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Original Article

ANTIOXIDANT ACTIVITY, PHYTOCHEMICAL ANALYSIS AND TOTAL POLYPHENOLICS CONTENT OF ESSENTIAL OIL, METHANOL EXTRACT AND METHANOL FRACTIONS FROM *COMMELINA NUDIFLORA*

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ABSTRACT

Objective: In the present study, the essential oil, methanol extract, and methanol fractions (n-hexane, chloroform, ethyl acetate, and n-butanol) obtained from *Commelina nudiflora* were investigated for the free radical scavenging effects and phytochemical analysis.

Methods: The antioxidative effect of the essential oil, methanol extracts and methanol fractions were evaluated using 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Total phenolic and flavonoid contents were determined using Folin -Ciocalteau and aluminium chloride reagents respectively. The phytochemical analyses of the essential oil, methanol extracts and methanol fractions were performed by gas chromatography and mass spectrometry (GCMS).

Results: The antioxidant, total phenolic and total flavonoid contents of butanol, ethyl acetate and chloroform fractions were higher followed by methanol extract, hexane fraction and essential oil. Phytochemical analysis indicated the presence of alkaloid, saponin, steroid, phytosterols, triterpenoids and tannins etc. The identified bioactive constituents of essential oil, methanol extract and methanol fractions of *C. nudiflora* were indole, 2-methoxy-4-vinylphenol, 2-pentadecanone, 6,10,14-trimethyl, phenol, benzyl alcohol, eugenol, phenol, 2, 4-bis (1,1-dimethylethyl), hexadecanoic acid, ethyl ester (palmitic acid ester), n-hexadecanoic acid (palmitic acid), 9, 12-octadecadienoic acid, (linoleic acid) and phytol. All identified bioactive compounds and their derivatives were generally reported with antimicrobial, antioxidant, anti-inflammatory and antitumor properties.

Conclusion: The obtained data suggest that the essential oil, methanol extract and methanol fractions of *C. nudiflora* possess remarkable antioxidant activities and vital phytochemicals. Thus the plant can be a utilized as a potential source of nutraceutical with antioxidant activity.

Keywords: *Commelina nudiflora,* Phytochemical compounds, Antioxidants, Methanol extract

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INTRODUCTION

Synthetically prepared drugs are utilized worldwide for the treatment of various diseases. However, these drugs have several side effects such as gastric ulcer, kidney damage as well as cardiac abnormities [1]. Plants have been used as an important source of medicine for centuries. In recent years a large number of researchers have focused on medicinal plants due to fewer side effects and high efficacy. The natural product obtained from medicinal plants include flavonoids, polyphenols and alkaloids show immense pharmacological significance such as anti-inflammatory, antimicrobial, antioxidant and anticancer activities [2-4].

Commelina nudiflora, herbal plant and member of family Commelinaceae. The English name is "Day Flower" while the local Malay name is "Rumpur Aur". The glabrous and fleshy stem grows up to 15-30 cm in length with roots at the nodes. The plant is consists of simple green leaves and pink or purple color small flowers [5]. *C. nudiflora* is edible by humans. In India, the young stem of the plant is steamed and eaten as a vegetable in daily diet [6] while the young leaves and small flowers are consumed fresh in salads or boiled with butter [7, 8]. The plant is also used for the treatment of the variety of diseases such as diarrhea, hemorrhoids, tuberculosis, eyes, ear, and throat diseases, mumps, boils, leprosy, antidote, gonorrhea and infertility treatments [9]. As the plant is used for the treatment of various diseases but no research has been done to explain the phytochemical compassion and free radical scavenging effects of the essential oil and solvent extract. Thus In the current study, the essential oil, methanol extract, and methanol fractions obtained from *Commelina nudiflora* were investigated for the free radical scavenging effects and phytochemical analysis.

MATERIALS AND METHODS

Chemicals

α, α-diphenyl-picrylhadrazyl (DPPH), and gallic acid were purchased from Sigma. Extraction solvents, methanol, n-hexane, butanol, chloroform and ethyl acetate (HPLC grade) were purchased from Merck (Darmstadt, Germany). Lead acetate, sodium carbonate, aluminum chloride, potassium acetate, potassium sulfate, sodium hydroxide, sodium nitroprusside, hydrogen peroxide, sulfanilic acid, glacial acetic acid were all obtained from Merck, USA.

Sample collection

The fresh leaves of *C. nudiflora* were collected from the lower land of Papar, Sabah, Malaysia. The plant was harvested and packed in polyethylene bags. The identification of plants was confirmed by Mr. Julius Kulip and Mr. Johnny Gisil from the Institute of Tropical Biology and Conservation, University Malaysia Sabah. A voucher specimen (MDS003) was deposited at the Tropical Biology and Conservation Herbarium, University Malaysia Sabah.

Extraction and fractionation

Sixty grams of powder plant was extracted with 300 ml of methanol using soxhlet method (50-60 °C and 72h). The methanol residues were removed from the extract using a vacuum rotary evaporator. The extract was further suspended in distilled water and successfully extracted with n-hexane, ethyl acetate, chloroform and butanol solvent in a separatory funnel. The solvents were removed from the extracts under vacuum. The samples were kept at at-80 °C for 24 h and then lyophilized using a freeze drier. The freeze-dried samples were then stored in the freezer for further analysis [10].

Isolation of essential oil

The *C. nudiflora* leaves (100 g) were subjected to hydrodistillation for 5 h using a distillation type apparatus. The essential oils from the leaves were dried over anhydrous sodium sulfate and further extracted with diethyl ether.

Determination of total phenolic content

The total phenolic content was determined by Folin-Ciocalteu method [11] with slight modification. A stock solution of 1 mg/ml was prepared. Folin-Ciocalteu reagent was prepared by 10 fold dilution (ratio 1:9). Briefly, 1.5 ml of Folin-Ciocalteu reagent was mixed with 0.2 ml of assay samples and mixed vigorously. After 5 min 1.5 ml sodium carbonate (60 g/l) was added to the mixture. Finally, the mixture was allowed to stand for 90 min in the dark at room temperature. The absorbance was measured at 725 nm against a blank. Gallic acid was used as a standard for the quantification of the phenolic compound. Concentrations of 0.01, 0.02, 0.04, 0.08 and 0.1 mg/g of gallic acid were used to plot the slandered calibration curve. The concentration of the total phenolic content was estimated as mg of gallic acid equivalent by using an equation obtained from the gallic acid calibration curve. The determination of phenolic compounds in all the fractions was carried out in triplicate.

Determination of total flavonoid content

The total flavonoid content was determined by usin g the aluminum chloride colorimetric method [12] with some modi fications. 0.25 ml of sample was mixed with 1.25 ml of distilled water and 0.075 ml of sodium nitrate (5%) in a tube. The mixture was mixed vigorously and left in the dark for 6 min. Further 0.15 ml aluminum chloride (10%) was added to the mixture, shaken vigorously and left for 5 min at room temperature. Finally, 0.5 ml of sodium hydroxide (4%) was added to the mixture, followed by the addition of distilled water to obtain a final volume of 2.5 ml. The mixture was thoroughly mixed by vortex. The absorbance was measured by spectrophotometer at 510 nm against the blank. Catechin was used as a standard with concentration at 0.02, 0.04, 0.06, 0.08, 0.1 and 0.2 mg/g. The calculation of total flavonoids in the extracts was done in triplicate and the results were averaged. The total flavonoid content was expressed in mg of catechin equivalents per gram of plant extract.

Radical scavenging activity using the DPPH method

The antioxidant activity of the essential oil, methanol extract and fractions was determined by DPPH assay [13]. A stock solution of 1 mg/ml was prepared. Six concentrations (0.012, 0.025, 0.050, 0.1 and 0.5 mg/g) of essential oil, extract and fractions were used. During the process, 0.3 ml of our diluted sample was mixed with 2.7 ml DPPH (150 µM) in absolute methanol and left in the dark for 60 min. Absolute methanol was used as a blank. The absorbance was measured at 512 using spectrophotometer. Ascorbic acid was used as a standard. The radical-scavenging activity was calculated according to the formula summarized below.

$$\% \text{ RSA} = A_{B \text{ control}} \left[\frac{- A_{A \text{ sample}}}{A_{B \text{ control}}} \right] \times 100$$

% RSA: Percentage of radical scavenging activities.

$A_{A:}$ Absorbance values of the extracted sample

$A_{B:}$ Absorbance values of the control sample

Determination of the effective concentration (EC_{50})

The EC_{50} values express the amount of essential oil, methanol extract and methanol fractions required to reduce the absorbance of DPPH by 50% [14]. The values were determined by using the slope of the linear regression.

Preliminary phytochemical screening

The preliminary phytochemical screening of the methanol extract and methanol fractions (I mg/ml) of *C. nudiflora* was carried out to determine the presence and absence of different phytochemical compounds. The plant extracts were screened for alkaloids, steroids, flavonoids, triterpenoids, saponins, tannins, anthraquinones and phytosterol [15, 16].

Gas chromatography-mass spectrometry (GCMS) analysis

Essential oil, methanol extract and methanol fractions of *C. nudiflora* were injected into a GCMS system consisting of an Agilent 7890A gas chromatograph system coupled with an Agilent 5975C mass spectrometry detector. A capillary column HP-5MS (30 m×0.25 mm) of 0.25 µm film thickness of the coated material was used. The injector temperature was set at 250 °C, the temperature arrangements were as follow: initiate at 40 °C and hold for 3 min; from 40 to 200 °C at 3 °C/min and then hold for 3 min. A post-run of 5 min at 200 °C was set for the next injection. Helium gas (99.9% pure) was utilized as a carrier gas and maintained at 1.0 ml/min constant flow rate. Split-fewer modes were used. Identification of phytochemicals was done out by referring to National Institute of Standards and Technology (NIST) library and the compositions were computed with reference to the abundance of the compounds in the chromatogram. Each analysis was carried out in triplicate.

Data analysis

The experimental results were expressed as mean±SD. All the assays were performed in triplicate.

RESULTS

The percentage yield, total phenol and flavonoid contents of *C. nudiflora* methanol extract and methanol fractions

Table 1 shows the percentage of extraction yields, total phenolic and flavonoid contents of methanol extracts and methanol fractions (hexane, ethyl acetate, chloroform, and butanol) of *C. nudiflora.* There were significant differences among the methanol extract and methanol fractions.

Table 1: The percentage yield, total phenol and flavonoid contents of *C. nudiflora* methanol extract and methanol fractions

Extract/fractions	Percentage (%) yield	Total phenolic content (mg/g)	Total flavonoid contents.(mg/g)
Methanol extract	11.22±0.58	42.67±1.78	31.54±2.23[a]
Hexane fraction	1.33±0.08	37.67±1.34	24.32±2.06[b]
Ethyl acetate fraction	0.64±0.10	59.33±0.76	52.78±2.20 [c]
Chloroform fraction	0.35±0.01	43.50±2.42	34.6±2.55
Butanol fraction	0.43±0.06	71.50±1.85[e]	66.19±1.97[e]

Results are expressed as mean±SD (n=3)., Different letters with each mean a statistical difference.

DPPH scavenging activity of *C. nudiflora* essential oil, methanol extracts and methanol fractions

The DPPH free radical scavenging activity of the *C. nudiflora* essential oil, methanol extracts and methanol fractions was estimated at various concentrations ranging from 0.012-0.5 mg/g. DPPH scavenging elevated with the concentration increase of essential oil, extract and fractions of our samples (fig. 1).

Data presented as mean±standard error of mean (SEM) value of three replicates

Calculated effective concentration (EC50) of *C. nudiflora* essential oil, methanol extract, and methanol fractions

Table 2 shows the EC_{50} values of the standard (ascorbic acid), *C. nudiflora essential* oil, methanol extracts and methanol fractions.

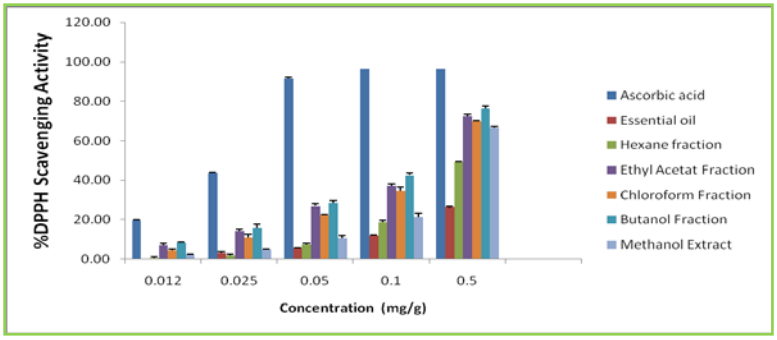

Fig. 1: Radical scavenging activity of *C. nudiflora* essential oil methanol extract and methanol fractions

Table 2: The EC50 values of ascorbic acid, essential oil, methanol extract and methanol fractions

Extract/Fractions	EC_{50} (mg/g)
Ascorbic acid	0.054±0.002
Essential oil	0.958±0.008
Hexane friction	0.496±0.004
Ethyl acetate friction	0.299±0.005
Chloroform friction	0.321±0.001
Butanol friction	0.277±0.005
Methanol extract	0.364±0.006

Results are expressed as mean±SD (n=3)., Different letters with each mean a statistical difference.

Phytochemical analysis of *C. nudiflora* methanol extracts and methanol fractions

The preliminary phytochemical screening of *C. nudiflora* methanol extracts and fractions indicated the presence of bioactive components such as alkaloids, flavonoids, phytosterols, saponins, tannins, triterpenoids, and steroids while anthraquinones were absent as shown in table 3.

Gas chromatography and mass spectrometry analysis of *C. nudiflora* essential oil, methanol extract, and methanol fractions

The phytochemical compounds exist in the essential oil, methanol extract and methanol fractions of *C. nudiflora* were identified by GC-MS (fig. 2 to 7).

Table 3: The analysis of phytochemical in the methanol extract and methanol fractions of *C. nudiflora*

Biochemical	Extract/Fractions				
	Hexane	Ethyl acetate	Chloroform	Butanol	Methanol
Alkaloids	++	+	+	+	++
Anthraquinones	-	-	-	-	-
Flavonoids	+	++	++	++	++
Phytosterol	+	++	++	++	+
Saponins	-	+	-	-	+
Tannins	-	+	+	+	+
Triterpenoids	+	+	+	+	++
Steroids	+	+	+	+	+

+= present; ++= Strong presence; –= absent.

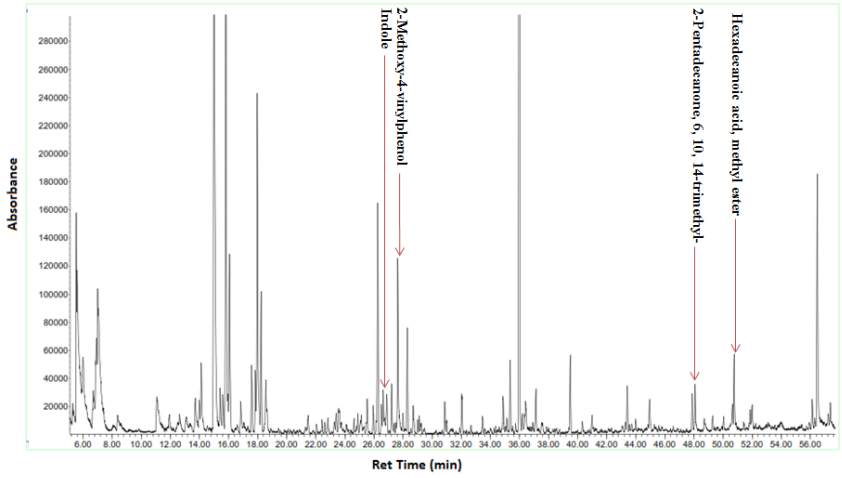

Fig. 2: A typical gas chromatogram of the chemical constituents of essential oil of C. nudiflora

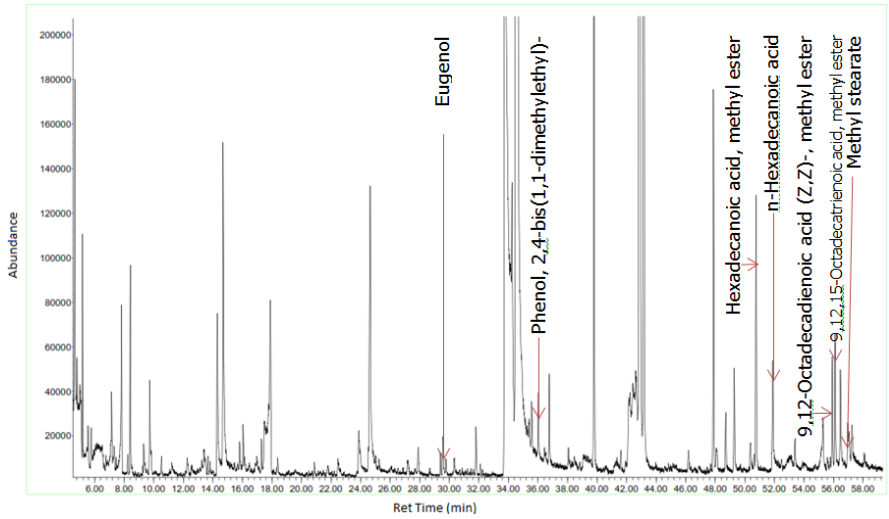

Fig. 3: GC-MS chromatogram of the hexane fraction of methanol extract of *C. nudiflora* plant

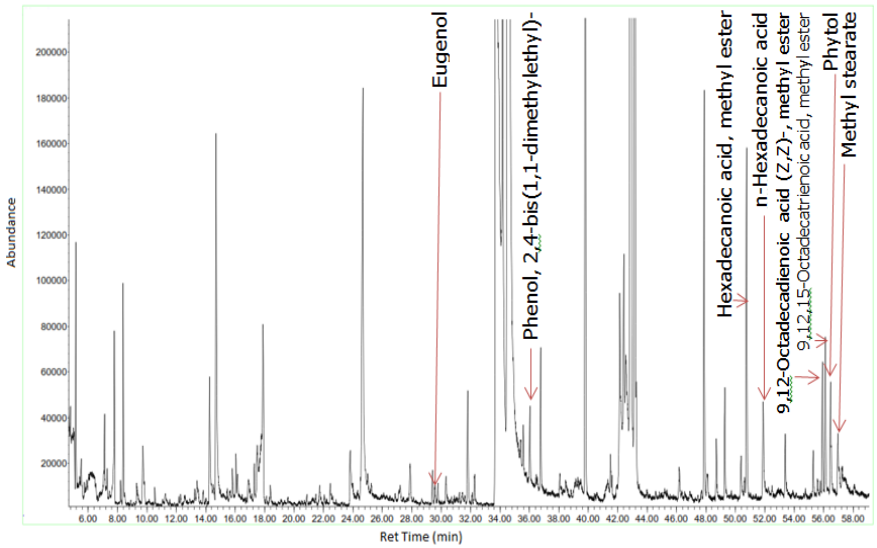

Fig. 4: GC-MS chromatogram of the ethyl acetate fraction of methanol extract of *C. nudiflora* plant

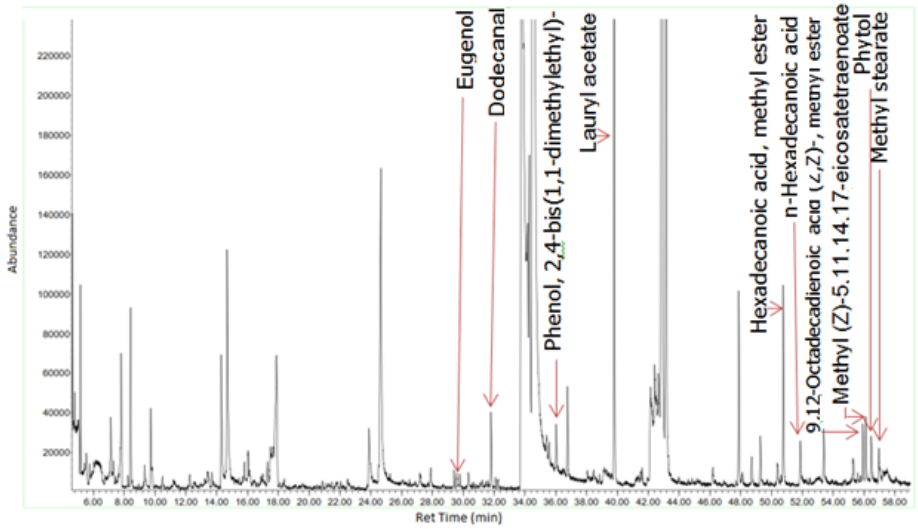

Fig. 5: GC-MS chromatogram of the chloroform fraction of methanol extract of *C. nudiflora* plant

Table 4: The phytochemical compounds detected in the essential oil, methanol extract and methanol fractions of *C. nudiflora*

E. oil/extract/fractions	No	Compound name	RT	Area (%)
	1.	Indole	26.72	4.30
E. oil	2.	2-Methoxy-4-vinylphenol	27.63	48.30
	3.	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	34.87	10.55
	4	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1. alpha.,4. beta.,4a. beta.,8a. beta.)]-	40.98	5.34
	5.	2-Pentadecanone, 6,10,14-trimethyl-	48.06	13.55
	6.	Hexadecanoic acid, methyl ester	50.75	17.95
	1.	Undecane	17.29	2.12
n-Hexane	2.	2H-Pyran-2-one, 4,6-dimethyl-	22.47	0.99
	3.	Eugenol	29.57	2.68
	4.	2-Fluorobenzoic acid. heptadecyl ester	35.58	7.47
	5.	Phenol, 2,4-bis(1,1-dimethylethyl)-	36.03	7.50
	6.	Cyclododecane	39.79	35.33
	7.	Hexadecanoic acid, methyl ester	50.76	16.27
	8.	n-Hexadecanoic acid	51.90	8.61
	9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	55.92	6.12
	10.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	56.11	7.85
	11.	Methyl stearate	56.97	2.78
	12.	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	57.05	2.28
Ethyl acetate	1.	N-. alpha.,N-. omega.-Di-cbz-L-arginine	14.52	1.65
	2.	2H-Pyran-2-one, 4,6-dimethyl-	22.46	1.08
	3.	Eugenol	29.57	1.34
	4.	Phenol, 2,4-bis(1,1-dimethylethyl)-	36.03	9.69
	5.	1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	42.14	23.73
	6.	Hexadecanoic acid, methyl ester	50.76	21.27
	7.	n-Hexadecanoic acid	51.89	7.92
	8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	55.92	7.83
	9.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	56.10	10.79
	10.	Phytol	56.46	8.48
	11.	Methyl stearate	56.96	6.20
Chloroform	1.	Eugenol	29.56	1.45
	2.	Dodecanal	31.8	6.14
	3.	Phenol, 2,4-bis(1,1-dimethylethyl)-	36.03	8.73
	4.	Lauryl acetate	39.79	42.19
	5.	Hexadecanoic acid, methyl ester	50.75	15.86
	6.	n-Hexadecanoic acid	51.86	4.49
	7.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	55.92	4.85
	8.	Methyl (Z)-5,11,14,17-eicosatetraenoate	56.11	6.70
	9.	Phytol	56.46	5.33
	10.	Heptadecanoic acid, 16-methyl-, methyl ester	56.96	4.20
	1.	Phenol, 2,4-bis(1,1-dimethylethyl)-	36.02	4.01
Butanol	2.	Fluoroacetic acid, dodecyl ester	39.79	37.80
	3.	Hexadecanoic acid, methyl ester	50.75	27.06
	4.	n-Hexadecanoic acid	51.86	3.80
	5.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	55.91	7.15
	6.	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	56.10	10.88
	7.	Phytol	56.46	4.96
	8.	Methyl Stearate	56.96	4.26
	1.	Acetic acid	3.39	1.60
Methanol	2.	Phenol	12.54	0.71
	3.	Benzyl alcohol	14.35	1.62
	4.	Undecane	17.29	2.00
	5.	Caprolactam	24.77	6.83
	6.	Eugenol	29.06	0.64
	7.	Cyclododecane	33.83	36.17
	8.	1-Decene	34.2	9.30
	9.	Phenol, 2,4-bis(1,1-dimethylethyl)	36.06	0.77
	10.	2(4H)-Benzofuranone, 5,6,7,7a-tetr ahydro-4,4,7a-trimethyl-, (R)	36.48	0.52
	11.	Dodecanoic acid	38.1	0.70
	12.	Lauryl acetate	39.83	5.64
	13.	Pentafluoropropionic acid, undecyl ester	42.66	3.32
	14.	Hexadecanoic acid ethyl ester	50.79	2.27
	15.	n-Hexadecanoic acid	51.95	3.15
	16.	9,12-Octadecadienoic acid, methyl ester	55.95	1.14
	17.	Cyclooctene, 3 ethenyl	56.15	1.25
	18.	Phytol	56.71	2.61
	19.	Methyl stearate	57.01	0.48
	20.	9,12-Octadecadienoic acid (Z,Z)-	57.10	0.66

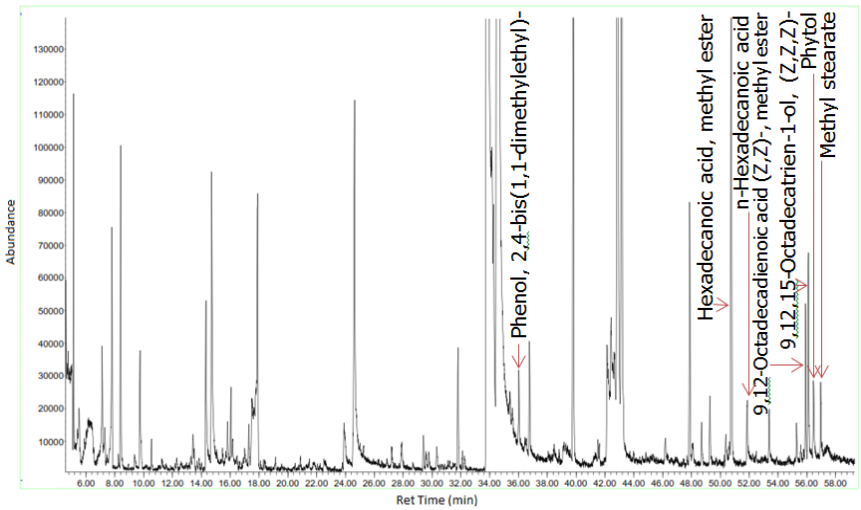

Fig. 6: GC-MS chromatogram of the butanol fraction of methanol extract of *C. nudiflora* plant

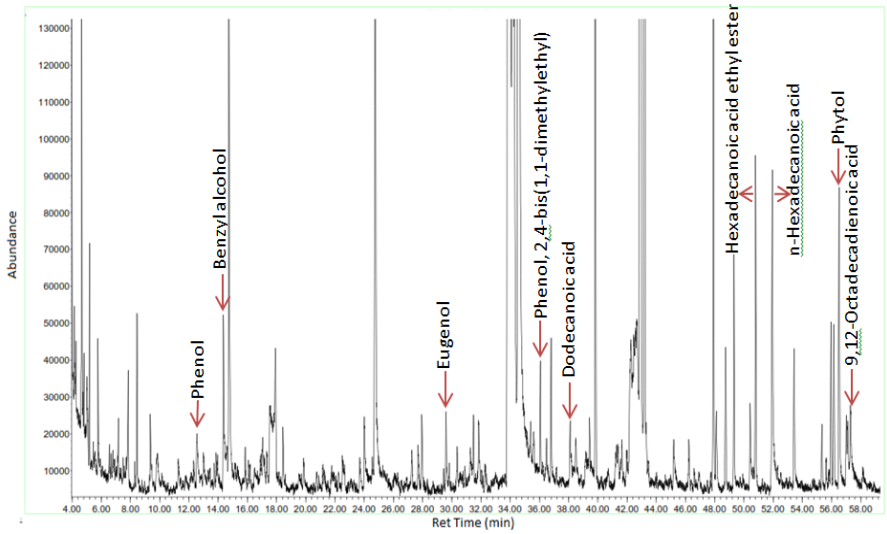

Fig. 7: GC-MS chromatogram of the methanol extract of *C. nudiflora* plant

The phytochemical compounds detected in the essential oil, methanol extract and methanol fractions of *C. nudiflora*

The list of the compound detected in the essential oil, methanol extract and methanol fraction of C. nudiflora is shown in the table along with retention time and area percentage as shown in table 4.

DISCUSSION

C. nudiflora is consumed as a traditional remedy for the treatment of various types of disease. Previously, there has been no study of *C. nudiflora* relevant to its antioxidant activity and phytochemical analysis.

Phenolics are important metabolites of plants with antioxidant potential due to their redox properties. The compounds are essential for decomposition and neutralization of free radicals [17]. The data recorded in the current study showed that the phenolic compound level in butanol, ethyl acetate, chloroform fractions and methanol extract of *C. nudiflora* is very high. Flavonoids are important and diverse phenolic substances, isolated from a large number of plants. These compounds possess antioxidant, antiviral, anti-inflammatory and anti-allergenic activities [18]. Thus it was important to evaluate the amounts of flavonoids in methanol extract and methanol fractions. The hexane fractions of methanol extract showed lower flavonoid values (4.61±0.16 and 24.32±2.06) as compared to Butanol, (66.19±1.97), ethyl acetate (52.78±2.20) and chloroform fractions (34.65±2.55 mg/g) (table 1).

The antioxidantive effect on DPPH is believed to be due to hydrogen donation ability [19]. The essential oil, methanol extract, and fractions of *C. nudiflora* indicated less DPPH radical scavenging activities in comparison to ascorbic acid as shown in fig. 1. Our results showed that the essential oil, methanol extract and methanol fractions of *C. nudiflora* possess proton donating potential and could play a crucial role in free radical scavenging and inhibition. The phytochemical analysis of methanol extract and methanol fractions of *C. nudiflora* is shown in table 3 indicates the presence of medicinally active compounds such as alkaloids, tannins, steroids, phytosterol, saponin, triterpenoids, and flavonoids. Studies have shown that these compounds exhibit several pharmacological properties such as anticholinergic, vasodilating, anti-hypertensive, anti-bacterial, antiviral and anti-tumour [19, 20].

In the essential oil, methanol extract and methanol fractions of *C. nudiflora* several bioactive compounds have been noticed. The identified bioactive compounds include indole, an aromatic heterocyclic organic compound. The derivatives of the compound have been reported with antimicrobial and anti-inflammatory properties [21]. 2-Methoxy-4-vinyl phenol has been also reported in the extract of *Pergularia daemia with* anti-microbial, analgesic, anti-oxidant and anti-inflammatory by Sridevi *et al.* [22]. 2-Pentadecanone, 6,10,14-trimethyl-has been reported with antimicrobial properties by Govindappa *et al.* [23], phenol, which is a sample phenolic compound with one hydroxyl group. This compound has been reported in

the *Ficus religiosa linn* ethanol bark extract with antioxidant, antiseptic and anti-bacterial properties [24]. Benzyl alcohol, an aromatic alcohol compound with a benzene ring, has also been reported in basil leaves (*Ocimum basilicum L*) with antioxidant and anti-microbial properties [25-27]. Eugenol is a phenolic compound and has been found in the extract of basil leaves (*Ocimum basilicum L*) and *Eugenia caryophyllata* with antioxidant and anti-inflammatory properties [25, 28, 29]. Phenol, 2,4-bis (1,1-dimethylethyl) is an alkylated phenolic compound and has also been reported in the extracts of *Plumbago zeylanica* and *Tephrosia tinctoria* with antioxidant, anti-cancer and anti-microbial properties [30, 31]. Dodecanoic acid is also known as lauric acid. It is a saturated fatty acid and has been found in the extract of *Vitex altissima L* with anti-microbial, antioxidant, anti-viral and hypo-cholesterolemic properties [32-34]. Hexadecanoic acid and n-Hexadecanoic acid have been found in the extract of *Vitex negundo and Centaurea aladagensis* and have been reported with antioxidant and hypocholesterolemic properties [35, 36]. Phytol, a diterpene alcohol, has also been noticed in the *Hybanthus enneaspermus* and reported with anti-microbial, anti-cancer, anti-inflammatory, hepatoprotective and diuretic activities [35, 37]. 9,12-Octadecadienoic acid is commonly known as linoleic acid. It is an unsaturated fatty acid. The compound has also been reported in *Scotia brachypetala* with anti-inflammatory, anti-cancer and hepatoprotective properties [35, 38, 39].

CONCLUSION

Our current data suggest that the essential oil, methanol extract and methanol fractions of *C. nudiflora* possess remarkable antioxidant activities and vital phytochemicals with antioxidant, anti-inflammatory, antimicrobial and antitumor activities. Thus the plant can be a used as a potential source of nutraceutical with antioxidant activity. This study was the first report about the antioxidant activities and phytochemical analysis of *C. nudiflora* essential oil, methanol extract and methanol fractions. Further investigation to determine antioxidant activity by the *in vivo* method is in progress in our laboratory.

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AUTHORS CONTRIBUTIONS

Muhammad Dawood Shah-Conducted the experiment and prepared the manuscript. Dr. Mohammad Iqbal-Helped in designing and conducting the experiment.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

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