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Study on the antioxidant potential of *Catharanthus species* and its thermal stability in sunflower oil model system

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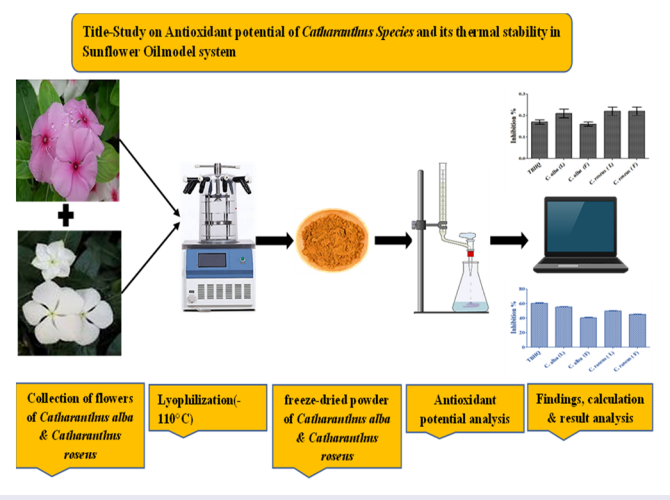
ABSTRACT

The studies analyze the efficiency of *Catharanthus alba* and *Catharanthus roseus* cultivars as a natural antioxidant measuring antioxidant potential through two methods. In the first method, antioxidant activity was estimated through the sunflower oil model system in which the Peroxide value (PV) and Thiobarbituric acid (TBA) tests were performed. While in the second method, antioxidant activity estimation is done through the assay method in which 2, 2-Diphenyl- 1-picrylhydrazyl (DPPH) radical scavenging method and ammonium thiocyanate method has been performed. The thermal stability of the leaves & flowers of *Catharanthus alba* and *Catharanthus roseus* were analyzed by mixing of powdered medicinal plant and sunflower oil. Further results indicate the higher antioxidant activity of flower *Catharanthus alba* as compared to flower *Catharanthus roseus*. Relatively among the leaves, *Catharanthus roseus* demonstrated slightly higher antioxidant potential than the leaves of *Catharanthus alba*. The antioxidant activity of *Catharanthus alba* (Flower)> *Catharanthus roseus* (Flower)> Catharanthus roseus (leaves)> Catharanthus alba (leaves) has been reported. The dehydrated powders of leaves and flowers of *Catharanthus alba* and *Catharanthus roseus* have shown good antioxidant activity at 0.5% level in sunflower oil while increase in concentration up to 1% and 1.5% does not show enhanced antioxidant activity in sunflower oil. The flowers and leaves of both cultivars of Catharanthus have shown good thermal stability when heated at 80°C continuously for 24 h considering all the parameters with statistical significance of ($p \leq 0.001$).

GRAPHICAL ABSTRACT



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PUBLIC IMPACT STATEMENT

The observation of the experiment reflects efficiency of Catharanthus species as a source of natural antioxidant. Two methods were employed to measure the antioxidant potential of two cultivars of Catharanthus. In the first method, antioxidant activity was estimated through Peroxide value and Thiobarbituric acid test. While in the second method, DPPH radical scavenging and ammonium thiocyanate method has been performed. Thermal stability of the flowers and leaves of the medicinal plant was also analysed by mixing 0.5,1.0 and 1.5 concentrations in sunflower oil. The findings of study depict that the flowers and leaves of Catharanthus alba and Catharanthus roseus exhibit good potential as natural based antioxidants to inhibit peroxidation induced by lipids. The order of antioxidant potential was found to be *Catharanthus alba* (Flower)> *Catharanthus roseus* (Flower) > *Catharanthus roseus* (Leaves) > *Catharanthus alba* (Leaves) when it was analyzed by 2, 2- Diphenyl- 1-picrylhydrazyl (DPPH) and linoleic acid peroxidation method. The leaves and flowers of *Catharanthus alba* and *Catharanthus roseus* exhibit good potential as natural-based antioxidants to inhibit peroxidation induced by lipids. They can replace the use of synthetic antioxidants.Introduction

Antioxidants are substances that can protect or reduce the destruction of cells induced by free radicals and unstable molecules produced by the body as a reaction to environmental and other pressures (Jug et al., 2021). They are known as 'free radicals' scavengers'. The DNA damage which depends on oxygen is due to the oxidation of phenolic compounds by a copper-redox cycle mechanism; it may be a significant factor in the mechanism of 1, 4-HQ & DNA damage in target cells by the effect of phenolic compounds (Li & Trush, 1994). Many observational analyses reveal that elevated consumption of food products high in natural antioxidants accelerates the antioxidant power of the plasma blood and decreases the possibility of diseases such as cancers, clogged arteries, and apoplexy. Various kinds of fragrant, spicy, and other categories of medicinal plants are well known to contain chemical compounds that represent high antioxidant properties and exhibit protective effects (Prior & Cao, 2000). The five indigenous rice bran varieties are evaluated for their antioxidant properties by the estimation of Total phenolic content, a linoleic acid system, which exhibits antioxidant activity, chelating ability by the metals, DPPH radical scavenging activity, ABTS cation radical & conjugated dienes (Iqbal et al., 2005).

Isoflavones, flavonoids, anthocyanin, Catechin, flavones, and other phenolics substances, exhibit antioxidant potential (Kähkönen et al., 1999) together with processes involving both metal chelation and free radical scavenging (Lien et al., 1999). The antioxidant capability of *Psidium guajava* leaves which is dried was extracted by water and aqueous ethyl alcohol 50% in the ratio (1:10), and the total phenolic content in the extracts of dried leaves was determined spectrophotometrically as per the Folin- Ciocalteu's phenol method and calculated as gallic acid equivalent (GAE) (El Khadem & Mohammed, 1958). Antioxidants can be obtained from natural as well as artificial sources. The genus of *Catharanthus* belongs to the flowering plants in the dogbane family known to be Apocyanaceae. The *Vinca* genus is a well-known synonym for *Catharanthus*, commonly known as periwinkles (WFO plant list). *Catharanthus* name is derived from the Greek word which means pure flower`. Two species of *Vinca,* including *Vinca major* and *V. minor*, are extensively cultivated as a flowering evergreen ornamental plants. The roots and leaves reportedly contain antineoplastic alkaloids, namely Vincristine and Vinblastine antihypertensive alkaloids such as ajmalicine, serpentine & reserpine (Mishra et al., 2001). In India, the plant is known assadaphuli meaning 'ALWAYS FLOWERING'. The tannin content of food products was determined utilizing immobilized proteins; the principles were selected for designing small-scale columns of sepharose-BSA to separate two categories of components (Hoff & Singleton, 1977).

Vegetable oils and fats are recognized as essential compounds in our diet. Oils & fats are oxidized very rapidly. To control this problem, synthetic antioxidants like Butylated hydroxyl anisole, butylated hydroxyl toluene, and tert – Butyl hydro quinine are well-known used as food additives (Raudsepp et al., 2013). A current study shows that the above compounds induce health hazards like inflammatory disorders and cardiovascular diseases. Concerning human health today, there is a need to replace these synthetic antioxidants with natural substances, namely natural antioxidants substances. Flavonoids and terpenoids, known as planted' secondary metabolites, play a crucial role in defense mechanisms against free radicals (Raudsepp et al., 2013). The data obtained from the leaves of *Coleus aromaticus* reflects the antioxidant potential of freeze-dried extract of *Coleus aromaticus*. The antioxidant potential of

Japanese knotweed rhizome bark extracts was analyzed by preparing eight different solvents mixtures using a 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (Jug et al., 2021). The elevated antioxidant effect in the buffered water infusion was analyzed with the barriers of blue honeysuckle. As the results are analyzed for different plant infusions, Siberian rhubarb roots exhibit excessive antibacterial activity against all bacterial species examined (Thanga Revathi & Joys Selva, 2018). Phenolic antioxidants can inhibit free radical formation & interrupt the propagation of autoxidation. Tea & grapes seeds extract and skins contain catechins, epicatechins, phenolic acids, proanthocyanidins, and resveratrol, contributing to antioxidative activity (Brewer, 2011). Phenolic acids and flavonoids exhibit antioxidant power due to their redox properties, along with the ability to chelate metals and quenching of singlet oxygen (Rice-Evans et al., 1996).

In the earlier studies, the antioxidant value of medicinal plants was estimated, but no one calculated their thermal stability in oil-based systems. In this paper, we have performed various experimental tests on the leaves and flowers of *Catharanthus roseus* and *Catharanthus alba,* which fill the gap for the conventional method of medicinal plants for their thermal stability in sunflower-based oil systems. Thus, these natural antioxidants may act as an alternative to synthetic antioxidants.

Materials and methods

The flowers and leaves of medicinal plants, namely *Catharanthus roseus* and *Catharanthus alba,* respectively, were collected from Defence Food Research Laboratory (Mysore), Karnataka, India. Collected flowers and leaves of medicinal plants were cleaned and aerated for surface moisture and microbial load removal. The entire sample for analysis is performed in triplicates to validate empirical data. Thus, the relative differences in data from the three samples can be measured and compared. It was then subjected to lyophilization at -110^{0}C. Dried samples were powdered, sealed, and stored in deep freezers at around 20 °C to preserve their texture and chemical properties for a longer time and further analysis.

Antioxidant activity estimation through the sunflower oil model system

Sample preparation

Powdered medicinal plants at different concentrations (0.5%, 1.0%, and 1.5%) were added to 100 gm of antioxidant-free sunflower oil. Samples were incubated at 37 °C for 40 days. A control sample was prepared by incubating pure oil without antioxidants, neither synthetic nor plant sample. TBHQ (Tert – Butyl Hydro Quinone) was employed as a reference synthetic antioxidant. TBHQ sample was prepared by adding 200 ppm of TBHQ to 100 gm of sunflower oil and incubated with the other samples. In order to estimate the antioxidant activity of selected medicinal plants, the oxidation of oil samples was followed through peroxide value and TBA value at a time interval of 10 days.

Peroxide value

Peroxide value was determined as per AOCS (1973) methods. About 4 gm of the sample was mixed with 20 ml of chloroform and 30 ml of glacial acetic acid. 1 ml of saturated potassium iodide was added to the mixture and incubated for 20 min. 50 ml of distilled water and 2–3 ml of the starch indicator were added to the mixture and titrated against 0.02 N sodium thiosulphate. The peroxide value was calculated based on the below-mentioned formula.

$$\text{Peroxide value}(PV) = \frac{(TV\ sample - TV\ blank) \times Normality\ of\ sodium\ thiosulphate \times 1000}{Weight\ of\ the\ sample} \quad (1)$$

Thiobarbituric acid test

10 gm of the sample was mixed with 20 ml of 0.67% aqueous Thiobarbituric acid solution (TBA) and 25 ml of benzene. The mixture was shaken for two h, and the aqueous layer was separated and collected. The collected aqueous layer was kept in a water bath for 35 minutes. Then it was cooled, and 0.D was measured at 540 nm. TBA value was calculated as mg malonaldehyde per sample using the below-mentioned formula.

$$TBA\left(\begin{matrix} mg\ malonaldehyde\ / \\ kg\ sample \end{matrix}\right) = \frac{O.D. \times 3.2}{0.15 \times sample\ weight} \quad (2)$$

Antioxidant activity estimation through assay method

Sample preparation

0.5 g of plant powder was mixed with 20 ml of methanol and subjected to shaking for 2 h. It was then filtered to remove the powder residues. The volume was collected, and the filtrate was made up to 50 ml with methanol and stored at 4 °C.

Ammonium thiocyanate method

A sample of 0.5 ml is added to 2.5 ml emulsion of Linoleic acid and a phosphate buffer solution of 2 ml. The above-mentioned mixture is then kept at the temperature of 37 °C for the incubation period. 0.1 ml of sample was taken from incubated samples at regular intervals, and the degree of oxidation was measured by sequentially adding 0.1 ml sample, 4.7 ml of ethanol, 0.1 ml of 30% ammonium thiocyanate, and 0.1 ml ferrous chloride. The mixture remained untouched for 3 minutes, and peroxidation was determined by reading absorbance at 500 nm. Control was performed with Linoleic acid without test samples. The percentage inhibition of oxidation by the sample was estimated by the following:

$$\%\ Inhibition = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample) \times 100}{Absorbance\ of\ control} \quad (3)$$

DPPH radical scavenging method

DPPH radical scavenging activity was measured according to the method (Braca et al., 2001). A methanolic solution of the amount of 5 ml prepared for DPPH was mixed with 100 µl of methanolic extract from the sample. The mixture was shaken vigorously and incubated for 30 min, and the OD was measured at 517 nm. Control was performed with methanol in place of test samples. The following formula determines the percentage inhibition of oxidation:

$$\%\ Inhibition = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample) \times 100}{Absorbance\ of\ control} \quad (4)$$

Thermal stability study of medicinal plants

The thermal stability of the leaves and flowers of *Catharanthus roseus and Catharanthus alba* were analyzed by mixing 0.5% powdered medicinal plant with 100 gm of sunflower oil. Mixed samples were incubated at 80 °C for a period of 24 h. A control sample was prepared by incubating pure oil without antioxidants, neither synthetic nor sample. TBHQ (tert – Butyl hydroquinone) was employed as a reference synthetic antioxidant. TBHQ sample was prepared by adding 200 ppm of TBHQ to 100 gm of sunflower oil and incubated with the other samples. Thermal stability was analyzed by following the oxidation trend of incubated oil samples through peroxide value and TBA value at a time interval of 8 h.

Statistical analysis

For the statistical analysis, both cultivars were tested in triplicate. Data were subjected to an analysis of variance (ANOVA) test using Minitab Statistical Software (Minitab Inc., USA).

Results and discussion

Antioxidant activity estimation through the sunflower oil model system

The effect of dehydrated powders of leaves and flowers of *Catharanthus alba* and *Catharanthus roseus* at 0.5, 1.0, and 1.5% levels on the rate of auto-oxidation in sunflower oil stored at 37 °C for 40 days in comparison to TBHQ is represented in Tables 1 and 2. The control sample free from antioxidants showed higher peroxide (PV) value and thiobarbituric acid (TBA) values than the samples containing dehydrated powders of the leaves and flowers of medicinal plants. *Catharanthus alba* flower showed comparatively reduced amounts of peroxide and malonaldehyde formation, followed by the flowers and leaves of *Catharanthus roseus* and *Catharanthus alba* leaves in sunflower oil during storage at 37^0C. The addition of flowers and leaves at a 0.5% level has shown a higher reduction in the oxidation of sunflower oil as measured by PV and TBA during storage for up to 40 days. An increase in concentration up to 1% level has not shown any effect in further retardation of oxidation during storage. The antioxidant power of higher plants has been demonstrated in in vitro experiments to protect against oxidative damage by reducing or quenching free radicals and reactive oxygen species. Some results reinforce the limited antioxidant activity effect in vivo conditions for various classes and subclasses of higher plant metabolites (Larson, 1988).

Antioxidant activity in sunflower oil at 80 °C

The effect of flowers and leaves of *Catharanthus roseus* and *Catharanthus alba* on peroxidation inhibition of sunflower oil at an elevated temperature of 80 °C during storage for 24 h are indicated in Table 3. The results showed that the flower of *Catharanthus alba* had shown higher thermal stability equivalent to that of TBHQ. The inhibition of oxidation for other

Table 1. Changes in peroxide value (PV, meq O2/kg fat) of *Catharanthus alba* (leaves and flower) as well as *Catharanthus roseus* (leaves and flower) powders in sunflower oil while storage at 37 °C.

	Concentration	10 Days	20 Days	30 Days	40 Days
Control		27.38± 0.25	60.46± 0.82	120.57± 0.48	212.05 ± 1.64
TBHQ	200 ppm	5.58± 0.58	15.88± 0.22	26.55± 0.60	35.82± 0.50
Catharanthus alba (leaves)	0.5	16.55± 0.21	43.42± 0.56	85.26± 0.74	156.58± 0.91
	1	15.74± 0.43	48.61± 0.70	90.19 ± 0.87	160.39± 0.95
	1.5	19.41± 0.37	55.34± 0.62	93.53 ± 0.76	169.38 ± 0.90
Catharanthus alba (flower)	0.5	14.19± 0.33	29.34 ± 0.78	59.53± 0.44	113.21 ± 0.81
	1	14.24± 0.12	35.71 ± 0.52	68.43± 0.43	125.15± 0.34
	1.5	15.16± 0.17	40.31 ± 0.52	65.67± 0.41	135.86± 1.34
Catharanthus roseus (leaves)	0.5	18.16± 0.44	40.05± 1.05	80.08± 0.49	147.11± 1.13
	1	20.01± 0.17	44.73± 0.43	85.42± 0.48	153.32± 1.00
	1.5	22.94 ± 0.25	49.81± 0.61	90.32± 0.59	159.37± 1.93
Catharanthus roseus (flower)	0.5	20.08± 0.14	36.32 ± 0.11	70.63± 0.75	139.06± 1.49
	1	22.39± 0.27	40.26± 0.84	74.28± 0.97	141.82± 0.89
	1.5	22.29± 0.64	44.98± 0.69	84.93± 0.67	150.39± 1.51

All values represented as mean + standard deviation ($n = 3$) are statistically significant ($p \leq 0.001$).

Table 2. Changes in thiobarbituric acid value (TBA, mg malonaldehyde/kg sample) of *Catharanthus alba* (leaves and flower) as well as *Catharanthus roseus* (leaves and flower) powders in sunflower oil during storage at 37 °C.

	Concentration	10 Days	20 Days	30 Days	40 Days
Control		0.23 ± 0.04	0.55 ± 0.11	0.94 ± 0.11	1.44 ± 0.07
TBHQ	200 ppm	0.17 ± 0.04	0.25 ± 0.05	0.44 ± 0.15	0.58 ± 0.08
Catharanthus alba (leaves)	0.5	0.19 ± 0.04	0.40 ± 0.06	0.65 ± 0.07	0.91 ± 0.09
	1	0.17 ± 0.03	0.44 ± 0.07	0.70 ± 0.06	1.05 ± 0.16
	1.5	0.21 ± 0.05	0.48 ± 0.06	0.70 ± 0.12	1.12 ± 0.19
Catharanthus alba (flower)	0.5	0.12 ± 0.04	0.27 ± 0.05	0.55 ± 0.07	0.77 ± 0.11
	1	0.13 ± 0.05	0.31 ± 0.03	0.60 ± 0.08	0.82 ± 0.12
	1.5	0.16 ± 0.05	0.33 ± 0.06	0.64 ± 0.06	0.96 ± 0.13
Catharanthus roseus (leaves)	0.5	0.19 ± 0.05	0.37 ± 0.06	0.62 ± 0.05	0.89 ± 0.14
	1	0.23 ± 0.05	0.41 ± 0.05	0.67 ± 0.07	0.93 ± 0.07
	1.5	0.22 ± 0.06	0.46 ± 0.06	0.72 ± 0.10	1.01 ± 0.14
Catharanthus roseus (flower)	0.5	0.17 ± 0.03	0.35 ± 0.06	0.58 ± 0.09	0.85 ± 0.09
	1	0.21 ± 0.03	0.41 ± 0.05	0.65 ± 0.08	0.90 ± 0.08
	1.5	0.22 ± 0.04	0.39 ± 0.04	0.71 ± 0.08	1.04 ± 0.17

All values represented as mean + standard deviation ($n = 3$) are statistically significant ($p \leq 0.001$).

Table 3. The effect of leaves and flowers of *Catharanthus roseus* and *Catharanthus alba* on peroxidation inhibition of sunflower oil at an elevated temperature of 80 °C during storage for 24 h.

	After 8 h	After 24 h
TBHQ	2.24 ± 0.12	2.34 ± 0.20
Catharanthus alba (Leaves)	1.51 ± 0.31	1.59 ± 0.16
Catharanthus alba (Flower)	2.04 ± 0.77	2.50 ± 0.27
Catharanthus roseus (Leaves)	1.5 ± 0.12	1.54 ± 0.08
Catharanthus roseus (Flower)	1.99 ± 0.16	2.17 ± 0.14

All values represented as mean + standard deviation ($n = 3$) are statistically significant ($p \leq 0.001$).

samples followed a similar pattern observed in different methods. The medicinal plant *Catharanthus* has shown good thermal stability when heated at 80 °C continuously for 24 h.

The data in Table 4 shows that antioxidant potential is estimated for these two cultivars. The reported order of antioxidant activity was *Catharanthus alba* (Flower) 1.98 ± 0.16> *Catharanthus roseus* (Flower) 1.62 ± 0.09> *Catharanthus roseus* (Leaves) 1.54 ± 0.09 >*Catharanthus alba*1.44 ± 0.09 *(*Leaves).

Table 4. Antioxidant activity calculated for *Catharanthus.*

	Concentration	Antioxidant potential
TBHQ	200 ppm	2.36 ± 0.17
Catharanthus alba (leaves)	0.5	1.44 ± 0.09
	1	1.37 ± 0.06
	1.5	1.26 ± 0.07
Catharanthus alba (flower)	0.5	1.98 ± 0.16
	1	1.79 ± 0.09
	1.5	1.64 ± 0.07
Catharanthus roseus (leaves)	0.5	1.54 ± 0.09
	1	1.41 ± 0.09
	1.5	1.33 ± 0.05
Catharanthus roseus (flower)	0.5	1.62 ± 0.09
	1	1.37 ± 0.06
	1.5	1.39 ± 0.07

All values represented as mean + standard deviation ($n = 3$) are statistically significant ($p \leq 0.001$).

Antioxidant activity by using DPPH radical scavenging method

The radical scavenging activity for two cultivars of *Catharanthus* is determined by DPPH methods and compared with the synthetic antioxidant TBHQ (Figure 1). Flowers of *Catharanthus alba* and *Catharanthus roseus* have shown radical scavenging

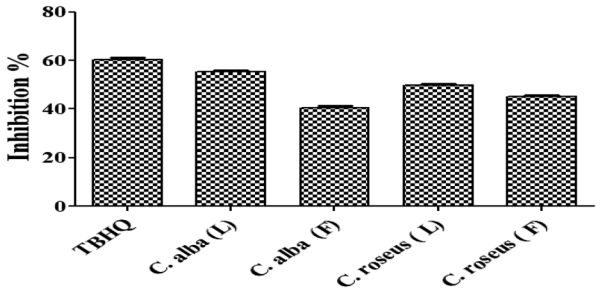

Figure 1. The antioxidant activity of the two cultivars was analyzed by DPPH radical scavenging method and compared with the synthetic antioxidant TBHQ.

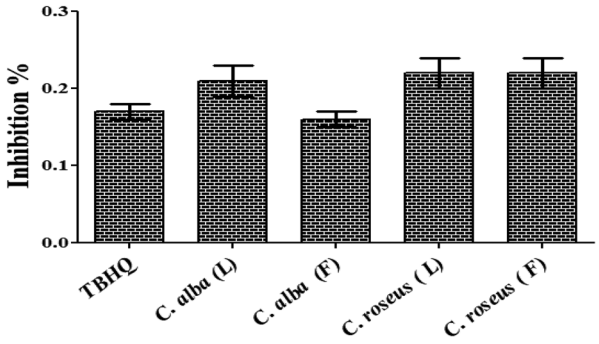

Figure 2. The antioxidant activity estimation is done by the linoleic acid peroxidation method. The order of inhibition of peroxidation has been found more in *Catharanthus alba (*flower) followed by *Catharanthus roseus* (flower), *Catharanthus roseus* (leaves), and *Catharanthus alba (*leaves), respectively.

activity similar to the activity of TBHQ. In contrast, among leaves, the leaves of *Catharanthus roseus* have shown slightly more radical scavenging activity than the leaves of *Catharanthus alba*. The ethanolic extracts of the roots of periwinkle cultivars extracts show good scavenging effects in all the radical scavenging assays, but *Catharanthus roseus* shows more antioxidant activity than *Catharanthus alba* (Bhutkar & Bhise, 2011). The roots' antioxidant activity was analyzed by applying DPPH, superoxide radical & nitric oxide radical. *Catharanthus roseus's roots* can be an antioxidant that promotes good health in various food and pharma industries (Pereira et al., 2010).

Between nonedible plant materials, high potential activities were supposed to be found in tree materials, chiefly in willow herb & meadowsweet. Additionally, peels of potato and beetroot extracts have exhibited good antioxidant potential (Kähkönen et al., 1999). Different phytochemicals and the antioxidant potential of various extracts of *Catharanthus roseus* were carried out by various chemical & spectroscopic methods. Water extract was found to have more power among different plant extracts as per the phytochemical analysis (Mir et al., 2018).

Antioxidant activity calculated by linoleic acid peroxidation method

The antioxidant activity, as determined by linoleic acid peroxidation (Figure 2), has shown a similar trend in peroxidation inhibition as observed by other methods. In this case, also, the order of inhibition of peroxidation has found more in *Catharanthus alba (*flower) followed by *Catharanthus roseus* (flower), *Catharanthus roseus* (leaves), and *Catharanthus alba (*leaves), respectively. The percentages of inhibition for peroxidation were almost similar, with slight variations. The results also showed that various plant-based food products and different types of beverages contain tannins which are predominantly worked as a biological antioxidant compound (Hagerman et al., 1998).

The comparatively higher antioxidant activity shown by the flower of *Catharanthus alba* by all the methods is due to more phenols and flavonoids. The antioxidant potential for the flower and leaves of *Catharanthus roseus* and *Catharanthus alba* also depends on the amount of phenols and flavonoids present in these two cultivars. The flower of *Catharanthus roseus* contained appreciable levels of antioxidant potential at various concentrations (Thanga Revathi & Joys Selva, 2018). Comparative studies were carried out on the general parameters of flowers & leaves of *Catharanthus alba* and *Catharanthus roseus*. The results showed that flowers of both cultivars compromise an astounding number of phenolic & flavonoid compounds than leaves (Sharma et al., 2017).

Conclusion

The dehydrated powders of leaves and flowers of *Catharanthus alba* and *Catharanthus roseus* have shown good antioxidant potential at 0.5% level in sunflower oil. The increase in concentration up to 1% and 1.5% has not enhanced antioxidant activity in sunflower oil. The flowers and leaves of both cultivars of *Catharanthus* have shown good thermal stability when heated at 80° C continuously for 24 h. However, the flower of *Catharanthus alba* has shown good thermal stability equivalent to the activity of synthetic antioxidant TBHQ. The order of antioxidant potential was found to be *Catharanthus alba* (Flower)> *Catharanthus roseus* (Flower) > *Catharanthus roseus* (Leaves) > *Catharanthus alba* (Leaves) when it was analyzed by 2, 2- Diphenyl- 1-picrylhydrazyl (DPPH)

and linoleic acid peroxidation method. The leaves and flowers of *Catharanthus alba* and *Catharanthus roseus* exhibit good potential as natural-based antioxidants to inhibit peroxidation induced by lipids. They can replace the use of synthetic antioxidants.

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Author contributions

Conceptualization, Y.K.A., R.B.S., K.D., S.M., and P.S.; methodology, Y.K.A., R.B.S., A.T., S.R.; software, M.K; validation, S.T., S.M., P.S.; formal analysis, S.M and J.A. ; investigation, S.M and P.S.; resources, M.K., S.T., data curation, A.T. and J.A.,.; writing—original draft preparation, Y.K.A., R.B.S., K.D.; writing—review and editing,; supervision, Y.K.A., R.B.S., S.M., P.S ; project administration, S.M., S.R., and P.S ; funding acquisition, P.S All authors have read and agreed to the published version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s)

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