***In-vitro* evaluation of bioactive compounds, anti-oxidant, lipid peroxidation and lipoxygenase inhibitory potential of *Citrus karna* L. peel extract**

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Abstract Many medicinal plants have been studied for their antioxidant and their pharmacological activity. *Citrus* species were well documented as potential antioxidant based therapy for cancer, inflammation, heart disease. *Citrus* seeds and peels have been shown to possess high antioxidant activity. Therefore, the present study to explore the antioxidant and lipid peroxidation & lipoxygenase inhibitory action of *Citrus karna* peel extracts were undertaken. Extraction was performed with different solvents of increasing polarity and yield was calculated. Peel extracts were also analyzed for the presence of phenols, flavonoids, vitamin C, and carotenoids. Then the *Citrus karna* peel extracts were evaluated for the antioxidant and lipid peroxidation & lipoxygenase inhibitory action In-Vitro. In further, the quantification of hesperidin and naringin was carried out by HPLC-DAD method. The results indicated the presence of phenols, flavonoids, vitamin C, carotenoids, hesperidin and naringin in *Citrus karna* peel extracts with maximum yield of (3.91% w/w). *Citrus karna* peel extracts were also found to have potential antioxidant and lipid peroxidation & lipoxygenase inhibitory action. Therefore, *Citrus karna* peel extracts could be used for the future therapeutic medicine due to presence of potential bioactive compounds.

Keywords *Citrus karna* · Naringin · Hesperidin · Free radical · Lipoxygenase

Introduction

Our body is bombarded daily with health hazards and irritants in the form of air pollutants, synthetic drugs, cigarette smoke and additives in the food. These are highly toxins associated with free radical generation in the cellular systems which can cause alteration of cellular function due to the changes in cell membrane, cytosolic enzymes, nuclear factor, gene expression in the human and animal body (Smith et al. 2009). Indeed, various herbal origins have reported to play a potential role in the pharmacological studies related to various oxidative disorders such as diabetes & its complication, obesity, asthma, Parkinson's disease, hepatotoxicity, and peptic ulcer (Lee et al. 2010; Watcho et al. 2010).

Many medicinal plants have been studied for their antioxidant activity such as *Ocimum sanctum*, *Artemisia dracunculus*, *Withania Somnifera* and *Citrus* peels (Das et al. 2010; Muthuraman et al. 2008; Watcho et al. 2010; Oboh and Ademosun 2011). Further, *Citrus* fruits are well documented as potential pharmacological action i.e., cancer, inflammation, heart disease etc. *Citrus* fruits or juice are widely used as potential health drink and it's also used for the ameliorations of certain diseases (Joshi-pura et al. 2001). *Citrus* juices are considered to be a rich source of anti-oxidants including flavonoids, vitamin C, phenolic compounds, carotenoids, limonoids and pectin which are responsible for their health benefits (Hamdan et al. 2010; Oboh and Ademosun 2011). However, most of the people throw away the peels and seeds after enjoying *Citrus* fruit, which is a huge waste. Even during the processing of

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Citrus fruit or juice in food industries, peels are the primary byproducts (Sood et al. 2009). *Citrus* seeds and peels have been shown to possess high anti-oxidant activity (Sood et al. 2010; Oboh and Ademosun 2011). Some authors have stressed the main role of hesperidin in the total anti-oxidant capacity of *Citrus* juices (Dhuique-Mayer et al. 2005). Our previous studies have experimentally proved that *Citrus decumana* peel extract possess anti-oxidant anti-inflammatory and ameliorative effect on stress induced peptic ulcer in rats (Sood et al. 2009, 2010). Other than anti-oxidant status, lipid peroxidation process is one of the major events in the acute and chronic disorder (Eleni and Dailianis 2010; Oboh and Ademosun 2011; Luzia and Jorge 2011). However, little information is available on the anti-oxidant and anti-lipid peroxidation potential of *Citrus karna* peels. Therefore, this study attempt to explore the phytochemical analysis and assessment of anti-oxidant & lipid peroxidation inhibitory activities of *Citrus karna* peel extracts *in-vitro*.

Materials and methods

Chemicals and reagents Gallic acid, butylated hydroxyanisole (BHA), adenosine diphosphate (ADP), ascorbic acid, soybean lipoxygenase and linoleic acid were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Himedia Laboratories Limited (Mumbai, India). All other chemicals used were of analytical grade. Naringin and hesperidin were purchased from the National Institute for the Control of Pharmaceutical and Biological Products in China. All other reagents were used as analytical grade.

Plant materials The fruits of *Citrus karna* were collected from northern region of India. The plant material was identified and authenticated in the P. G. Department of Horticulture, Khalsa College, Amritsar (Voucher No. HD-1108). The peels were removed manually and dried under shade at room temperature. The dried peels were grounded into a coarse powder in a mixer. The powder was sieved through a 1 mm metal sieve to achieve a standard size of particles.

Extraction The extraction process was carried out on powdered material by maceration method. Dry powder peel (100 g) was extracted twice with methanol (600 ml) for 3 days. The slurry obtained was filtered and combined methanolic extract were dried at rotary evaporator (Medica Instrument Mfg. Co. India) under reduced pressure. Water (60 ml) was added to concentrated resulting extract and obtained mixture was heated at 70°C. The aqueous extract

was then partitioned twice with different solvents of increasing polarity (i.e. hexane, dichloromethane, ethyl-acetate and n-butanol). The % w/w yield of extracts from the peels of *Citrus karna* was calculated.

Phytochemical screening

Determination of total phenolic content Total phenolic contents of the peels of *Citrus karna* extracts were measured by using a modified colorimetric method (Harborne 1973). Four (4) ml of distilled water and 0.5 ml of *Citrus karna* peel extract were placed in a test tube. Folin-Ciocalteu reagent (0.5 ml) was added to the solution and allowed to react for 3 min. The reaction was neutralized with 1 ml of saturated sodium carbonate. Absorbance at 760 nm was read after 2 h, using a spectrophotometer (Beckman Coulter DU-800, USA). Chlorogenic acid was used as standard and data were expressed as mg chlorogenic acid equivalents (CAE)/100 g of *Citrus karna* peel for the triplicate extracts. The values were expressed as mean±SD.

Determination of total flavonoid content The total flavonoid content of the peels of *Citrus karna* extracts were measured using a modified colorimetric method (Chen et al. 2010). A total of 4 ml of each sample extraction was pipetted into a 10 ml volumetric flask. The solution was treated with 0.40 ml of the 5% NaNO₂ solution for 6 min and evenly mixed, into which 0.4 ml of the 10% Al (NO₃)₃ solution was added and shaken up; then 6 min later, 4 ml of the 4% NaOH solution was added to it. The mixture was diluted to the volume with double distilled water, and allowed to stand for 15 min before analyzing against the blank solution. Absorbance at 420 nm was measured after 10 min of incubation at 40°C using a spectrophotometer (Beckman Coulter DU-800, USA). Rutin was used as the standard and total flavonoid content was expressed as mg rutin equivalents (RE)/100 g of *Citrus karna* peel for the triplicate extracts. The values were expressed as mean±SD.

Determination of vitamin C content The Vitamin C content of the peels of *Citrus karna* extracts were measured by using a reverse phase HPLC method (Harborne 1973) with slight modifications. Briefly, vitamin C in *Citrus karna* peel extracts treated with 10 ml of 1% (w/v) oxalic acid. After sonication (10 min) and centrifugation (10,000 grpm for 10 min at 4°C), the supernatant was injected into a reversed phase HPLC system with UV detection (Cyberlab Corporation, USA). The conditions of the system were: C18 column (4.6 X 250 mm), flow rate of 1.0 ml/min; injection volume of 20 µl; detector wavelength set

at 251 nm. Ammonium acetate buffer (0.02 M, pH 5.4) with 1 mM of octylamine (dissolved in methanol) was used as mobile phase. The vitamin C content was expressed as mg /100 g of *Citrus karna* peel for the triplicate extracts. The values were expressed as mean $\pm$ SD.

Determination of carotenoid content Total carotenoids content of the peels of *Citrus karna* extracts were determined by using the colorimetric method (Harborne 1973) with slight modifications. Four (4) ml of chloroform were added to a test tube containing 1 g of ground fruit tissue and 2 ml of methanol. Then, 2 ml of 10% sodium chloride were added and vortexed. The mixture was centrifuged at 8000 g for 15 min at 4°C and the coloured chloroform layer was collected in a new tube. The chloroform extraction was repeated three times until the chloroform layer became colourless. The chloroform layers were pooled and evaporated to dryness in a rotary evaporator. The residue was resuspended in 2 ml of 6% methanolic KOH solution (w/v) and samples were saponified for 30 min at 60°C. The samples were cooled in an ice bath and 4 ml of chloroform was added to each tube. The suspensions were washed thrice with 4 ml of water by vigorous vortexing, followed by centrifugation at 8000 g for 15 min at 4°C. Then chloroform layer was carefully separated and absorbance at 450 nm was measured using a spectrophotometer (Beckman Coulter DU-800, USA). The carotenoid content was expressed as mg β -carotene equivalents (BCE)/100 g of *Citrus karna* peel for the triplicate extracts. The values were expressed as mean $\pm$ SD.

Identification and determination of naringin and hesperidin content *Citrus* fruits are known to be a rich source of flavonoids. High performance liquid chromatography with diode-array detection (HPLC-DAD) technique, using either normal or reverse phase column has been used to identify and quantify these flavonoids in *Citrus* species (Hamdan et al. 2010). The crude extracts were analyzed for alkaloids, tannins, saponins, flavonoids, steroids, terpenoids and phenolic acids using standard procedures of analysis (Harborne 1973). However, crude extracts were quantitatively determined the bioactive constituents such as total phenol, total flavonoid, vitamin C and carotenoid. The presence of flavonoids has been identified in the extracts of ethyl acetate and n-butanol.

Based on the above results, the present study to analyse the bioactive flavonoid (i.e., naringin and hesperidin) contents in ethylacetate and butanol extracts of *Citrus karna* peels (EtCK and BuCK) by using reversed-phase HPLC-DAD. The chromatographic separation was performed on an C18 column (4.6 $\times$ 150 mm). The mobile phase consisted of 75 mM citric acid and 25 mM ammonium acetate in double-distilled water (A) and

methanol (B) with a ratio of 60:40 (v/v) at a flow rate of 1 ml/min. The injection volume was 20 μ l and the detection wavelength was set at 282 nm. The retention times and spectra were compared to those of authentic standards. The contents of naringin and hesperidin were expressed in mg/100 g of ethylacetate and butanol *Citrus karna* peel for the triplicate extracts. The values were expressed as mean $\pm$ SD.

Determination of reducing power of *Citrus karna* peel extract The reducing power of the *Citrus karna* peel extracts and the synthetic standard (i.e butylated hydroxyanisole; BHA) were determined as described method of Yen and Chen (1995). The test sample (EtCK and BuCK) containing 5–20 μ g of gallic acid equivalents (GAE) was made up to 500 μ l with 0.2 M phosphate buffer (pH 6.6), mixed with 1 ml of potassium ferricyanide (0.1%) and the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (500 μ l, 10%) was added to the reaction mixture and centrifuged at 3000 g for 10 min. The supernatant obtained was mixed with equal volume of distilled water, 300 μ l of 1% ferric chloride was added and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power. The anti-oxidant activity of the extract was compared with BHA.

Determination of DPPH radical scavenging activity of *Citrus karna* peel extract The free radical scavenging activity of the ethyl acetate and butanol extracts of *Citrus karna* was measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) using the method of Brand-Williams et al. (1995). A 100 mM solution of DPPH in methanol was prepared and ethyl acetate and butanol extracts of *Citrus karna* peel (200 μ l) containing 1–5 μ g of GAE was mixed with 1 ml of DPPH solution. The mixture was shaken vigorously and left in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured at 517 nm. The control contained all the reagents except peel extract/BHA. The capacity to percentage scavenging of DPPH radical was calculated by the following equation.

$$\text{Scavenging activity (\%)} = 1 - \left(\frac{\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{control}}}{\text{Absorbance}_{\text{control}}} \right) \times 100$$

The results were plotted as the % of scavenging activity against concentration of the sample. The inhibition concentration (IC₅₀) was defined as the amount of GAE required for 50% of free radical scavenging activity. The IC₅₀ value was calculated from the plots as the anti-oxidant concentration required for providing 50% free radical scavenging activity.

Determination of hydrogen peroxide radical scavenging activity of *Citrus karna* peel extract Hydrogen peroxide scavenging activity was measured using the modified

method of Ruch et al. (1989). One ml (10–100 µg /ml of EtCK and BuCK) of test sample with different concentrations was added to 2.4 ml of 0.1 M phosphate buffer (pH 7.4) and mixed with 0.6 ml of 43 mM solution of hydrogen peroxide. After 10 min, the absorbance at 230 nm of the reaction mixture was recorded. The control contained all the reagents except peel extract/BHA. All experiments were run in triplicate. Ascorbic acid was used as reference compound. The capacity to percentage scavenging of hydrogen peroxide was calculated by the following equation:

$$\text{Scavenging activity(\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

The results were plotted as the % of scavenging activity against concentration of the sample. The inhibition concentration (IC₅₀) was defined as the amount of GAE required for 50% of free radical scavenging activity. The IC₅₀ value was calculated from the plots as the anti-oxidant concentration required for providing 50% free radical scavenging activity.

Determination of microsomal lipid peroxidation inhibitory action of *Citrus karna* peel extract The liver microsomal lipid peroxidation was performed as described method of Miller and Aust (1989) with some modifications. Liver microsomes was prepared and the protein content was determined by using commercially available protein kits. Peel extract (100 µl) containing 1–5 µg of GAE were added to 1 ml of microsomes (100 µg protein). Lipid peroxidation was induced by adding 25 µl ADP (2 mM), 25 µl of FeSO₄ (4 mM) and 25 µl of ascorbic acid (0.1 mM). After incubation for 1 h at 37°C, the reaction was stopped by adding 2 ml of 0.25 N HCl containing 15% tri-carboxylic acid (TCA) and 0.375% thiobarbituric acid. The reaction mixture was boiled for 15 min, cooled, centrifuged and absorbance of the supernatant was measured at 532 nm. Appropriate blanks and controls were run along with the test samples. The blank contained all the reagents and peel extract/BHA except microsomes while control contained all the reagents and microsomes except peel extract. The capacity to percentage inhibition of lipid peroxidation was calculated by the following equation:

$$\text{Inhibition lipid peroxidation (\%)} = 1 - \frac{(\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{control}})}{\text{Absorbance}_{\text{control}}} \times 100$$

The percentage of inhibition was plotted against the concentration of the peel extract that was expressed in terms of GAE. The half-inhibition concentration (IC₅₀) value was defined as the amount of GAE required for inhibition of 50% of lipid peroxidation. The IC₅₀ value was calculated from the plots as the anti-oxidant concentration required for providing 50% inhibition of lipid peroxidation.

Determination of lipoxygenase inhibitory action of *Citrus karna* peel extract: Lipoxygenase inhibiting activity was measured according to the method described earlier by Shobana and Naidu (2000) with some modification. The enzymatic lipid peroxidation was measured spectrophotometrically following an increase in absorbance of lipid hydroperoxide formation at 234 nm. The 1 ml reaction mixture contained 250 µM linoleic acid substrate solution, 5 nM soybean lipoxygenase and 50 mM Tris buffer (pH 9.0). Different concentrations of mango peel extracts (2.5–40 µg of GAE) were incubated with soybean lipoxygenase for 2 min prior to the initiation of the reaction with linoleic acid substrate. Control contained all the reagents except peel extract/BHA. The decrease in hydroperoxide formation in presence of peel extract or BHA was calculated by the following equation:

$$\text{Inhibition(\%)} = 1 - \frac{(\text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{control}})}{\text{Absorbance}_{\text{control}}} \times 100$$

The percentage of inhibition was plotted against the concentration of the peel extract that was expressed in terms of GAE. The IC₅₀ value was determined from plotting a graph with concentration of peel extract required for 50% percentage of inhibition of lipid peroxidation.

Statistical analysis

All analysis were performed in triplicate and data were reported as means ± SD. Duncan's new multiple range test was used to determine the difference of means and $P < 0.05$ was considered to be statistically significant as described by the method of Steel and Torrie (1980).

Results and Discussion

Determination of % yield of extracts of *Citrus karna* peels Extracts of *Citrus karna* peels were prepared by using different solvent systems on the basis of polarity. Percentage yield was calculated i.e. hexane (1.28% w/w), dichloromethane (0.99% w/w), ethyl acetate (2.31% w/w) and butanol (3.91% w/w). Results indicated that maximum yield was obtained in the ethyl acetate and butanol extract of *Citrus karna* peels.

Quantification of bioactive constituents in extracts of *Citrus karna* peels Phenolics are secondary metabolic products, which mainly include flavonoids, tannins phenolic acids, stilbenes, and coumarins (Pereira et al. 2009). In addition to vitamin C and carotenoids, a variety of phenolic compounds are present in *Citrus* fruit as bioactive compounds (Cano et

al. 2008). Naringin and hesperidin (*Citrus* flavonoids), are two major flavanone glycosides present in *Citrus* fruits (Yoshida et al. 2010). HPLC-DAD, using either normal or reversed-phase columns, has reported to identify and quantify flavonoids in *Citrus* (Kanaze et al. 2004). In this study, flavonoids (Naringin and hesperidin) content of EtCK and BuCK were analyzed by a modified reversed-phase HPLC-DAD system. The chemical structures and HPLC-DAD profiles of naringin and hesperidin are shown in Fig. 1. The active constituents of *Citrus karna* peel extracts were determined by earlier described method. The results shown in Table 1 indicates that BuCK possess the maximum yield active constituents as compared to the all other extracts.

Reducing power of *Citrus karna* peel extract The reducing power of a *Citrus karna* peel extract is known as marker of anti-oxidant activity *In-Vitro*, is related to electron transfer ability (Ajila et al. 2007). Figure 2 shows the reducing power of the *Citrus karna* peel extracts. The reducing power increased with the concentration of peel extract. The reducing power of butanol extract of *Citrus karna* was more compared to other extracts and BHA. Phenol, vitamin C and carotenoids are present in the peel which is good electron donors and could reduce Fe^{3+} /ferricyanide complex to ferrous form, which indicates the anti-oxidant activity (Ajila et al. 2007; Oboh and Ademosun 2011).

DPPH radical scavenging activity of *Citrus karna* peel extract The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm induced by anti-oxidants. Due to rapid hydrogen accepting ability of DPPH, it reacts with anti-oxidants and gets converted into 1,1-diphenyl-2-picrylhydrazine and hence shows decrease in absorbance. The degree of discoloration indicates the scavenging potential of the anti-oxidant extract, which is due to the hydrogen donating ability (Ajila et al. 2007). The extracts showed significant radical scavenging activity. The maximum activity was found in butanol extract followed by ethyl acetate, dichloromethane and hexane. BuCK anti-oxidant activity was found to be comparable to that of BHA. The IC_{50} values (μg of GAE) of DPPH indicated in Fig. 3a.

Hydrogen peroxide (H_2O_2) radical scavenging activity of *Citrus karna* peel extract The measurement of H_2O_2 scavenging activity is one of the useful method for determining the ability of anti-oxidants to decrease the level of pro-oxidants such as H_2O_2 (Ajila et al. 2007; Sood et al. 2009; Oboh and Ademosun 2011). Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells (Rey et al. 2010). The extracts showed significant radical scavenging activity. The maximum activity was found in

Fig. 1 HPLC chromatogram of EtCK & BuCK extracts and standard compounds. HPLC peak in (Fig. 1a, b and c) indicates EtCK, BuCK and reference samples *i.e.* naringin & hesperidin, respectively. Further, Fig. 1a and b indicates the presence of naringin & hesperidin in both extract (R_t values for naringin and hesperidin are 32 and 32.5 s, respectively). N; Naringin and H; Hesperidin

Table 1 Contents of total phenolics, total flavonoids, carotenoids vitamin c and naringin and hesperidin in *Citrus karna* peel extracts

Extraction solvent	Total phenolics ¹	Total flavonoid ²	Vitamin C ³	Carotenoid ⁴	Naringin ³	Hesperidin ³
Hexane	779±1.26 ^b	274±1.57 ^b	14.2±0.73 ^b	0.14±0.02 ^b	NA	NA
Dichloromethane	779±0.94 ^b	278±1.25 ^b	15.7±0.92 ^b	0.16±0.06 ^b	NA	NA
Ethyl acetate	801±1.32 ^a	332±1.69 ^b	32.6±0.58 ^a	0.34±0.01 ^b	1.74±0.03	0.51±0.05
Butanol	916±1.56 ^a	713±2.83 ^a	35.2±0.47 ^a	0.62±0.03 ^a	2.81±0.07 ^a	2.25±0.01 ^a

All data are the mean±SD of three replicates. Means with different letters within the same column represent significant differences at $p<0.05$

¹ mg chlorogenic acid equivalents (CAE) / 100 g of peel extract (PE)

² mg rutin equivalents (RE) / 100 g of PE

³ mg of Vitamin C/ 100 g of PE

⁴ mg b-carotene equivalents (BCE) / 100 g of PE

butanol extract followed by ethyl acetate, dichloromethane and hexane. BuCK anti-oxidant activity was found to be comparable to that of BHA. The IC₅₀ values (μg of GAE) of hydrogen peroxide indicated in Fig. 3b.

Microsomal lipid peroxidation inhibitory effect of *Citrus karna* peel extract Lipid peroxidation of biological cell membrane leads to generate a large number of degradation products. Malonaldehyde (MDA) is one of the important mediators of cell membrane destruction and cell damage (Eleni and Dailianis 2010). It has been extensively used as an index for lipid peroxidation and as a marker for oxidative stress. The reaction of MDA with thiobarbituric acid has been widely accepted as a sensitive assay method for lipid peroxidation (Miller and Aust 1989). The extracts showed significant lipid peroxidation inhibitory action. The maximum activity was found in butanol extract followed by ethyl acetate, dichloromethane and hexane. BuCK anti-oxidant activity was found to be comparable to that of BHA.

The IC₅₀ values (μg of GAE) of lipid peroxidation indicated in Fig. 3c.

Lipoxygenase inhibitory effect of *Citrus karna* peel extract Lipoxygenase process is an important event for many diseases like asthma, atherosclerosis, cancer, tumor, angiogenesis, peptic ulcer, neuropathic and inflammatory pain. It acts as major biological target for certain disorders (Muthuraman and Sood 2010a, 2010b). Lipoxygenase (E.C. 1.13.11.12) is a family of non-heme containing dioxygenase group of enzymes that are widely distributed in plants and animals. In mammalian cells, these enzymes have key role in the biosynthesis of a variety of bioregulatory compounds such as hydroxyeicosatetraenoic acid, leukotrienes, lipoxins, hepoxylines (Keereetaweep et al. 2010; Luzia and Jorge 2011). Therefore, lipoxygenases are potential targets for the acute & chronic inflammatory disease and autoimmune disorders (Keereetaweep et al. 2010). Anti-oxidants

Fig. 2 Reducing power of the *Citrus karna* peel extracts. All data are the mean±SD of three replicates. BHA; butylated hydroxyanisole, GAE; gallic acid equivalents

Fig. 3 IC_{50} values of *Citrus karna* peel extracts on (a) DPPH activity, (b) H_2O_2 activity, (c) lipid peroxidation activity and (d) lipoxygenase activity IC_{50} values were calculated from the dose responses curves. All data are the mean $\pm$ SD of three replicates

interact with lipoxygenase by scavenging of free radical (Keereetaweep et al. 2010; Oboh and Ademosun 2011).

All the extracts showed significant inhibition of lipoxygenase action. The maximum activity was found in

butanol extract followed by ethyl acetate, dichloromethane and hexane. BuCK anti-oxidant activity was found to be comparable to that of BHA. The IC₅₀ values (µg of GAE) of lipid peroxidation are shown in Fig. 3d.

Conclusion

Citrus karna peel extracts documented the presence of phenols, flavonoids, vitamin C, and carotenoids. These extracts exhibited good anti-oxidant activity by effectively reducing Fe³⁺/ferricyanide complex, scavenging of various free radicals such as DPPH· and H₂O₂ radicals. Moreover, these extracts were also found to have the inhibitory action of liver microsomal lipid peroxidation and lipoxygenase activity *In-Vitro*. Hence, it may be concluded that the anti-oxidant and lipid peroxidation & lipoxygenase inhibitory action of *Citrus karna* peel extracts would be used for the future therapeutic medicine due to presence of potential bioactive compounds.

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