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Phytochemical screening for antimicrobial and antioxidant activity of periwinkle

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ABSTRACT

To perform preliminary phytochemical screening, investigate total flavonoids, phenolic, and antioxidants, and evaluate the antimicrobial potential of *Catharanthus roseus*, the chemical composition of the various crude extracts from the leaves was determined by GC-MS analysis. The dried leaves of *C. roseus* were powdered and subjected to extraction using three different solvents. All prepared extracts were then subjected to detect flavonoid and phenolic contents as per Folin-Ciocalteu and aluminium chloride methods, respectively. Various assays like SOD, POD, CAT, ABTS, and DPPH were used to analyze in vitro antioxidant activity. All plant extracts were further used to determine antimicrobial activity against five selected bacterial strains. Each section was subjected further to GC-MS. Preliminary phytochemical analysis showed that the maximum phytoconstituents were found in methanolic extract. The lowest IC50 value was exhibited by the methanolic extract, followed by the ethanolic extract. The activity levels of antioxidant enzymes in the plant extracts were satisfactory. The antimicrobial potential of the plant extracts was found to be positively correlated with the total phenolics and flavonoid contents. Fifteen different phytoconstituents were in methanolic, seven in ethanolic, while five compounds were identified in the petroleum ether extract by GC-MS. Some of these compounds are known to possess antimicrobial and antioxidant properties.

Keywords: *Catharanthus roseus,* antimicrobial, antioxidant, phytoconstituents.

INTRODUCTION

Periwinkle {*Catharanthus roseus* (L.) G. Don}, commonly known as Madagascar periwinkle belonging to the family Apocynaceae, is an important species having a long history. It has been mentioned in folk medicinal literature as early as 2nd B.C. as diuretic, anti-dysenteric, anti-haemorrhagic and wound healing. *Catharanthus roseus* is an easy growing perennial evergreen sub-shrub plant that grows up to one meter in height. It is a native of Madagascar and spread to India, Indonesia, Indochina, Philippines, South-Africa, Israel, USA and other parts of the world. USA is the world's largest user of raw material and Hungary has been one of the major consumers of the leaves (Lubbe and Verpoorte, 6). It has countless horticultural uses like annual ground cover, bedding, border, container gardening, decoration, edging, hanging basket, mass planting, naturalizing and wall cascade. Surprisingly, the plant is naturalised for pharmaceutical purposes in different parts of the world. The plant is also a potential source of compounds like sterols, flavonoids, sesquiterpene lactones, terpenoids and lupeol acetate which largely contribute to various biological properties in traditional as well as in some modern therapeutic principles.

Almost all parts of this plant possess different active alkaloids with an indole moiety. Approximately, 130 indole alkaloids, collectively known as terpenoid indole alkaloids (TIAs), have been extracted from *C. roseus*. Two pharmacologically and commercially significant cytotoxic dimeric alkaloids of *C. Roseus viz*., vincristine and vinblastine, have been extensively used for cancer treatment. These alkaloids possess various pharmacological activities including antibacterial, anticancer, antiarrhythmic, analgesic, antimalarial, antihyperglycemic activities, *etc*. Because of their effective pharmacological effects, alkaloids are the source for many drugs (Wang *et al.,*15). The main alkaloids present in the plant are catharanthine, isositsirikine, leurosine, lochrovicine, leurocolombine, tetrahydroalstonine, vincaleukoblastine, vincarodine, vincoline, viramidine, vincathicine, vincolidine, vindoline, lochnerine and vindolinine. Ajmalicine, reserpine and serpentineare the important root alkaloids while tabersonine, tetrahydroalstonine, vindorosidine and vincristine dominate in the flower (Kapoor and Rani, 4). Looking at the ethnomedicinal values and scanty information available in literature, the current study was conducted to investigate different plant extracts of *C. Roseus* for their phytocompounds, antioxidant and antimicrobial properties and to identify the phytoconstituents with the help of GC-MS also.

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MATERIALS AND METHODS

The leaves of *C. roseus* were collected from plant conservatory of Punjabi University, Patiala, Punjab (India) during 2015-19 and identified by a botanist/ herbarium curator of Punjabi University, Patiala. The experimental soil was sandy loam in texture with adequate drainage and optimum water holding capacity, slightly acidic in reaction (6.65 pH), medium in organic carbon (0.51%), low in available nitrogen (102.63 kg/ha), high in available phosphorus (41.74 kg/ha) and medium in available potassium (127.55 kg/ ha). After thorough washing and shade drying at room temp for 6-7 days, the dried leaves were crushed to very fine powder and kept in airtight glass bottles, till further use. Leaf powder (50 gm) was extracted in a Soxhlet apparatus by 500 mL of different solvents *i.e.* petroleum ether, ethanol and methanol. The plant extracts were filtered through a Whatman filter paper and solvent was evaporated by using a rotary evaporator. The extracts were preserved in airtight glass bottles and stored at 4ºC till further use. These plant extracts were screened qualitatively for the presence of different phytochemicals as per some standard methods (Onwukaeme *et al*., 7). Total flavonoid content (TFC) in leaf crude extracts was estimated by the procedure of Park *et al*. (8). Briefly 1 mL of plant extract was added to 0.2 mL of 10% aluminium chloride, 3 mL of methanol, 5.6 mL of distilled water and 0.2 mL of 1M potassium acetate and kept at room temperature for 30 min to complete the reaction. The absorbance was measured at 420 nm against blank. Quercetin was used as standard of 10–100 µg/mL range from 1 mg/mL stock solution. All the tests were performed in triplicates and results are expressed as mg of quercetin equivalent (QE)/ gram dry weight of sample.

Phenolic components of plant extracts react with phosphomolybdic acid in Folin-Ciocalteu reagent and produced a blue-coloured complex which can be observed spectrophotometrically at 650 nm. A stock solution of extracts was prepared to 1 mg/ mL of solvent. Plant extract (1 mL) was added with 2 mL of sodium carbonate (7.5%, w/v) and 5 mL of Folin-Ciocalteu (diluted 1:10 with distilled water). The solution was mixed and incubated for 15 min. in the dark with intermittent shaking. Blank consisted of 2 mL of Na_2CO_3 solution, 5 mL of FC reagent and 1 mL of solvent. Tannic acid was used as a standard of 10-100 µg/mL range from a stock solution of 1 mg/mL. Results for total phenolic content (TPC) are expressed in terms of mg of tannic acid equivalent (TAE)/gram dry weight of sample (Singleton and Rossi, 13). Nutrient agar (25 g) was dissolved in 1000 mL distilled water and then autoclaved at 121°C for 15 minutes and left to cool at room temperature. Once the nutrient medium was cooled, it was poured into petri dishes. Each petri dish was left at room temperature for 30-45 min. till solidification. An amount of 25 µL of overnight grown culture was spread onto petri dishes by using a sterile L-shaped spreader and allowed to dry for about 5 minutes. Wells were punched using sterile gel puncher and the extracts were added at various concentrations (50, 75 and 100 mg/100 mL of DMSO). Measured quantity (100 µL) of different test extracts was added to the well. Chloramphenicol 2 mg/10 mL of DMSO was used as positive control while DMSO was used as negative control. The plates were incubated for 24 hours at 37ºC. The diameter of inhibitory zones formed around each well was measured in mm and recorded.

Antioxidant potential of plant extracts was measured using DPPH (1, 1-diphenyl-2-picryl-hydrazyl) assay as per Blois (2). All crude extracts or standard ascorbic acid solution of 1 mL at 100 µg/ mL concentration were taken in separate test tubes. A solution of 1.0 mmol/L DPPH (2 mL) prepared in methanol, was added to each test tube. The solution was mixed and placed for 30 min. in dark at 37°C. Similarly, the blank solution was prepared without ascorbic acid or extract. The decrease in absorbance was measured at 517 nm using UV-VIS spectrophotometer. The percentage of free radical scavenging activity of extracts and control ascorbic acid was calculated by using the following formula:

$$\text{Free radical scavenging activity (\%)} = \frac{\text{Absorbance at 517 nm (control)} - \text{Absorbance at 517 nm(sample)}}{\text{Absorbance at 517 nm (control)}} \times 100$$

Scavenging activity was determined by ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay as per method described by Shirwaikar*et al*., (12). ABTS radical cation was formed by reacting 7 mM ABTS solution with 2.45 mM ammonium per sulphate and allowing the mixture to stand at room temperature in the dark for 12-16 hours. Ascorbic acid was used as standard in ABTS assays and absorbance was measured at 745nm by UV-VIS spectrophotometer (SHIMADZU 1800, Japan). Experiments were performed in triplicates and mean values were recorded. Antioxidant potential was calculated as per the formula given below:

$$\text{Free radical scavenging activity (\%)} = \frac{\text{Absorbance at 745 nm (control)} - \text{Absorbance at 745 nm (sample)}}{\text{Absorbance at 745 nm (control)}} \times 100$$

Plant extract was prepared by homogenization of freshly collected plant material (0.5 g) in 5 mL of cold extraction buffer containing 100 mM potassium phosphate buffer (pH7.0) and 0.1 mM sodium ethylenediaminetetraacetic acid (EDTA). The homogenate was filtered through three layers of muslin cloth, and filtrate was centrifuged at 14,000 rpm

for 20 minutes at 4ºC. The supernatant obtained was used for all the enzymatic assays. The assay mixture containing 1.9 mL of sodium carbonate buffer (50 mM, pH 10.0), 750 μL Nitro blue tetrazolium dye (NBT), 240 mM and 150 μL of 0.3% Triton X-100 was added to the test cuvette. The reaction was initiated by the addition of 150 μL of hydroxylamine hydrochloride (10 mM, pH 6.0). After 2 minutes, 70 μL enzyme samples from the plant tissue were added. The percentage inhibition in the rate of NBT reduction was recorded as increase in absorbance at 560 nm (Kono, 5). The percent inhibition of NBT reduction was calculated as below:

$$\frac{\text{Change in absorbance/minute (Control) - Change in absorbance/minute (Test)}}{\text{Change in absorbance/minute (Control)}} \times 100$$

X% of inhibition was produced by 70 μL of sample.
Hence, 50% inhibition was produced by

$$\text{Y } \mu\text{L of sample} = \frac{50 \times 70}{X} \times 100$$

The reaction mixture composing of 3 mL phosphate buffer (0.1 M), 30 μL guaiacol solution (20 mM), 100 μL enzyme sample and 30 μL H_2O_2 solution (12.3 mM) was taken in the test cuvette. The rate of formation of guaiacol dehydrogenation product was determined spectrophotometrically at 436 nm (Putter, 9). One unit of enzyme activity was expressed as units/minutes/gram tissue. Enzyme activity was calculated as follows:

$$\text{Enzyme activity} = \frac{\text{Change in absorbance/minute} \times \text{Total volume}}{\text{Extinction coefficient} \times \text{Volume of sample taken}} \times 100$$

Where extinction co-efficient = 26.60 $mM^{-1}cm^{-1}$

Catalase (CAT) enzyme activity was estimated as per method by taking 300 μL of enzyme extract in a test tube and adding 1.5 mL phosphate buffer (0.1 M) and 1.2 mL of hydrogen peroxide (150 mM). The absorbance was measuredat 240 nm and decrease in absorbance per minute was recorded (Aebi, 1). One unit of enzyme activity was calculated as the amount of enzyme required to liberate half the peroxide oxygen from H_2O_2 and calculated from the following equation:

$$\text{Units/minute/gram tissue} = \frac{\text{Change in absorbance/minute} \times \text{Total volume}}{\text{Extinction coefficient} \times \text{Volume of sample taken}} \times 100$$

Where extinction coefficient = 39.4 $mM^{-1}cm^{-1}$

GC analysis was carried out at Central Instrumentation Laboratory/SAIF, Panjab University, Chandigarh. This technique is important for the characterization of different phytochemicals of medicinal plants. For GC-MS analysis, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at a flow rate of 1 mL/min and 1 μL of plant extract was employed (split ratio of 10:1), at injector temperature 250°C, ion-source temperature of 280°C and total running time for a sample of about 76 min. Interpretation of GC-MS was conducted using the database of National Institute of Standards and Technology (NIST), U.S. having >6200 patterns. The spectrum of the unknown component was compared with the spectrum of the known component in the repository of NIST library. The retention time, molecular weight, molecular formula and composition percentage of the sample material were recorded. A statistical analysis of data was performed in accordance with the standard procedure and was analysed as per completely randomized design to test the significance of differences between the treatments. Antioxidant activity (IC_{50} values) and TPC/TFC were correlated using Pearson's correlation coefficient.

RESULTS AND DISCUSSION

The plant extracts of *C. Roseus* prepared in three different solvents were evaluated for the presence of different phytochemicals. All the three plant extracts showed the presence of alkaloids and glycosides. Methanolic extract showed maximum number of phytoconstituents followed by ethanolic and petroleum ether extracts. Flavonoids, phenols and tannins were present only in methanol and ethanol extracts. Proteins, saponins and quinones were absent in all extracts. The result of the analysis in various solvents has shown a remarkable variation in the presence of phytochemical compounds and our findings match with the experimental results of Hussein (3).

The TPC of the different crude extracts is expressed in terms of TAE and presented in Fig. 1. The TPCs were calculated using the linear regression equation obtained from the standard plot of tannic acid. The maximum phenolic content was exhibited by methanolic extract (120.4± 0.14), followed by ethanolic extract (71.38± 0.32).The flavonoid content of different leaves extracts is expressed in term of QE and is presented in Fig. 2. Linear regression equation obtained from standard curve of quercetin is $y=0.0047x+0.0391$, $R^2 = 0.996$. Similar to the TPC, the highest amount of TFC was also obtained in methanolic plant extract (92.93 ± 0.41) followed by ethanolic extract (86.59 ± 0.21). Plant extracts prepared in methanolic extract has also been reported to be rich sources of phenolic and flavonoid content by several workers (Roby *et al.*,11). Agar well diffusion assay was performed for all three plant extracts against five different bacterial

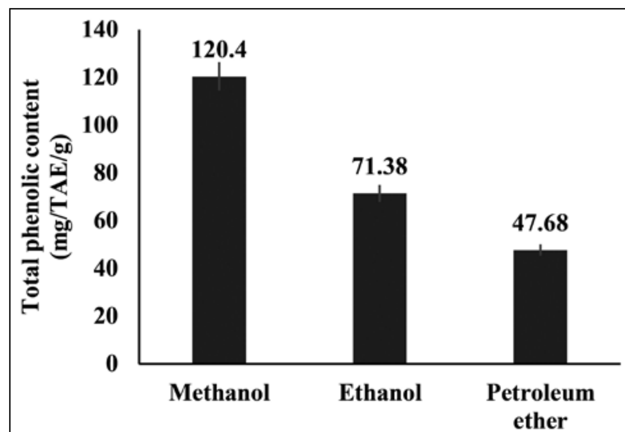

Fig. 1. Total phenolic content of *C. roseus*.

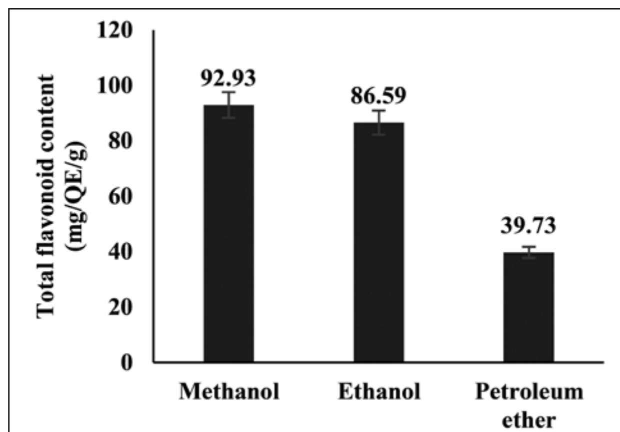

Fig. 2. Total flavonoid content of *C. roseus*.

strains and zone of inhibition (in mm) obtained are graphically represented in Fig. 3. Out of all three extracts studied, ethanolic extract showed maximum inhibitory effect of *Escherichia coli* (16.32±0.19), followed by *Bacillus subtilis* (15.32± 0.16) and lowest was showed against *Mycobacterium smegmatis* (9.33± 0.33). Methanolic extract showed maximum zone of inhibition against *B. subtilis* (15.53±0.09) followed by *E. coli* (15.48±0.24). Petroleum ether extract exhibited lowest inhibitory effect against all tested strains. *Staphylococcus aureus* and *E. coli* were found to be more susceptible whereas *Pseudomonas aeruginosa* and *M. smegmatis* were more resistant. Among the different concentrations (50, 75 and 100 mg/mL of DMSO), 100 mg/mL produced highest inhibitory activity against all the tested microorganisms.

The outcomes indicate that antibacterial activity of plants depends on the solvent used for extraction and bacterial strain. The inhibitory activity was found directly dependent upon the concentration of plant extract used.

DPPH and ABTS assays of all the three extracts are graphically represented in the Fig.4 and 5).The highest antioxidant activity was obtained with the methanolic extract in both the assays and showed percentage scavenging activity from 13.84 to 92.03% for DPPH and 38.29 to 69.10% for ABTS, respectively, for different concentrations.Ethanolic extract also showed good antioxidant activity. Least antioxidant potential was obtained with the petroleum ether extract.

The IC_{50}values were calculated for all the three extracts and are represented in the Fig. 6 and 7. A low IC_{50} value shows high antioxidant activity and *vice*

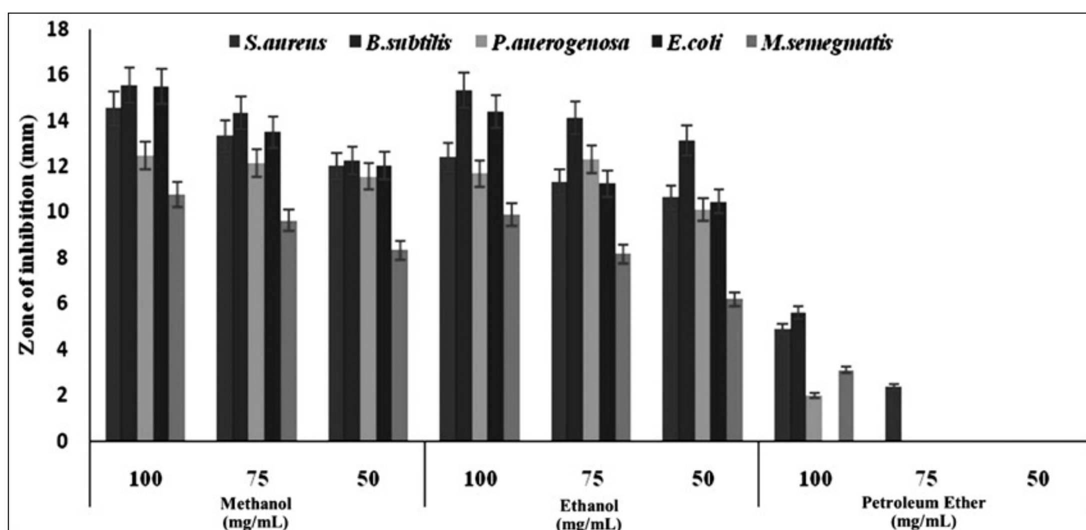

Fig. 3. Zone of inhibition obtained with different extract of *C. Roseus* and positive controls against different bacterial strains.

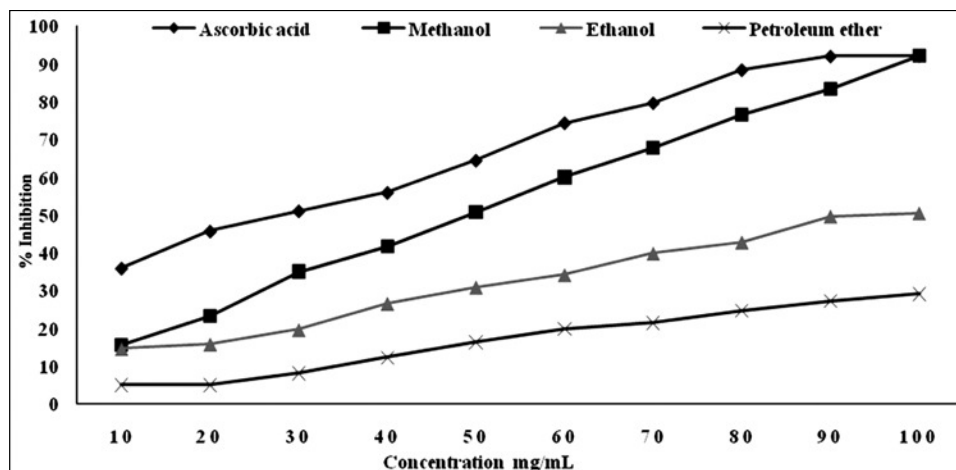

Fig. 4. Percentage inhibition of DPPH radical by different plant extracts of *C. roseus*.

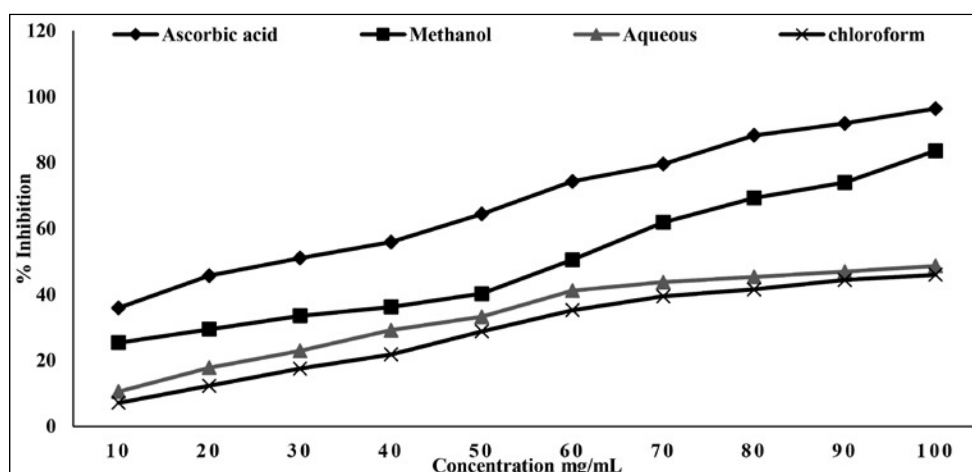

Fig. 5. Percentage inhibition of ABTS radical by plant extracts of *C. roseus*.

versa. Methanolic extract showed the least value of IC_{50} (47.93μg/mL, DPPH and 42.91μg/mL, ABTS) in both the assays, hence showed highest antioxidant activity and the poorest antioxidant activity was shown by petroleum ether extract having highest IC_{50}value of 139.05μg/mL DPPH and IC_{50}143.96μg/mL value.

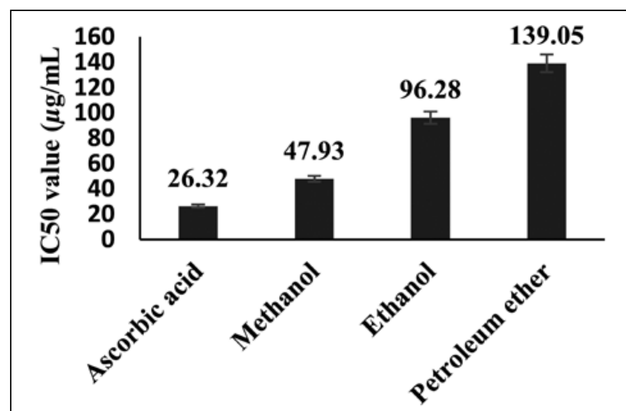

Fig. 6. IC_{50} values of *C. roseus* extracts by DPPH.

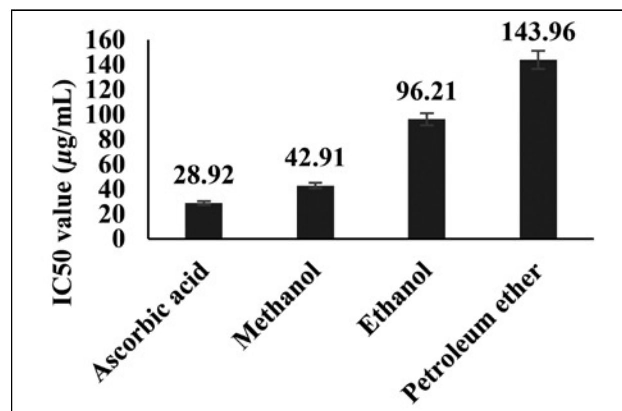

Fig. 7. IC_{50} values of *C. roseus* extracts by ABTS.

Correlation values (Pearson correlation coefficient =r) between the antioxidant activities(IC_{50}values) and total phenolic and total flavonoid content of the plant extracts are shown in the Table 1.

Perusal of data given in Table 1 reflects that the two variables *i.e.* TPC/TFC and IC_{50} values are negatively correlated with each other which means plant extracts having high TPC/TFC values showed lower IC_{50} values, lower IC_{50} values means higher antioxidant potential. From the results, it is also clear that TFC may have contributed more for the antioxidant potential than the TPC as it showed very good correlation ($R > 0.9$) than the TPC ($R < 0.9$). The antioxidant activity results obtained in case of antioxidant enzymes namely, catalase, gluthione S-transferase andsuperoxide dismutase are shown in Table 2.

The enzyme activity was found to be 12.07±0.33*μ*mole of H_2O_2 per minute fresh weight (FW) for catalase, 15.46±0.17μmol of GS-DNB conjugate/ min FW for glutathione-S-transferase and 50.94±0.31 SOD (Unit) FW for superoxide dismutase.

GC-MS analysis of different plant extracts, with their retention time (RT), molecular formula and peak area (%) are presented in Table 3 to 5. Methanolic extract exhibited fifteen phytoconstituents, ethanolic extract showed seven and petroleum ether extract showed minimum five phytoconstituents.

Many significant biological active components estimated by GC-MS were Myo-inositol (2.38), 9,12,15-octadecatrienoic acid, methyl ester (2.70%), 1-4H-pyran-4-one (4.73%), 5-hydroxymethylfurfural (30.86%), n-hexadecanoic acid (6.58%), phytol (17.17%), 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (23.64%) and 2,20-cycloaspidospermidine-3-carboxylic acid (4.95%). Antimicrobial and antioxidant activities are mainly shown by various detected compounds such as 4H-pyran-4-one, benzofuran, myo-inositol, 4-C-methyl-hexadecanoic acid, methyl ester, pentadecanoic acid and phytol.

Among the present phytoconstituents, 5-hydroxymethylfurfuryal and mannitol are sugar derivatives and exhibited antioxidant and antineoplastic activity formerly reported by Rani and Kapoor (10). 9,12,15-octadecatrienoic acid is a linoleic acid and

Table 1. Correlation between IC_{50} values, TPC and TFC.

Pearson Correlation Coefficient (r)				
TPC & TFC	TPC & IC_{50} ABTS	TFC & IC_{50} ABTS	TPC & IC_{50} DPPH	TFC & IC_{50} DPPH
0.818	-0.986	-0.902	-0.986	-0.900

Correlation is significant at the 0.05 level (2 tailed).

Table 2. Antioxidant enzymatic activities of *C. roseus*.

Antioxidant enzymatic activities	
Superoxide dismutase	50.94±0.31
Glutathione peroxidase	15.46±0.17
Catalase	12.07±0.33

Values are expressed as mean (n=3) ± SD.

Table 3. GC-MS results of methanolic leaf extract of *C. roseus*.

RT	Name of the compound	MF	MW	Peak area %	Biological properties
5.75	4H-Pyran-4-one	$C_6H_8O_4$	144	0.63	Antimicrobial, Antioxidant
6.53	Benzofuran, 2,3-dihydro	C8H8O	120.1	0.61	Antioxidant, Analgesic
6.77	1,2,3-Propanetriol, 1-acetate/acetin	$C_5H_{10}O_4$	134.1	0.84	Antimicrobial
7.24	1,3-Diazacyclooctane-2-th	$C_6H_{12}N_2S$	144.2	0.82	Antimicrobial, Antioxidant
8.73	Sucrose	$C_{12}H_{22}O_{11}$	342.2	3.14	Antioxidant, Anti-hiccup
10.05	1,2,3,5-Cyclohexanetetrol	$C_6H_{12}O_4$	148.1	5.12	Antioxidant, Antineoplastic
10.94	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	194.1	30.45	Antimicrobial, Antioxidant
12.29	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	370	0.97	Antioxidant
12.64	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	10.41	Antioxidant, Anti-cancerous
14.22	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4	0.67	Antioxidant, Anti-cancerous
14.32	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.2	2.70	Anti-cancerous
14.50	Phytol	$C_{20}H_{40}O$	296.5	1.38	Antioxidant, Antimicrobial
14.86	9-Octadecynoic acid	$C_{18}H_{32}O_2$	280.4	3.24	Inhibit fibrosis
14.99	Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	278.4	30.04	Antioxidant
23.76	2,20-Cycloaspidospermidine-3-carboxylic acid	$C_{21}H_{42}N_2O_2$	336	1.05	Anti-cancerous

Table 4. GC-MS results of ethanolic leaf extract of *C. roseus*.

RT	Name of the compound	Molecular formula	MW	Peak area %	Pharmacological actions
5.78	4H-Pyran-4-one	$C_6H_8O_4$	144	4.73	Antioxidant, Antimicrobial
6.67	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	30.86	Antioxidant, Antiproliferative activity
12.43	n-Hexadecanoic acid	$C_{17}H_{34}O_2$	256	6.58	Antioxidant
13.38	Phytol	$C_{20}H_{40}O$	296	17.17	Antimicrobial, Anticancer
13.57	9,12,15-Octadecatrienoic acid,	$C_{18}H_{30}O_2$	278	23.64	Anti-cancerous
16.08	2,20-Cycloaspidospermidine-3-carboxylic acid Vindolinine	$C_{21}H_{42}N_2O_2$	336	4.95	Anti-cancerous
20.57	Aspidospermidine-3-carboxylic acidVindoline	$C_{22}H_{28}N_2O_5$	400	12.09	Anti-cancerous

Table 5. GC-MS results of petroleum ether leaf extract of *C. roseus*.

RT	Name of the compound	MF	MW	Peak area %	Pharmacological actions
10.62	Myo-inositol	$C_7H_{14}O_6$	194.1	37.77	Antimicrobial, antioxidant
12.41	n-Hexadecanoic acid	$C_{17}H_{34}O_2$	370	7.58	Antioxidant
13.30	9,12,15-Octadecatrienoic acid,	$C_{27}H_{52}O_4Si_2$	496.2	4.55	Anti-inflammatory, Anti-cancerous
13.36	Phytol	$C_{20}H_{40}O$	296.5	9.79	Antioxidant,Antimicrobial
16.39	2,20-Cycloaspidospermidine-3-carboxylic acid	$C_{21}H_{42}N_2O_2$	16.39	7.96	Anti-cancerous

RT- Retention time; MF- molecular formula;MW- molecular weight

showed anticancer, anti-arthritic, anti-inflammatory, hepatoprotective, antihistaminic and insectifuge properties. Hexadecanoic acid methyl ester is also known as palmitic acid ester and effectively used as an antioxidant, pesticide, anti-androgenic, nematicide, flavouring agent, hypocholesterolemic, and lubricant.

In the present study, a direct relationship between the TPC, TFC and antioxidant activity was observed. Many other studies have also reported positive correlation between the total phenolics and antioxidant activity. Similar to our observation, other workers have also reported *C. roseus* to exhibit good antioxidant properties carried out by non-enzymatic assays (Suriyavathana and Sivanarayan, 14). As the plant parts of *C. Roseus* possess important bioactive compounds in the different plant extracts, which can be used for various pharmaceutical applications especially the methanolic extract. The

Three plant extracts prepared in different solvents from leaves of *C. roseus* were analysed for their qualitative, quantitative phytochemical analysis, antimicrobial and antioxidant potential. Out of the three extracts, methanolic extract of *C. roseus* showed maximum properties. The extract also showed highest phenolic, flavonoid content and antimicrobial properties. The antioxidant potential of the extract was also found to be highest as it showed minimum IC_{50} values in both cases DPPH as well as ABTS methods. GC-MS analysis further showed that the methanolic extracts also contain maximum number of components. Finally, it is anticipated that this investigation may help researchers at the global level to select this plant for detailed pharmacological and phytochemical investigations. Further investigations, when successfully implemented on *C. roseus* would hopefully lead to isolation of new phytoconstituents with a potential to develop effective phyto-drugs.

AUTHORS' CONTRIBUTION

Conceptualization of research (MK, JR, AK, SG); Designing of lab/field experiments (MK, JR, AK, SG); Contribution of experimental materials (MK, JR); Execution of lab/field experiments and data collection (MK, JR); Analysis of data and interpretations, (MK, JR, AK, SG); Preparation of manuscript, reviewing and editing (MK, JR, AK, SG).

DECLARATION

The authors do not have any conflict of interest.

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REFERENCES

1. Aebi, H. 1983. Catalase. **In**: *Methods of Enzymatic Analysis*, H.U. Bergmeyer (Ed.). Verlag Chemie, Weinhan. 673-84.

2. Blois, M.S. 2002. Antioxidant determination by the use of a stable free radical. *Nature*. **26**: 11991200.

3. Hussein, H.M. 2016. Analysis of trace heavy metals and volatile chemical compounds of *Lepidium sativum* using atomic absorption spectroscopy, gas chromatographymass spectrometric and fouriertransform infrared spectroscopy. *Res. J. Pharm. Biol. Chem. Sci*. 252955.

4. Kapoor, M. and Rani, J. 2019. Determination of vindoline and rutin content in five different morphotypes of *Catharanthus roseus* leaves using HPLC. *Int. J. Pharm. Pharm. Sci*. **11**: 52-57.

5. Kono, Y. 1975. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Arch. Biochem. Biophys*. **186**: 18995.

6. Lubbe, A. and Verpoorte, R. 2011. Cultivation of medicinal and aromatic plants for specialty industrial materials. *Ind. Crops. Prod*. **34**: 785-801.

7. Onwukaeme, D.N., Ikuegbvweha, T.B. and Asonye, C.C. 2007. Evaluation of phytochemical constituents, antibacterial activities and effect of exudates of *Pycnanthusangolensis* Weld Warb (*Myristicaceae*) on corneal ulcers in rabbits. *Trop. J. Pharm. Res.* **6:** 725-30.

8. Park, Y.S., Jung, S.T., Kang, S.G., Heo, B.G., ArancibiaAvila, P., Toledo, F., Drzewiecki, J., Namiesnik, J. and Gorinstein, S. 2008. Antioxidants and proteins in ethylenetreated kiwifruits. *Food. Chem.* **107**: 64048.

9. Putter, J. 1975. Peroxidases. **In**: *Methods of Enzymatic Analysis*, H.U. Bergmeyer., (Ed.)., Verlag Chemie-Academic Press. New York, NY, USA. pp. 685-90.

10. Rani, J. and Kapoor, M. 2019. Gas chromatography-mass spectrometric analysis and identification of bioactive constituents of *Catharanthus roseus* and its antioxidant activity. *Asian. J. Pharm. Clin. Res*. **12**: 461-65.

11. Roby, M.H.H., Sarhan, M.A., Selim, K.A-H. and Khalel, K.I. 2013. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Ind. Crops. Prod*. **43**: 827-31.

12. Shirwaikar, A., Rajendran, K. and Barik, R. 2006. Effect of aqueous bark extract of *Garuga pinnata* Roxb. instreptozotocin-nicotinamide induced type-II diabetes mellitus, *J. Ethnopharmacol*. **107**: 2285-90.

13. Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *J. Enol. Vitic.***16**:14458.

14. Suriyavathana, M. and Sivanarayan, V. 2013. Comparative analysis of phytochemical screening and antioxidant activity of some medicinal plants. *Int. J. Pharm. Sci. Rev. Res.* **18**: 72-76.

15. Wang, L., Zhang, Y.H., He, H.P., Zhang, Q., Li, S.F. and Hao, X.J. 2011. Three new terpenoid indole alkaloids from *Catharanthus roseus*. *Planta Medica.* **77**: 754-58.

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