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Extraction, Estimation and Utilization of Curry Leaf (*Murraya koenigii*) Stalks as a Potential Source of Antioxidants

Sai Geetha Pyla[1], Kore Jahnavi[1], Yamuna Sagubandi[1], Ambati Padmaja[1]

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ABSTRACT

Background: Processing wastes that are generated at industrial and house hold level are a huge concern. Utilization of these by-products and their bioactive compounds is a blooming industry at present. This study has been designed to unleash the potential of such by-product (stalks) and their possible application.

Methods: In order to use plant by-products as a potential source of antioxidants, the methanolic, aqueous methanolic and aqueous extracts of the curry leaf stalks were studied. The nutrient, anti-nutrient, phytochemical content and *in-vitro* antioxidant activities were assessed for the stalks. The plant extracts were then incorporated in sunflower oil and tested for stability of oil upon repetitive deep frying.

Result: Total phenolic content and flavonoids followed the order of aqueous methanolic>aqueous > methanolic extracts. The phytochemical analysis of the extracts for total flavanols followed the order aqueous >aqueous methanolic > methanolic. *In vitro* antioxidant activities of the extracts in DPPH, FRAP, reducing power and CUPRAC assays followed the order aqueous methanolic> methanolic> aqueous extracts. Therefore, aqueous methanol is a suitable solvent among the three solvents for extracting most active components with higher antioxidant activity from the stalks. Findings from the present study infer that Curry leaf stalks can be a potential source of natural antioxidants and can be used in foods and pharmacological formulas.

Key words: Antioxidant activity, By-products, Curry leaf, SFO, Stability studies, Stalk.

INTRODUCTION

The reusable plant wastes of good market value are termed as ‘by-products’ (Sánchez-Zapata *et al*., 2009). Plant by-products occurring at various stages of processing at home scale and industrial scale have attracted many technical and health professionals. Their ecological impact on the planet due to the increase in waste generation has become a matter of great concern. Disposal of these wastes under Government regulations could cost huge amounts (Gowe, 2015). The idealistic solution for this problem is utilization and has become immensely popular to overcome this issue (Zhao *et al*., 2012). The increasing awareness on the role of food processing by-products, in particular plants, makes it necessary to explore and review the benefits in relation to human well-being. Usually, the plant by-products contain a good source of carbohydrates and bioactive molecules such as proteins, vitamins, minerals and antioxidants (Grigoraş Cristina-Gabriela *et al*., 2012). The bioactive compounds and fiber may be extracted or recovered from food processing by-products and incorporated into foods as natural food additives, colorants and food preservatives. In addition, they can be a vital inclusion in pharmacological formulas and plant-based supplements providing nutritional and economic benefits. Estimation, extraction, quantification and utilization of these compounds and assessment of their effects have become a key research area in the process of finding antioxidant, anticancer, anti-inflammatory and antimicrobial properties.

Green leafy vegetables are excellent sources of natural antioxidants such as polyphenols, tocopherol, Vitamin C and are known for their radical scavenging activity. Curry leaf is one such, among the medicinally important plants aiding in disease mitigation and has various biologically active compounds present in it. The phyto constituents isolated from the leaves are coumarin glycoside like scopotin murrayanine (Onayade and Adebajo, 2000), O-methyl mahanine (Tachibana *et al*., 2003). The by-products of curry leaves processing are the stems, stalks, flowers, fruits, *etc*. However, there are very limited studies on the various parts of the plant unleashing their potential as natural antioxidants.

The present study was designed to analyze and compare the methanolic, aqueous methanolic and aqueous extracts of curry leaf stalks (CS) for nutritional, mineral, anti-nutrients and quantitative analysis of phytochemicals and *in vitro* antioxidant activities.

[1]Department of Food and Nutritional Sciences, Sri Sathya Sai Institute of Higher Learning, Anantapur-515 001, Andhra Pradesh, India.

Corresponding Author: Ambati Padmaja, Department of Food and Nutritional Sciences, Sri Sathya Sai Institute of Higher Learning, Anantapur-515 001, Andhra Pradesh, India.
Email: ambatipadmaja@sssihl.edu.in

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MATERIALS AND METHODS

The work was carried out as a part of M.Sc. academic project work in Sri Sathya Sai Institute of Higher Learning, Anantapur from July 2022-April 2023.

Procurement and processing of samples

Curry leaf stalks were procured from hostel kitchen of Sri Sathya Sai Institute of Higher Learning, Anantapur Campus andhra Pradesh. The stalks were cleaned, washed, cut into desired size and dried in food dehydrator. They were pulverised and then stored for further processing.

Nutrient analysis of CS

The nutrient analysis of CS was carried out by adopting the standard procedures. Moisture content (AOAC, 2012), Protein (Tulin's KjelTRON based on kjeldhal principle), Fat content (AOAC, 2012), Fibre content (Tulin's FibroTRON), Ash content (AOAC, 2012), Iron and calcium content (Raghuramulu, 2003) and Phosphorus (AOAC, 2012) were carried out.

Extraction of active components from CS

Three solvents, methanol, aqueous methanol (Sultana *et al*., 2009) and aqueous (Kaneria *et al*., 2012) were taken for extraction, extracts were concentrated separately using Rotary evaporator (Superfit Rotavap Model: PBU-6D) and solvent free powders were stored at refrigerated temperatures.

Phytochemical analysis of CS

Curry leaf stalk extract was assessed for total phenolic content (Singleton and Rossi, 1965), total flavonoids (Chang *et al*., 2002), total flavanols (Kumaran and Karunakaran 2006) and total carotenoids (Raghuramulu *et al*., 2003).

In vitro antioxidant assessment of CS

To assess the antioxidant potential of CS extract, 2, 2-diphenyl-1-picyl hydrazyl (DPPH) radical scavenging activity (Sreejayan and Rao (1996), Reducing power Oyaizu (1986), Ferric reducing antioxidant power Pellegrini *et al*. (2003), CUPRAC (Apak, 2017) were carried out.

Incorporation of sample extracts into edible oils

The aqueous methanolic extract of CS was incorporated into additive free refined sunflower oil. For comparison, standard (TBHQ) and control (without any additive) were assessed, by repetitive deep frying. The standard and the extracts were incorporated at the level of 200 ppm in sunflower oil.

Deep frying of incorporated oils

The extracts of sample which proved to be best in terms of TPC and antioxidant assays were incorporated into the SFO. Hence the aqueous methanolic extract of CS was taken in comparison with a standard (TBHQ) and control (without any additives) to study the efficiency in SFO.

Stability studies

To test the stability of oils during frying, two types of foods *i.e*., high moisture, Mc Cain's French fries and dehydrated foods, Fryums, were taken. SFO was subjected to frying of selected foods and batches of products were fried with five-minute interval to test the effect of repetitive frying on the oil composition. This procedure was followed for three days and each day the samples were assessed for colour, refractive index, Acid value and Thiobarbituric acid.

Assessment of SFO

Deep fried SFO was tested for colour (Lovibond Tintometer), RI (Abbe's Refractometer), Acid Value (Raghuramulu *et al*., 2004) and Thiobarbituric acid value (Pokorny and Dieffenbacher, 1989).

Statistical analysis

The analysis of samples was carried out in triplicate. Values were expressed as means of three independent samples analysed in triplicate ± standard deviation. The results obtained were subjected to one-way, two-way analysis of variance (ANOVA) and the significance between the means was calculated. The data analysis was done using Microsoft Excel Software 2019.

RESULTS AND DISCUSSION

Nutrient analysis

Proximate analysis is the quantitative analysis done to estimate macromolecules which are present in foods. Under nutrient analysis, moisture, fibre, protein, fat, ash and mineral contents of CS were estimated.

Moisture, fibre, protein, fat, ash of sample was estimated to be 63.42%, 5.47%, 7.12% and 4.32% respectively.

The calcium, iron and phosphorus content of the CS were 150 mg/100 g, 6.27 mg/100 g and 120.35 mg/100 g respectively. Total carotenoid, which could be responsible of antioxidant activity was found to be 2100 µg/100 gm of CS.

Total phenolic content

Phenolics are the principal compounds that are present in plants which have hydroxyl functional groups that have redox potential which are responsible for activity of antioxidants. Total phenolics were measured using Folin-Ciocalteau reagent and the results derived were expressed in terms of Gallic acid equivalent (GAE). As shown in Fig 1, AMCS had higher TPC of 4516.7 mg GAE/100 gm. Followed by ACS 3783.0 mg GAE/100 gm and MCS 2738.9 mg GAE/100 gm. The decreasing order of TPC of the three solvent extracts of the sample was: AMCS>ACS>MCS.

Flavonoids

Flavonoids are the class of secondary plant metabolites which have great importance due to presence of low molecular weight compounds that have polyphenolic structure present in vegetables and fruits. Its subgroups are flavanols, isoflavones, chalcones and flavones. Flavonoid content is expressed in terms of quercetin equivalents. (Panche *et al*., 2016). AMCS had the highest flavonoid content of 3640 mg QE/100 gm followed by ACS with 3356.6 mg QE/100 g and MCS with 2666.6 mg QE/100 gm.

The decreasing order of flavonoid content of the three solvent extracts of the sample was: AMCS>ACS>MCS.

Flavanols

Flavanols are the sub class of flavonoids which has alcohol as the major functional group. They are also antioxidants that had potency to scavenge free radicals. MCS had the highest flavonol content of 733.33 mg/100 gm followed by AMCS with 513.6 mg/100 g and ACS with 480.6 mg/100 gm. The decreasing order of flavonoid content of the three solvent extracts of the sample was: MCS>AMCS>ACS.

Assessment of *in vitro* antioxidant activity of extracts

Highly reactive oxygen species, which contain a lone unpaired electron creates oxidative stresses, which results in degradation of foods and induces various unwanted reactions like oxidation of lipids which causes rancidity in foods (Aryal *et al*., 2019). Antioxidants are those substances which scavenge the free radicals that are produced during oxidation process and helps in preventing/slowing down the deterioration of foods by reducing the oxidative stress on them. The stalks contain good amount of polyphenols which acted as antioxidants. Many studies proved that natural antioxidants are more effective than synthetic antioxidants like BHT, BHA, TBHQ which are commonly used to prevent oxidation in foods (Al-Weshahy and Rao, 2012). Thus, antioxidant assays were conducted to know the efficiency of the extracts that have the ability to decolourize in DDPH or develop coloured compounds in FRAP, CUPRAC, reducing power based on the type of assay used.

DPPH radical scavenging activity

It is a colorimetric assay which helps in measuring the radical scavenging capacity of an antioxidant. DPPH is a stable organic nitrogen radical which is long lived and when mixed in solvent forms deep purple solution which turns to yellow upon reacting with antioxidants by forming 2,2-diphenyl-1-picrylhydrazine (Kaneria *et al*., 2012). DPPH radical scavenging activity of different extracts of CS was dose dependent and depicted in Fig 2.

Reducing power

Reducing power is used to assess the antioxidant activity of the extract by reducing the Fe^{3+} ion in ferric/ferricyanide complex into the ferrous (Fe^{2+}) form (Jayanthi and Lalitha, 2011b). Based on the reducing power of extracts, the test solution's yellow colour changed into various shades of blue and green, which was measured at 700nm to measure the concentration of ferrous ions (Jayanthi and Lalitha, 2011a). In all the three different solvent extracts, reducing power was dose dependent. AMCS has shown the highest reducing power and aqueous extracts have the least reducing power as shown in Fig 3.

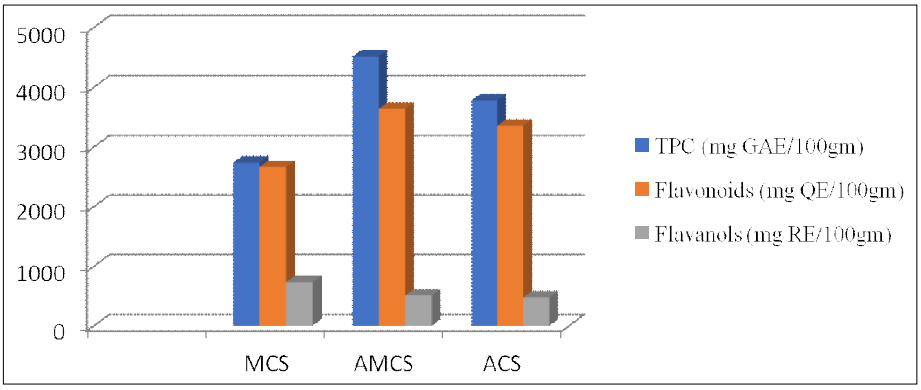

Fig 1: Phytochemical composition of CS.

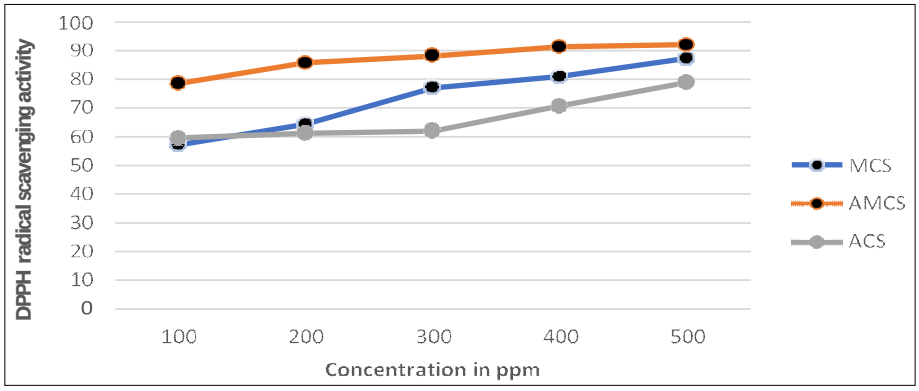

Fig 2: DPPH radical scavenging activity of CS.

CUPRAC

The CUPRAC assay is based on reduction of cupric complex present in neocuprin to copper form by antioxidants present in the samples (Multescu *et.al*., 2022). The AMCS (457mg/g of extract) was having the highest activity followed by MCS (424 mg/g of extract) and ACS (261 mg/g of extract).

FRAP

In this antioxidant assay, Fe2+-TPTZ (2,4,6-tri (2-pyridyl)-1,3,5 - triazine) is formed due to reduction of Fe^{+3}-TPTZ by antioxidants. The formed Fe^{+2} ions then bind with ligands to give intense navy-blue colour, which was measured at 600nm (Xiao *et al*., 2020). The ferric reducing activity of CS extracts was: MCS (48.47 mg BHTE/g)>AMCS (39.45 mg BHTE/g)>ACS (19.8 mg BHTE/g).

Stability of SFO

Deep fat frying is a complex frying process where heat and mass transfer occurs between the food fried and frying medium simultaneously. Apart from these many physico-chemical changes occur in both oil and food products.

Quality of the frying oil is adversely affected by two main reactions, thermo-oxidative and hydrolytic. Using the same oil repeatedly for frying without changing the oil or adding fresh oil will affect both the shelf life of the food product and indirectly increases the rancidity of the oils. Addition of antioxidants will slow down this degradation of oils.

To study these frozen French fries, a moisture dense product and fryums, a low moisture product was selected as the moisture content of the food being fried also affects the degradation velocity of oil.

Frying was done at 170°C for 4 times with a timelapse of 5 minutes for 3 days this was done to both French fries and fryums. Each day samples were collected and analysed for different parameters like acid value, TBA value, colour and refractive index.

Acid value

Acid value of SFO used for frying HM and LM foods is represented in Fig 4. Day 1 control HM had shown a high acid value of 0.558. TBHQ HM and AMGSHM both have showed an acid value of 0.451 Control LM sample of day 1 had shown an acid value of 0.561 which was high among all samples and AMCS showed 0.537 higher than TBHQ.

Day 2 control HM had shown a higher value of 1.423. Standard TBHQ HM had an excellent effect which resulted in a low acid value of 0.471. AMCS had a high acid value of 1.243. Acid value of control LM was 1.52 which was high found on day 2. TBHQ had shown great effect in retarding formation of value of 0.51.

Day 3 control HM had shown a greater acid value of 1.51 because of repeated frying. AMCS had shown less acid value compared to TBHQ. Due to repeated usage of oil for frying on day 3 control LM acid value had raised up to 1.58.

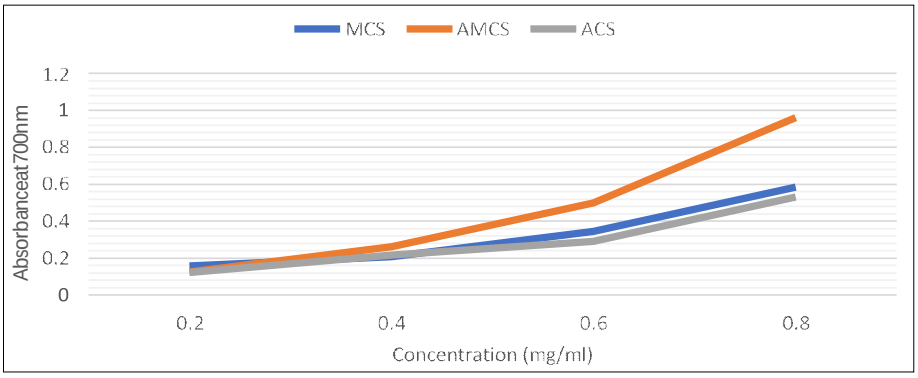

Fig 3: Reducing power of MCS, AMCS and ACS.

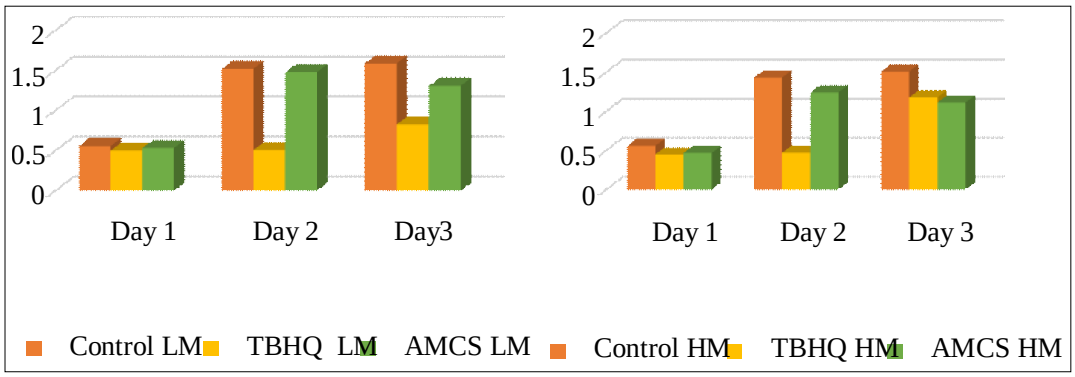

Fig 4: Acid value of LM and HMSFO.

Standard TBHQ had shown its tremendous effect in preventing the formation of FFA resulted in low acid of 0.82. AMCS showed 1.312 which is visibly higher than TBHQ.

TBA value

When oils are exposed to high temperatures and oxygen then autoxidation of the lipids occur resulting in formation of monoaldehyde, a 3-carbon compound that is a major carbonyl compound formed during the decomposition process.

So, detection of this monoaldehyde is possible with the help of TBA test where TBA value is found using TBA reagent where colour developed is read at 538 nm and results are shown in Fig 5.

Day 1 control HM had shown a high value of TBA of 0.78. AMCS HM had shown a low TBA value of 0.39 and TBHQ HM had shown a TBA value of 0.468. Day 1 control LM showed the highest TBA values of 0.515 followed by TBHQ with 0.468 and AMCS showed the least TBA value of 0.39.

Day 2 control HM showed a significant increase to 0.89. TBHQHM and AMCSHM were 0.483 and 0.468 respectively. Day 2 control LM showed TBA value of 0.759. AMCS showed less TBA value than TBHQ.

Day 3 control HM had a drastic increase from the previous day with value 1.46. AMCS HM had efficiently prevented the formation of MDA and thus showed less TBA value of 0.468 and TBHQ showed 0.624. Day 3 control LM had TBA values of 0.858 and TBHQ had similar values. AMCS again exerted a great effect on the oil by having low TBA of 0.39.

Refractive index (RI) of oils

RI can be used to determine the quality of frying oils. Formation of conjugated dienes, conjugated trienes and non-volatile carbonyl compounds during frying elevates the RI of oils. Antioxidants help in preventing the formation of these compounds. Therefore, it maintains the RI fairly stable. The results of the refractive index in the current study are represented in the Fig 6.

Refractive index of sample extract incorporated oils, Standard (TBHQ) and control (no additives added) were assessed after frying each day for 3 days. Control and TBHQ significantly showed the increase in RI each day and they were maximum on Day-3 for both, high moisture and low moisture foods were fried. Whereas, the sample incorporated oils showed a gradual and minimal increase, inferring that they affected the oil parameters positively by minimal increase in the Refractive index. Control and standard exceeded the limit of RI (1.470-1.476), as specified by Swern (1964) (Goburdhun and Jhurree, 1995) but AMCS was within the limits specified.

Color of the oils

The color of oils was analyzed after frying each day for three days to the sample incorporated, standard (TBHQ) and control oils.

Among all the oils, control showed the most increase in color followed by TBHQ. Although the color of oils with high moisture foods fried in them had similar values, the

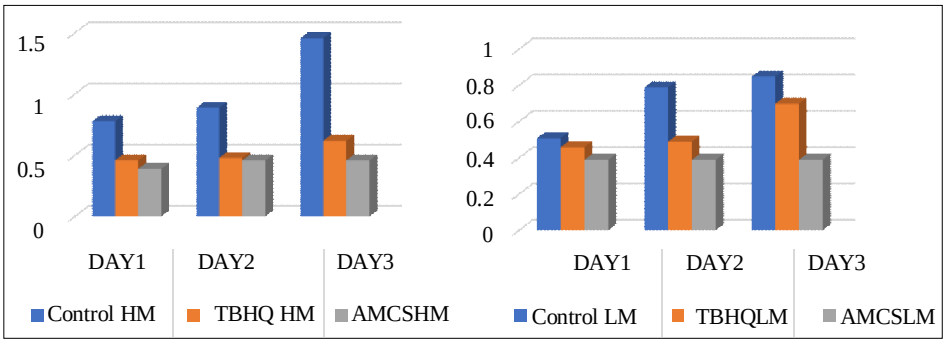

Fig 5: TBA value of LM and HM of SFO.

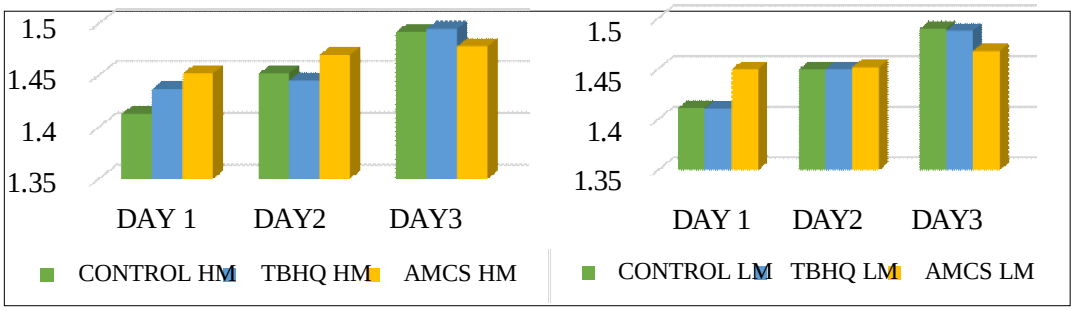

Fig 6: Refractive index of HM and LM foods fried SFO with extracts in comparison with control and synthetic antioxidants.

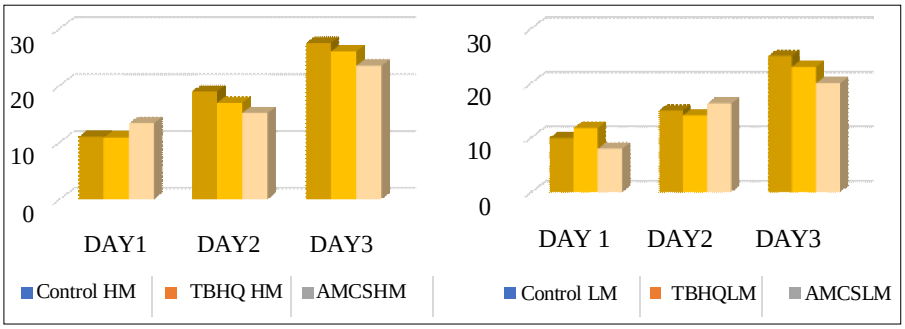

Fig 7: Color of HM and LM foods fried SFO with extracts in comparison with control and synthetic antioxidants.

low moisture fried oils were considerably less. Results of colour of SFO, as shown in Fig 7, also deciphers that the sample incorporated oils had protected the color of oils compared to TBHQ incorporated and control oils. The color of 3rd day oils had shown the deep color development due to repetitive frying. The significant increase in the R and Y values may be because of the reaction of metal ions during the frying process (Li *et al*., 2021). In preliminary comparative studies conducted by Sulieman *et al*. (2006) on the antiradical performance and physicochemical characteristics of vegetable oil upon frying of French fries, the gradual increase in darkness of the oil was observed during the frying period (Nayak *et al*., 2016).

CONCLUSION

The main objective of the study show that the underutilized stalks of curry leaves can be a potential source of natural antioxidants. The various antioxidant assays have revealed the potent antioxidant potential of CS extracts. The application of the CS extracts in oil and the effects on repetitive frying are positive. The various parameters like colour, RI, acid value and TBA values have shown desirable and advantageous values for AMCS when compared to standard synthetic antioxidants. This infers that, CS has a promising future in the field of food additives and pharmacological formulas. Due to the increasing concerns with synthetic antioxidants, underutilized plant by-products like CS can be explored in new light. It is in the quest of finding alternative solutions for cancerous synthetic antioxidants, the plant sources in specific, the by-products can be viable means of antioxidants.

Conflict of interest

On behalf of all the authors, I state that there is no conflict of interest in the work which is submitted in manuscript.

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