard cytotoxic compound, served as the positive control. Cytotoxic activity was calculated by using a equation:

$$\text{Cytotoxic activity (\%)} = 100 - \left(\frac{\text{Absorbance of test sample}}{\text{Absorbance of negative control}} \times 100 \right)$$

Statistical analysis: The mean and standard deviations for each of the methods, namely, Total Phenolic Content (TPC), Total Flavonoids Content (TFC) and Total Antioxidant Capacity (TAC) were performed in triplicates (n = 3) whereas that of DPPH free radical scavenging was performed in duplicates (n = 2). In case of HPLC analysis, however, the content of polyphenolic compounds present in the extract was presented as the mean and standard deviation of five determinations (n = 5). Cytotoxic activity experiments were performed in duplicates. All the statistical analysis, involving calculation of mean and standard deviation and graphical representations were done using Microsoft Office Excel 2010.

RESULTS

Preliminary phytochemical screening of CVMLE: The current study performed a preliminary phytochemical screening of the CVMLE which qualitatively confirmed the presence of flavonoids, phenolic compounds and alkaloids among many other chemical constituents such as tannins, glycosides, resins, steroids and carbohydrates (Table 1).

Determination of antioxidant potential of CVMLE

DPPH free radical scavenging assay: Increasing the concentration, increased the inhibition (%) of DPPH free radicals by both CVMLE and ascorbic acid (standard) (Fig. 1). For the concentrations of 200, 400, 800 and 1200 µg mL^{-1}, the standard antioxidant ascorbic acid showed 96.05, 96.28, 96.36 and 96.52% of inhibition and CVMLE showed 24.0, 53.10, 86.07 and 86.07% of inhibition, respectively (Fig. 1). The IC_{50} value of ascorbic acid and CVMLE was found to be 0.15 and 0.53 mg mL^{-1}, respectively.

Total Antioxidant Capacity (TAC): The TAC of CVMLE was determined using the phosphomolybdenum method, where ascorbic acid was used as the standard. The calibration curve of ascorbic acid yielded an equation of y = 0.0046x+0.1094 (R^2 = 0.9954) as seen in Fig. 2. Ascorbic acid equivalents of 8.35, 19.58, 24.63 and 32.34 mg g^{-1} of dried extract were obtained for concentrations of 200, 400, 800 and 1200 µg mL^{-1}, respectively (Table 2).

Total Phenolic Content (TPC): The quantitative determination of the TPC of this extract was determined by using the Folin-Ciocalteu method which utilized gallic acid as the standard. The calibration curve of gallic acid yielded an equation of y = 0.0085x-0.1607 (R^2 = 0.9977) as seen in Fig. 3. The results showed that at concentrations of 200, 400, 800 and 1200 µg mL^{-1} of this extract, the TPC obtained were 34.78,

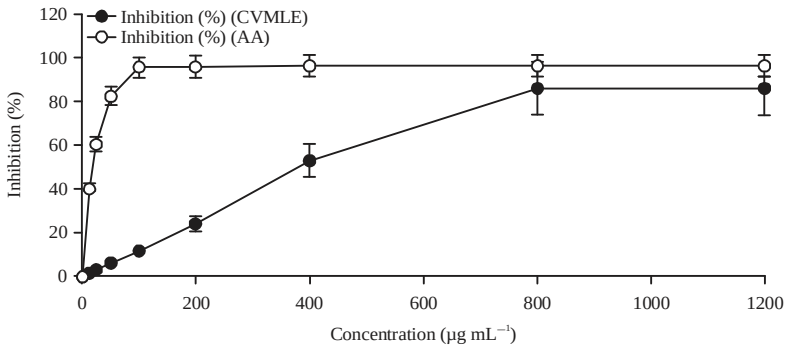

Fig. 1: Inhibition (%) of DPPH free radical scavengers by CVMLE (Black) and standard ascorbic acid, AA (White)

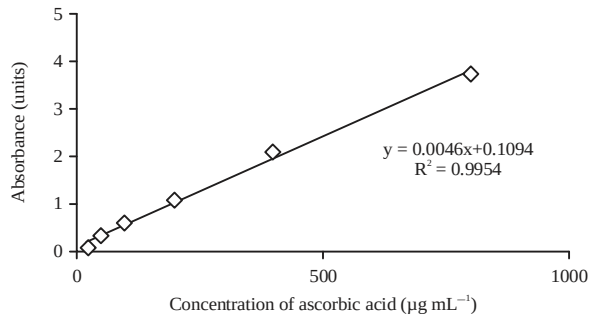

Fig. 2: Calibration curve of ascorbic acid measured at 695 nm

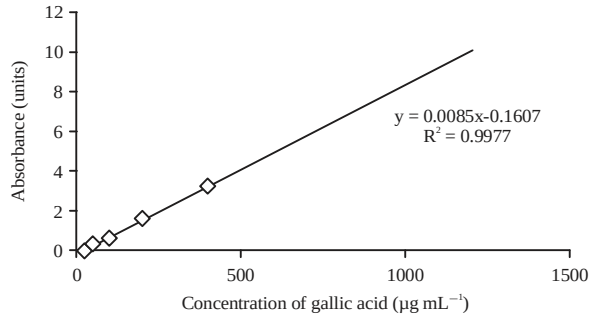

Fig. 3: Calibration curve of gallic acid measured at 765 nm

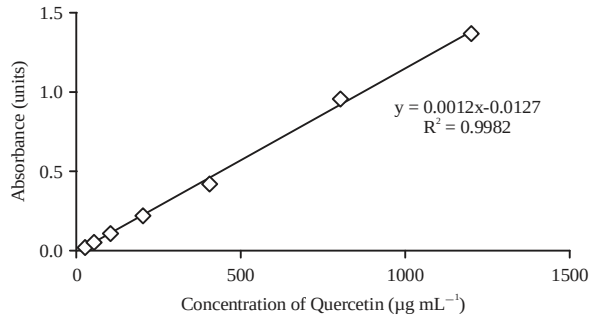

Fig. 4: Calibration curve of Quercetin measured at 415 nm

Table 1: Preliminary phytochemical screening of CVMLE

Class of compounds	Result
Alkaloids	+++
Flavonoids	++
Phenols/Phenolic compounds	+
Glycosides	+
Tannins	+++
Carbohydrates	+
Phytosterol	-
Resins	+
Steroids	+
Saponins	-

+: Means presence in a single method test, ++: Means presence experimented in two methods, +++: Means presence experimented in three methods, -: Means absence

59.72, 99.56 and 152.18 mg of GAE g^{-1} of dried extract, respectively (Table 2). It could thus be suggested that *C. verrucosa* possesses sufficient content of gallic acid to exhibit antioxidant properties.

Total Flavonoid Content (TFC): The TFC of CVMLE extract was investigated to deduce the quantity of flavonoids responsible for antioxidant activity by using Quercetin (QU) as the standard. The calibration curve of Quercetin yielded an equation of y = 0.0012x-0.0127 (R^2 = 0.9982) as seen in Fig. 4. Quercetin equivalents of 32.08, 78.94, 120.60 and 184.51 mg g^{-1} of dried extract were obtained for concentrations of 200, 400, 800 and 1200 µg mL^{-1}, respectively (Table 2).

Phytochemical analysis of CVMLE using HPLC-DAD: The identification and quantitation of individual polyphenolic compounds present in CVMLE were done using HPLC-DAD. The chromatographic separations of phenols in standard and CVMLE are shown in Fig. 5 and 6, respectively.

The content of polyphenolic compounds present in the extract was calculated from the corresponding calibration curve and presented as the mean of five determinations

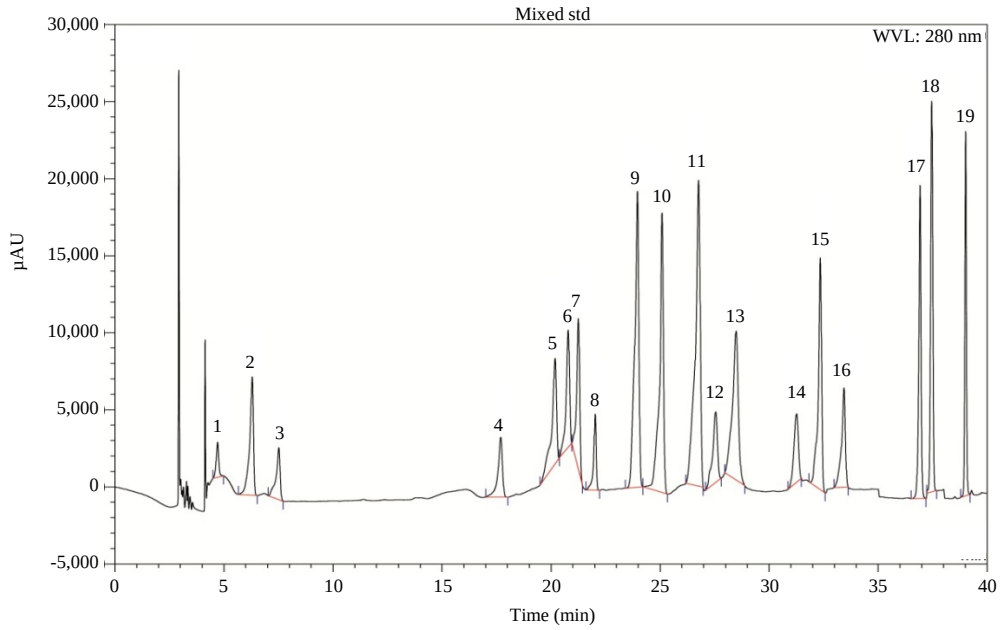

Fig. 5: HPLC chromatogram of standard mixtures of phenolic antioxidant compounds showing the presence of peaks
1: Arbutin, 2: Gallic acid, 3: Hydroquinone, 4: (+)-Catechin hydrate, 5: Vanillic acid, 6: Caffeic acid, 7: Syringic acid, 8: (-)-Epicatechin, 9: Vanillin, 10: p-Coumaric acid, 11: trans-Ferulic acid, 12: Rutin hydrate, 13: Ellagic acid, 14: Benzoic acid, 15: rosmarinic acid, 16: Myricetin, 17: Quercetin, 18: trans-Cinnamic acid, 19: Kaempferol

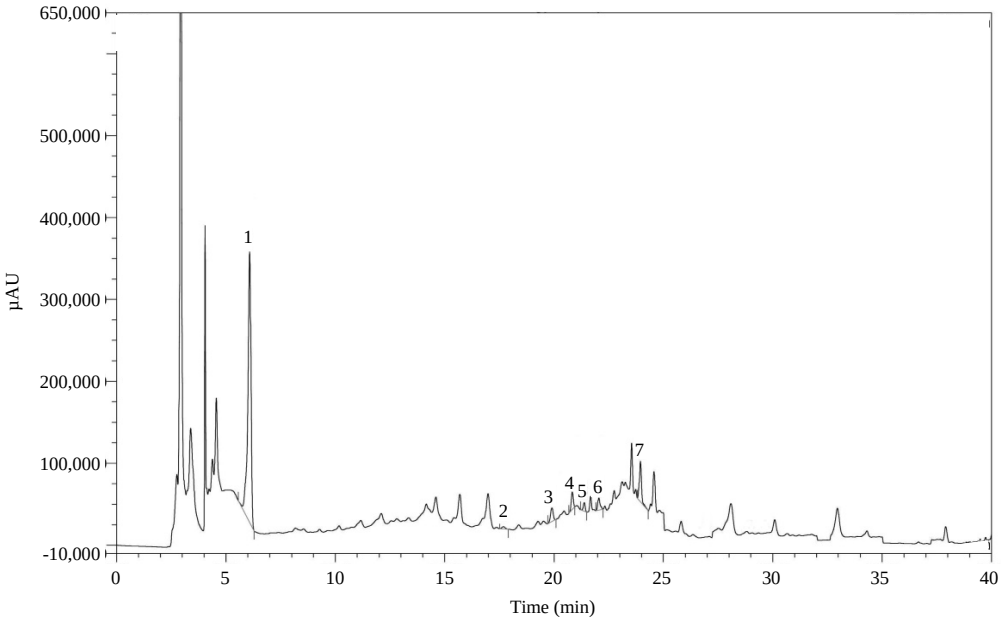

Fig. 6: HPLC chromatogram of CVMLE showing the presence of peaks
1: Gallic acid (GA), 2: (+)-Catechin hydrate (CH), 3: Vanillic acid (VA), 4: Caffeic acid (CA), 5: Syringic acid (SA), 6: (-)-Epicatechin (ECA), 7: Vanillin (VL)

(Table 3). The HPLC analysis of CVMLE identified the presence of gallic acid, (+)-Catechin hydrate, vanillic acid, caffeic acid, syringic acid, (-)-Epicatechin and vanillin among which gallic acid (19.53 mg/100 g of dried extract) was predominantly present (Fig. 9).

Cytotoxic activity of CVMLE: The cells were examined under a Trinocular Microscope with Camera (Olympus, Japan) and the effect of CVMLE on cell viability is shown in Fig. 7. Furthermore, the cytotoxic effect at different sample concentrations of 0.0025, 0.025, 0.25 and 2.5 mg mL^{-1} using

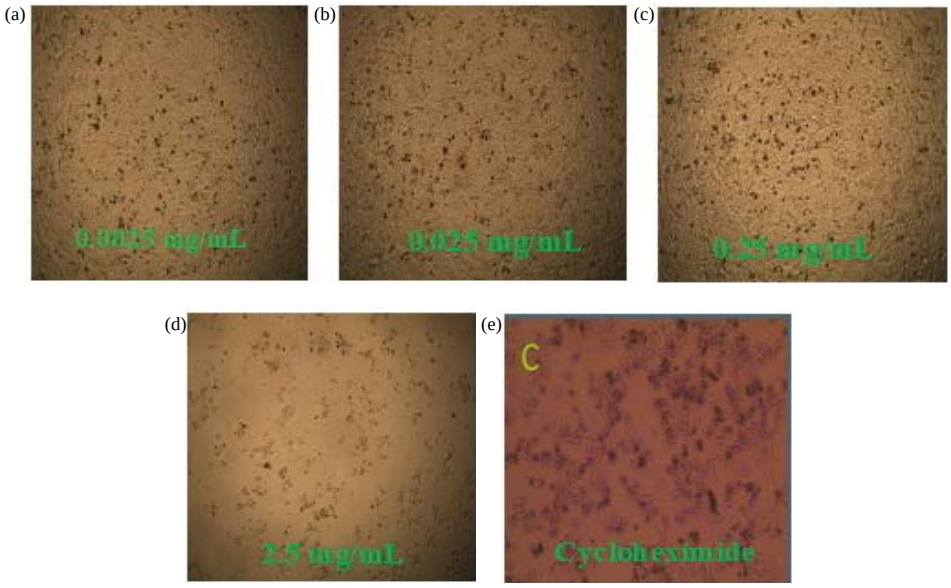

Fig. 7(a-e): (a-d) Cell viability of CVMLE at different concentrations and (e) Cycloheximide after incubating for 48 h, where cycloheximide (C) was used as a positive control

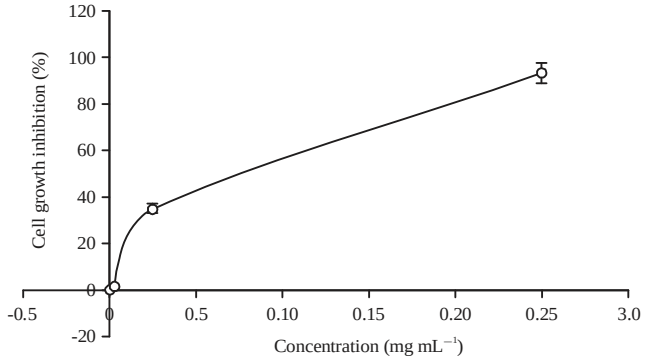

Fig. 8: Cytotoxic activity of CVMLE at different concentrations

Table 2: Antioxidant potential of CVMLE determined at four different concentrations by four separate tests namely, DPPH free radical scavenging assay, TAC, TPC and TFC

Concentration (μg mL^{-1})	DPPH free radical scavenging assay inhibition (%)	Total antioxidant capacity (mg of AAE g^{-1})	Total phenolic content (mg of GAE g^{-1})	Total flavonoid content (mg of QE g^{-1})
200	24.00±0.78	8.35±1.85	34.78±1.76	32.08±2.16
400	53.10±0.57	19.58±1.65	59.72±1.51	78.94±1.70
800	86.07±0.44	24.63±0.98	99.56±2.88	120.60±2.62
1200	86.07±0.36	32.34±0.13	152.18±1.21	184.51±0.78

Values are the average of triplicates of experiments and are represented as Mean±% of relative standard deviation

HeLa cell line were found to be 0, 1.3, 35 and 93.2%, respectively, as illustrated in Fig. 8; thus, implying that with increasing sample concentration, the inhibitory activity (%) also increased. The IC_{50} value of CVMLE was found to be 0.83 mg mL^{-1}, compared to the positive control, cycloheximide, which was 0.16 mg mL^{-1}.

DISCUSSION

The current study on the preliminary phytochemical screening of CVMLE qualitatively confirmed the presence of flavonoids, phenols and phytosterols amongst many other chemical constituents such as tannins, glycosides and resins

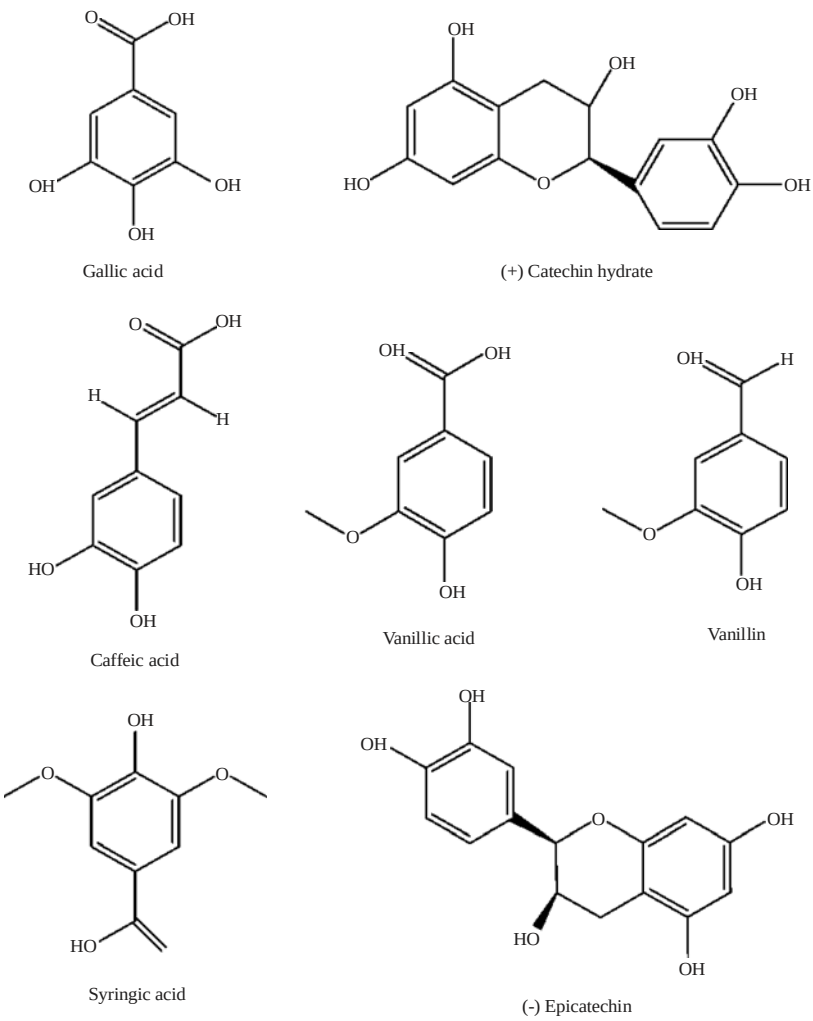

Fig. 9: The phytocompounds identified in HPLC analysis of CVMLE

Table 3: Quantity of polyphenolic antioxidant compounds in CVMLE using HPLC

	Methanol extract of *C. verrucosa*	
Phenolic compound	Content (mg/100 g of dried extract)	RSD (%)
GA	19.53	0.57
CH	5.08	0.14
VA	5.97	0.18
CA	6.22	0.22
SA	1.09	0.05
ECA	7.16	0.29
VL	8.05	0.35

Each of the values are the result of mean of five determinations (n = 5), RSD: Relative standard deviation, GA: Gallic acid, CH: (+)-Catechin hydrate, VA: Vanillic acid, CA: Caffeic acid, SA: Syringic acid, ECA: (-)-epicatechin, VL: Vanillin

(Table 1). Previous studies on phytochemical screening were performed on ethanolic extract of *C. verrucosa* that confirmed the presence of flavonoids and phytosterols in this plant[25-28]. Plant-derived compounds such as polyphenolic compounds and flavonoids, are responsible for a variety of positive biological effects, including antioxidative, anti-apoptosis, anti-aging, anticancer, anti-inflammatory and anti-atherogenic effects[37]. Antioxidant and cytotoxic potential of the plant may be attributed to these flavonoids and polyphenols identified in our study.

Because ROS are involved to cause a number of diseases such as cancer, atherosclerosis, cardiovascular diseases, neurodegenerative diseases, Alzheimer's disease and Parkinson's disease[1-11]. The antioxidant potential of secondary metabolites of plants particularly flavonoid and polyphenolic compounds describe their ability to scavenge free radicals, making them useful for studying health benefits of antioxidants oxidative stress-mediated diseases mentioned earlier[38].

The DPPH assay is based on electron transfer and involves reduction of the coloured oxidant, the purple $DPPH^{\cdot}$, 1,1-diphenyl-2-picryl hydrazine[38]. Using DPPH free radical scavenging (FRS) assay, it was found that the IC_{50} values of CVMLE and standard ascorbic acid were 0.53 and

0.15 mg mL^{-1}, respectively (Table 2). The measured activity was increased in a dose-dependent manner but were not as significant as the standard. It can be thus considered to possess a moderate antioxidant potential. Furthermore, other antioxidant assays such as TAC, TPC and TFC also showed moderate antioxidant potential compare to the standard compounds.

Conducting HPLC analysis of CVMLE, the presence and quantity of gallic acid, (+)-Catechin hydrate, vanillic acid, caffeic acid, syringic acid, (-)-Epicatechin and vanillin (Fig. 6) were determined (Table 3). Among those phenolic compounds, the experimental results indicate that gallic acid (19.53 mg/100 g of dried extract) was predominantly present. The metabolite profile is illustrated in Fig. 9.

Thus, the higher total phenolic content of CVMLE can be attributed to the presence of phenols such as gallic acid, syringic acid, caffeic acid and vanillin. Also, its higher total flavonoid content can be attributed to the fact that flavonoids such as (+)-Catechin hydrate and (-)-Epicatechin are present in the crude extract in addition to other unidentified compounds. The (+)-Catechin hydrate and (-)-Epicatechin are flavonoids and vanillic acid is a catechin-metabolite-all of which have shown high antioxidant activity in tea and green tea drinks[17,39]. Nonetheless, flavonoids are one of the largest class of polyphenolic compounds. Gallic acid (pseudo tannin), syringic acid, caffeic acid and vanillin are polyphenolic compounds, all of which demonstrate antioxidant activity with syringic acid claiming to possess higher antioxidant potential[16,40,41].

Furthermore, in our study, *in vitro* cytotoxicity activity was also examined on cervical cancer cell line (HeLa) where different concentrations of leaves extracts were used to determine the cell viability. It was observed that cell growth inhibition was concentration-dependent, cytotoxic activity also increases gradually from 35% for 0.25 mg mL^{-1} to 93.2% cell death at 2.5 mg mL^{-1} concentration. From previous studies, it is found that leaf extract of *C. verrucosa* contains alkaloids, flavonoids, glycosides, tannins, steroids and polyphenolic compound, etc. Literature studies revealed that plant-derived compounds such as alkaloids, flavonoids, tannins and terpenoids exhibit the significant cytotoxic activity on cancer cell[42]. Thus, the results ensured that the CVMLE has the potential to show significant cytotoxic activity against HeLa cancer cell line due to the presence of (+)-Catechin hydrate and (-)-Epicatechin (tannins), particularly. Therefore, the presence of such antioxidant substances in CVMLE may be responsible for its anticancer potential, particularly for cervical cancer. However, since the extract showed moderate antioxidant potential, it could be used as a folkloric remedy for oxidative stress-mediated diseases after purification of the extract.

CONCLUSION

This study found that CVMLE has moderate antioxidant potential and significant cytotoxic effect against cervical cancer cells, HeLa. It also identified and quantified a total of 7 phenolic compounds responsible for its moderate antioxidant potential using HPLC-DAD technique. They are gallic acid, syringic acid, caffeic acid, vanillin, (+)-Catechin hydrate, (-)-Epicatechin and vanillic acid, of which gallic acid was present in the highest quantity. Thus, the presence of these polyphenolic compounds extrapolates to its moderate antioxidant potential and strong cytotoxic potential while justifying its role in folkloric remedies.

SIGNIFICANCE STATEMENT

This study discovered strong cytotoxic activity against cervical cancer cells (HeLa) and moderate antioxidant potential of *C. verrucosa* leaf extract, thus justifying its role in folkloric remedies. This research also identified the presence of several metabolites such as gallic acid, syringic acid, caffeic acid, vanillin, (+)-Catechin hydrate, (-)-Epicatechin and vanillic acid having gallic acid as predominant phytocompound and these phytocompounds may be attributed to the screened cytotoxic and antioxidant potential. It is worth mentioning that this study will help future researchers to investigate any correlation between each metabolite to its cytotoxic and antioxidant activity after isolation of these pure metabolites. It will also open the domain for investigating *in vivo* antioxidant and *in vivo* cytotoxic activity to better assess the bioactivity potential of this medicinal plant. Thus, a new theory on it may be devised at.

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