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Original Article

In Silico PASS Predictions and Exploration of Antioxidant and Anti-inflammatory Activity of *Citrus Karna* Raf. Fruit

Citrus Karna Raf. Meyvesinin Antioksidan ve Anti-enflamatuvar Aktivitesinin İn Silico PASS Tahminleri ve Araştırılması

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ABSTRACT

Objective: Inflammation and oxidative stress are major factors in the development of many disorders. Natural antioxidants present in plants can interrupt, decrease, or reduce the oxidation of components sensitive to oxidative processes by scavenging free radicals and lowering oxidative stress. Most anti-inflammatory agents used in the management of inflammatory disorders diminish oxidative damage. The biological potential of *Citrus karna* Raf. remains undisclosed, despite its richness in several bioactive compounds.

Methods: The methanolic extract was evaluated for quantitative phytochemical analysis and antioxidative efficacy using 1,1-diphenyl-2-picrylhydrazyl radical and hydrogen peroxide scavenging activity. A carrageenan-induced paw edema model was employed to evaluate the anti-inflammatory potential as antioxidants exert anti-inflammatory effects. *In silico* prediction of activity spectra for substance predictions were performed to understand the possible mechanism of action of phytochemicals.

Results: *Citrus karna* methanolic extract (CKME) showed dose-dependent radical scavenging effects. The powerful scavenging activity of CKME could be due to the diverse polyphenolic compounds such as ascorbic acid, beta-carotene, and naringin. In addition, the percentage inhibition of paw edema and swelling was observed in CKME-treated rats and mice, which is the same as that of standard drug-treated groups. The Pa value of ascorbic acid, beta-bisabolene, linalool, and naringin is more than 0.7 which shows that these phytoconstituents might contribute to the anti-inflammatory action of extract samples such as CKME.

Conclusions: Our findings shows that CKME possess strong antioxidant and anti-inflammatory effects. The richness of plants in polyphenolics such as flavonoids might be a contributing factor for these potential effects.

Keywords: *Citrus karna* Raf., plant extract, antioxidant, anti-inflammatory, PASS prediction

ÖZ

Amaç: Enflamasyon ve oksidatif stres birçok hastalığın gelişimindeki önemli faktörlerdir. Bitkilerde bulunan doğal antioksidanlar, serbest radikalleri temizleyerek ve oksidatif stresi azaltarak oksidatif süreçlere duyarlı bileşenlerin oksidasyonunu durdurabilir, azaltabilir veya yok edebilir. Enflamatuvar bozuklukların tedavisinde kullanılan çoğu anti-enflamatuvar ajan oksidatif hasarı azaltır. *Citrus karna* Raf.'ın biyolojik potansiyeli, çeşitli biyoaktif bileşikler bakımından zengin olmasına rağmen açıklanmamıştır.

Yöntemler: Metanolik ekstrakt, kantitatif fitokimyasal analiz ve 1,1-difenil-2-pikrilhidrazil radikali ve hidrojen peroksit süpürme aktivitesi kullanılarak antioksidatif etkinlik açısından değerlendirilmiştir. Antioksidanlar anti-enflamatuvar etkiler gösterdiğinden, anti-enflamatuvar potansiyeli değerlendirmek için karragenan kaynaklı bir pençe ödemi modeli kullanılmıştır. Fitokimyasalların olası etki mekanizmasını anlamak amacıyla madde öngörüleri için aktivite spektrumlarının *in silico* tahmini yapılmıştır.

Bulgular: *Citrus karna* metanolik özü (CKME) doza bağlı radikal süpürücü etkiler göstermiştir. CKME'nin güçlü radikal temizleme aktivitesi askorbik asit, beta-karoten ve naringin gibi çeşitli polifenolik bileşiklerden kaynaklanıyor olabilir. Buna ek olarak, CKME ile tedavi edilen sıçan ve farelerde, standart ilaçla tedavi edilen gruplarla aynı olan pençe ödemi ve şişmesinin yüzde inhibisyonu gözlenmiştir. Askorbik asit, beta-bisabolen, linalool ve naringinin Pa değerinin 0,7'den fazla olması, bu fitokonstitüentlerin CKME gibi ekstrakt örneklerinin anti-enflamatuvar etkisine katkıda bulunabileceğini göstermektedir.

Sonuçlar: Bulgularımız, CKME'nin güçlü antioksidan ve anti-enflamatuvar etkilere sahip olduğunu göstermektedir. Bitkilerin flavonoidler gibi polifenolikler bakımından zengin olması, bu potansiyel etkilere katkıda bulunan bir faktör olabilir.

Anahtar kelimeler: *Citrus karna* Raf., bitki ekstresi, antioksidan, anti-enflamatuvar, PASS tahmini

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INTRODUCTION

Numerous diseases are largely influenced by inflammation processes and oxidative stress. Inflammation is the body's protective immunological response, which is inflated in conditions such as injury, infection, allergy, and other noxious stimuli[1]. Similarly, oxidative stress is one of the crucial mechanisms that plays an important role in the progression of diseases such as atherosclerosis, cancer, neurodegenerative diseases, diabetes mellitus, inflammatory diseases, and aging. By free radical scavenging and reducing oxidative mechanisms, antioxidant compounds can postpone, slow, or stop the oxidation of chemicals that might be able to undergo oxidation[2]. Natural antioxidants present in plants can interrupt, decrease, or reduce the oxidation of components sensitive to oxidative processes by scavenging free radicals and lowering oxidative stress. Most anti-inflammatory agents used for treating inflammatory diseases diminish oxidative damages[3].

Traditional herbal medicines constitute one of the most readily accessible treatment sources within the primary healthcare system. The medicinal use of plants dates back to ancient times. In many areas of developing countries, a significant portion of the population depends on traditional healthcare healthcare[4]. Currently, approximately 25% of bioactive chemicals have been identified from plant sources and are used as prescribed medicines[5]. *Citrus karna* Raf. is a wild species found in India and belongs to the family Rutaceae[6]. The various plant parts are employed for the preparation of traditional folk medicine as antiseptic, antimicrobial, anxiolytic, and astringent agents for the treatment of stomach ailments, headache, and constipation etc[7]. The health benefits of *Citrus karna* Raf. are attributed to its high levels of phytochemicals and bioactive compounds such as limonoids, coumarin flavonoid tannins, amino acids, phenols, saponins, phytosterols, terpenoids, minerals, and vitamins[8,9].

Based on the ethnopharmacological relevance of *Citrus karna* Raf., the work was performed to assess the total phenolic and flavonoid content and antioxidant potential using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and H_2O_2 radical scavenging assays. In addition, it has been assessed for its potential to reduce inflammation *in vivo* because compounds with antioxidant properties and free radical scavenger potential have anti-inflammatory properties[10]. The outline of the present study is given in Figure 1.

MATERIALS and METHODS

1. Collection of *Citrus Karna* Raf. Fruit

Citrus karna Raf. fruits that were fully matured were obtained from the area of Nanded district, Maharashtra, India during November. In addition, the taxonomical details of the plant materials were authenticated from the Botanical Survey of India, Pune, India (Reference No. BSI/WRC/Iden. Cer./2024/1311230000374), and a voucher specimen was deposited in the herbarium for further use.

2. Preparation of Peel Extracts

Fresh fruits were cleaned and dried at room temperature, and peels were removed (Figure 2). Peels were then carefully cleaned, dried, and ground into a powder using a grinder. The extraction of the peel powder was performed according to Modak et al.[11] with some modifications. The peel powder (500 g) was initially Soxhlet extracted for three days using petroleum ether (60-80 °C), and then it was extracted again with methanol (Figure 3). With the aid of a rotary evaporator, both petroleum ether extract (CKPE) and methanolic extract (CKME) were dried at 45 °C. The yield obtained

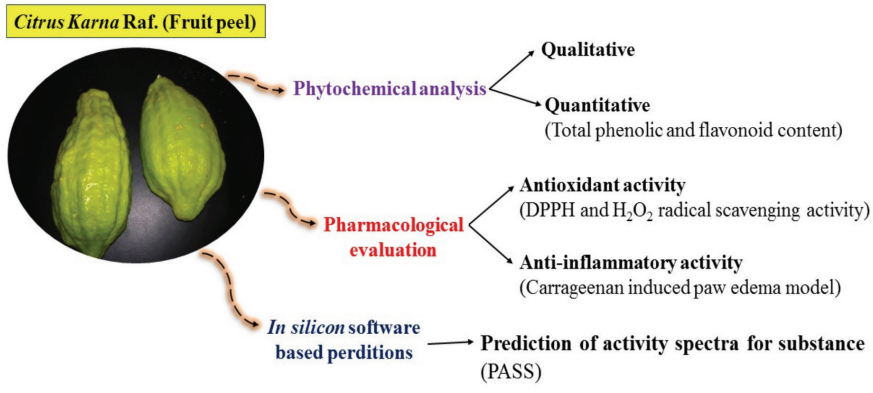

Figure 1. Outline of present research study.

was 63.01 g (12.60%) for CKPE and 88 g (17.6%) for CKME. Methanolic extract was further selected for study as it dissolves most polyphenolic phytochemicals.

3. Phytochemical Screening

Preliminary analysis of phytochemicals in CKME was performed as per standard methods[12]. CKME showed positive tests for glycosides, alkaloids, tannins, saponins flavonoid phenolsphytosterol carbohydrates, proteins, and amino acids.

Figure 2. Unripe and ripe fruit of *Citrus karna* Raf.

Figure 3. Soxhlet apparatus for extraction.

4. Chemicals and Reagents

Chemicals such as DPPH, b-carotene, linoleic acid, ferrous chloride, and Folin-Ciocalteu reagent were purchased from Hi-Media Lab. Pvt. Ltd., Mumbai, India. Methanol, dimethyl sulfoxide (DMSO), sodium hydroxide and H_2O_2 obtained from Rankem (India). Ascorbic acid (Oxford Laboratories), potassium dihydrogen phosphate (S. D. Fine, Mumbai), and indomethacin (Merck, Bangalore) were purchased from respective vendors. Other chemicals and analytical grade reagents were purchased from SD FineChem Ltd. k-Carrageenan used to produce inflammation was procured from Sigma Aldrich Chemicals, India.

5. Animals

Wistar rats of weight 100 to 150 g procured from the S. N. Institute of Pharmacy, Pusad. The rats were maintained in polypropylene cages at a temperature of 24±2 °C with 12 hours (h) day night cycles. In addition, 35-60% humidity was maintained, and rats were allowed to feed with proper fodder and water *ad libitum*. For experimentation, before 4 h, rats were not allowed to access food but were allowed to access water. Anti-inflammatory activity was evaluated at the Pharmacology Department of S. N. Institute of Pharmacy, Pusad, MS, India.

Experimental procedures and protocols were performed to complete the present work and were sanctioned by the Institutional Animal Ethics Committee of the Sudhakarrao Naik Institute of Pharmacy, Pusad (Ref. No. SNIOP/IAEC/2021-22/22, date: 21.05.2022). All procedures were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

6. Acute Toxicity Study

To assess the acute toxicity LD_{50} value of the CKME was calculated using the up and down method defined by Bruce[13]. Oral administration of CKME at 2000 mg/kg did not show any toxicity signs in rats and mice and was found to be safe.

7. Determination of the Total Phenolic and Flavonoid Content

7.1. Determination of the Total Phenolic Content

The amount of total phenolic compounds in CKME was evaluated using the Folin-Ciocalteu process, for which gallic acid was used as a standard. The blue color produced because of the polyphenols of the extract was determined at 660 nm with an ultraviolet spectrophotometer. Briefly, 0.1 mL of the extract was

added to 0.2 mL Folin-Ciocalteu phenol reagent, 2 mL water, and 1 mL (15% w/v) sodium carbonate. The mixture was incubated for 2 h for a period of 10 min at °C, and at 760 nm, absorbance was determined using a spectrophotometer (Shimadzu 2405). The total phenolic content of CKME was stated in terms of gallic acid equivalent (GAE) (µg) using the linear regression equation Y=0.005X + 0.137 and R^2=0.992 based on the calibration curve. In this equation, Y represents absorbance at 760 nm and X is the concentration of GAEs (µg/mL). The study was executed in triplicate, and the results were represented as microgram GAEs[14].

7.2. Determination of the Total Flavonoid Content

The total flavonoid content of CKME was determined using the colorimetric assay given by Rick-Leonid et al. (2011)[15]. The procedure depends on the development of flavonoid-aluminum complexation. 0.5 mL CKME, 1.5 mL ethanol, 0.1 mL aluminum chloride (10%), 0.1 mL CH_3COONa (1 M), and 2.8 mL water were carefully combined and maintained at room temperature for 40 min. Furthermore, the obtained reaction mixture absorbance was determined using a spectrophotometer (Shimadzu 2405) at 415 nm. The same process was repeated three times, and the results were obtained in triplicate. The standard curve plotted using quercetin was used to determine the total flavonoid content, and the results were represented as micrograms of quercetin equivalents per mg extract[15].

8. Antioxidant Activity

8.1. DPPH Radical Scavenging Assay

The radical scavenging potential of CKME was evaluated using a DPPH radical scavenging assay with some modification[16]. Initially, 0.2 mL of CKME was mixed with 10, 20, 40, 60 and 80 µg/mL ascorbic acid (standard antioxidant) and further mixed with freshly formed DPPH methanol solution (1 mL of 0.2 mM). The obtained reaction solution was mixed and maintained in the dark for 30 min at ambient temperature. The control solution used in the study has the same composition without CKME, and for the blank reading, DMSO was employed. The absorbance of the resultant mixtures was determined at 517 nm using a spectrophotometer (Shimadzu 2405). The following formula was employed for calculating the percentage of DPPH radical scavenging activity:

Radical scavenging activity (%) = $(A_0 - A_1/A_0) \times 100$

Where A_0 represents the control sample absorbance and A_1 represents the CKME and ascorbic acid absorbance. The graph of percentage inhibition against concentration of extract was plotted, and antioxidant results were represented as IC_{50} (micro molar concentration of sample needed for 50% inhibition of DPPH radical).

8.2. Hydrogen Peroxide Scavenging (H_2O_2) Assay

The hydrogen peroxide scavenging potential of CKME was evaluated according to the procedure defined previously by Sahoo et al.[17]. Phosphate buffer (pH 7.4) was used to prepare a solution of H_2O_2 (40 mmol/L). The absorbance was measured at 230 nm using a spectrophotometer (Shimadzu 2405) to evaluate the concentration of hydrogen peroxide. Aqueous 50, 100, 150, 200 and 250 µg/mL concentrations of CKME were mixed with 0.6 mL hydrogen peroxide solution (40 mmoL/L). The reaction mixture was kept for 10 min, and at 230 nm, the absorbance of hydrogen peroxide was measured against a blank solution having phosphate buffer but do not contains hydrogen peroxide. The hydrogen peroxide percentage scavenging of CKME and standard ascorbic acid was determined using the following equation:

Percent scavenging activity of H_2O_2 (%) = $(A_0 - A_1)/A_0 \times 100$

Where A_0 and A_1 represent the absorbance of control, and CKME and ascorbic acid, respectively.

9. *In vivo* Anti-inflammatory Activity

The anti-inflammatory potential of the folklore claims of *Citrus karna* Raf. fruit peel extract was validated in this study. The effect was assessed in rats and mice using two independent models based on the carrageenan-induced rat paw edema method.

9.1. Carrageenan-induced Rat Paw Edema Model (Using Plethysmometer)

In this model, the selected rats were separated into five groups, each containing six rats (n=6). Group I rats were treated with vehicle (DMSO) and group 2 rats were orally treated with diclofenac at a dose of 20 mg/kg. Group three to five rats were treated orally with CKME at doses of 50, 100, and 200 mg/kg, respectively. The right hind paw subplantar region of each rat was carefully cleaned, and 0.1 mL of carrageenan was injected. Further, foot volume was determined using a plethysmometer for durations of 0 min, 30 min, 60 min, 120 min, and 180 min. The percentage inhibition was estimated using following formula[18,19].

$$\text{Percentage inhibition} = \frac{(V_t - V_0)\ \text{control} - (V_t - V_0)\ \text{treated}}{(V_t - V_0)\ \text{control}} \times 100$$

Where V_0 and V_t represent the mean paw volume at 0 h and at particular time intervals, respectively.

9.2. Carrageenan-induced Rat Paw Edema Model (Using Vernier Calipers)

For this study, male Swiss albino mice of average weight 18 to 20 g were procured from a commercial supplier. Mice were maintained in the laboratory under standard conditions and separated into seven groups each containing six mice. CKME was mixed in DMSO and injected intraperitoneally at selected doses. Group I mice were administered 1% w/v carrageenan dissolved in DMSO in the right hind paw subplantar region. Negative control group (group II) mice were treated with normal DMSO. Group III mice were treated with standard indomethacin at a dose of 10 mg/kg. Mice in groups IV-VII were administered CKME at a dose of 5 mg/kg, 10 mg/kg, 20 mg/kg, and 40 mg/kg body weight. 0.1 mL carrageenan 1% (w/v) dissolved using DMSO was injected to induce acute edema in the paws of mice. After 60 min, 0.1 mL of carrageenan was injected into the subplantar region of the right hind paw. Over the course of 4 h, the linear paw circumference of mice was measured every h using a vernier caliper. After the administration of carrageenan, measurements were made between 0 and 4 h[20,21]. The anti-inflammatory effect of CKME was determined as;

% inhibition of edema = $(T - T_0)/ T \times 100$

Where T and T_0 represent the thickness of mouse paw edema of the control and CKME treated groups, respectively.

10. Statistical Analysis

Results are expressed as mean ± standard error of the mean. One-Way analysis of variance (ANOVA) followed by multiple Tukey's comparison tests was employed for the statistical analysis of the obtained data. The differences were supposed to be statistically significant at $^{a}p<0.05$ as compared to control.

11. *In Silico* Prediction of Activity Spectra for Substance Prediction Analysis

The possible biological activities of a chemical compound are predicted using the online software database program prediction of activity spectra for substance (PASS). This program aids in estimating the biological activities of chemicals such as organic chemicals (having molecular weight of 50 to 1250 Da) or plant chemicals. In this software, compounds that have to be evaluated for biological activities are analyzed for structural activity relationship using a training set containing approximately 205,000 chemical structures that show almost 3750 different biological activity[22,23]. The procedure was performed as described by Habibyar et al.[24] with some modifications. For the probable biological activity of the chemical, the first MDL mole file [V 3000] (*mol) structure was drawn using software such as ACD/ Labs chemsketch software 2021 (file version C10E41) and placed into the software. The software gives values in the form of Pa and Pi. Pa represents the active nature and Pi represents the inactive nature of the compound. If the Pa value is more than 0.7, then the possibility of experimental, biological, and pharmacological activity of the compound is high, and if the Pa value is 0.5<Pa>0.7, less is the pharmacological activity is less.

RESULTS

1. Total Phenolic Content

The total phenolic content of CKME was evaluated using the Folin-Ciocalteu process, and for comparison, gallic acid was used as a standard. The standard calibration curve of gallic acid for the determination of the total phenolic content of CKME is given in Figure 4. CKME contains a high level of phenolic content (86.6 µg GA/mg), which was calculated using a linear regression equation.

2. Total Flavonoid Content

Quercetin is a renowned flavonoid present in plants that has antioxidant, anti-inflammatory, and analgesic effects. Quercetin was used to plot the standard graph. The formula was used to calculate the total flavonoid content as quercetin (QA) equivalents (Y=0.0025 X + 0.674 and R^2=0.972) obtained from this standard graph and expressed µg of QA/mg of extract. The total flavonoid content of CKME was found to be 90 µg QA/ mg, as shown in Figure 5.

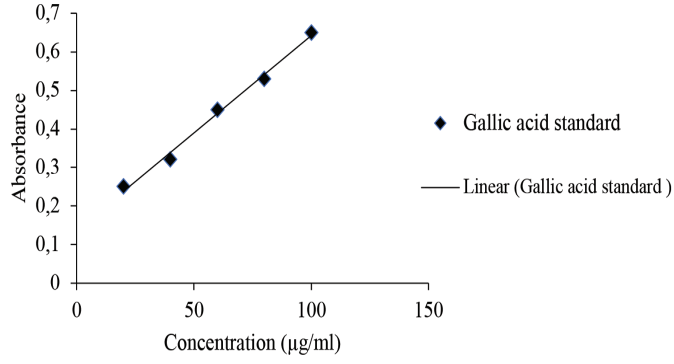

Figure 4. Standard calibration curve of gallic acid for the determination of total phenolic content of CKME.

CKME: *Citrus karna* methanolic extract

3. Antioxidant Activity

3.1. DPPH Radical Scavenging Assay

The scavenging capacity of CKME on DPPH mentioned in Figure 6 and compared with that of ascorbic acid. The scavenging effect of ascorbic acid and CKME on the DPPH radical was expressed as IC_{50}, which was found to be 105 µg/mL and 160 µg/mL, respectively. CKME showed strong antioxidant activity that increased with concentration, similar to that of ascorbic acid.

3.2. Hydrogen Peroxide Scavenging Activity

The polyphenol-rich CKME showed a concentration-dependent scavenging effect on H_2O_2 (R^2=0.973) (Figure 7) with an IC_{50} value of 150.82 µg/mL and for ascorbic acid 168.86 µg/mL for ascorbic acid (R^2=0.993). A prominent concentration-dependent hydrogen peroxide scavenging effect was exhibited by CKME similar to that of ascorbic acid (Figure 7).

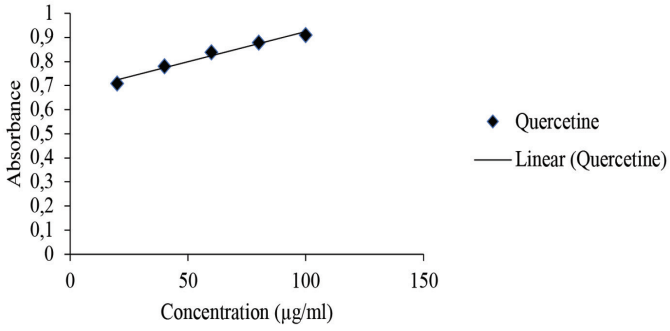

Figure 5. Standard calibration curve of quercetin for the determination of total flavonoid content of CKME.

CKME: *Citrus karna* methanolic extract

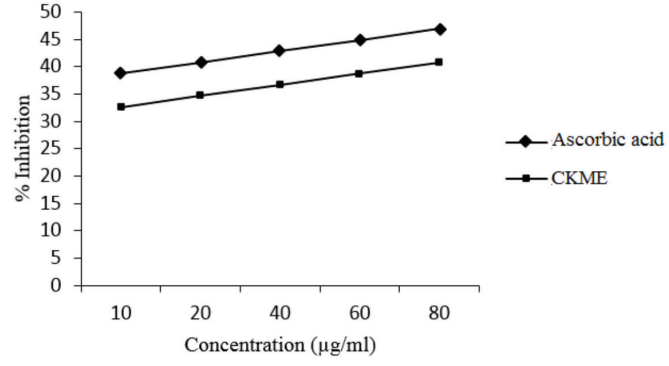

Figure 6. The dose response curves for percentage scavenging of DPPH by CKME and ascorbic acid.

DPPH: 1,1-diphenyl-2-picrylhydrazyl, CKME: *Citrus karna* methanolic extract

4. *In Vivo* Anti-inflammatory Activity

In comparison with the control group, the CKME demonstrated dose-dependent reduction of carrageenan-induced rat paw edema at doses of 50, 100, and 200 mg/kg from 0.5 to 3 h after drug administration. The highest percentage inhibition of paw edema by CKME was 40.65 at 200 mg/kg after 3 h of its administration. The results are the same as those of standard diclofenac, which showed a maximum percentage inhibition of 61.53% at 3 h after its administration (Table 1). In addition, CKME at 40 mg/kg bw showed dose-dependent percentage inhibition similar to that of standard indomethacin (Table 2).

5. *In Silico* PASS Prediction

Citrus karna Raf. fruit phytochemicals were assessed for antioxidant potential using PASS, and outcomes were employed in a flexible manner. The chemicals exerting more Pa values than Pi are mentioned in Table 3. Phytoconstituents such as ascorbic acid, beta-carotene, and naringin were found to have strong antioxidant activity because their Pa is much greater than Pi. The Pa values of ascorbic acid, beta-bisabolene, linalool, and naringine are more than 0.7 which shows that these plant extract chemicals (Table 4) may have anti-inflammatory properties.

DISCUSSION

Phenolic compounds, which mostly consist of flavonoids and phenolic acids, are secondary plant metabolites that are present in a wide variety of fruits. Phenolics are bioactive components found in plants and exhibit significant health-protective activity[25]. Natural polyphenols have the ability to counteract oxidative processes and are believed to play a defensive role in

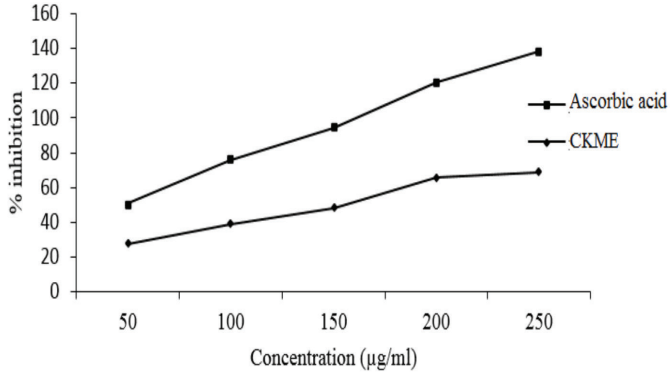

Figure 7. The dose response curves for percentage scavenging of H_2O_2 by CKME and ascorbic acid.

CKME: *Citrus karna* methanolic extract

oxidative diseases and disease complications, including inflammation[26].

Plants are a rich source of flavonoids, which are the main class of polyphenolic chemicals present in the human diet[27]. Under microbiological infection, plants produce hydroxylated phenolic compounds called flavonoids. The hydroxyl group is the most reactive group in flavonoids[28]. Several previous studies have shown that flavonoids exert strong antioxidant activity because of their radical scavenging action and ability of metal ion chelation, which is responsible for preventing lipid peroxidation[29]. Numerous phytochemical studies have documented the antioxidative properties of polyphenols obtained from plants. Because polyphenolic compounds may scavenge free radicals and active oxygen species such as singlets, superoxide free radicals, and hydroxyl radicals, they have a high level of antioxidant properties[28,29]. Hence, this findings indicate that the polyphenolic constituents of CKME are directly related to their potent antioxidant and anti-inflammatory activities.

It is crucial to enhance radical scavenging activities to prevent free radicals from negatively affecting living systems. Lipid oxidation is accelerated by the overabundance of free radical generation, thereby affecting cellular morphology. The ability of different antioxidant compounds to scavenge radicals has been extensively measured using DPPH radicals, including phytochemicals[30,31]. CKMEs have strong scavenging capacity because of their electron or hydrogen transfer ability[32].

Table 1. Effect of the CKME and diclofenac in comparison to carrageenan control in carrageenan induced paw edema in rat by means of plethysmometer.

Groups	Before	0 h	30 min	60 min	120 min	180 min
GI - Distilled water	0.93±0.04	1.01±0.03	1.62±0.04 (0.61)	1.91±0.03 (0.9)	2.01±0.06 (1)	1.92±0.04 (0.91)
GII - Diclofenac (20 mg/kg)	0.96±0.03	1.05±0.04	1.43±0.03* (37.70)	1.6±0.03* (38.88)	1.52±0.04* (53)	1.40±0.04* (61.53)
GIII - CKME (50 mg/kg p. o.)	0.93±0.04	1.05±0.06	1.60±0.03 (9.83)	1.82±0.03 (14.44)	1.85±0.02 (20)	1.80±0.03 (17.58)
GIV - CKME (100 mg/kg p. o.)	0.91±0.04	1.01±0.03	1.51±0.02 (18.03)	1.72±0.02* (21.11)	1.76±0.03* (25)	1.65±0.03* (29.67)
GV - CKME (200 mg/kg p. o.)	0.88±0.03	0.99±0.04	1.43±0.02* (27.86)	1.60±0.03* (32.22)	1.62±0.02* (37)	1.53±0.03* (40.65)

Values are expressed as the mean ± SEM, n=6 in each group; all the readings represent percentage inhibition. Results were analyzed using One-Way ANOVA. *p<0.05, as compared to the control group. SEM: Standard error of the mean, h: Hours, min: Minutes , CKME: *Citrus karna* methanolic extract, p. o.: Per oral

Table 2. Effect of CKME and indomethacin in comparison with carrageenan control in carrageenan induced paw edema model by means of vernier caliper.

Groups	0 h	30 min	1 h	2 h	3 h	4 h
GI - Carrageenan (1% w/v)	1.7±0.05	3.23±0.07	3.8±0.04	3.86±0.05	3.81±0.04	3.86±0.03
GII - DMSO	1.7±0.04	1.72±0.01	1.76±0.05	1.79±0.04	1.8±0.01	1.71±0.05
GIII - Indomethacin with carragenan (10 mg/kg bw)	1.7±0.05	3.1±0.06	2.91±0.07 (23.42%)	2.32±0.04 (38.89%)	2.3±0.06[a] (39.63%)	1.9±0.05[a] (50.77)
GIV - CKME (5 mg/kg bw)	1.75±0.01	3.3±0.01	3.4±0.05 (10.52%)	3.20±0.15 (17.09%)	3.15±0.01 (17.32%)	3.1±0.05 (19.68%)
GV - CKME (10 mg/kg bw)	1.75±0.12	2.7±0.01	3.2±0.12 (15.78%)	3.15±0.25 (18.39%)	3.1±0.01[a] (18.63%)	3.0±0.04[a] (22.27%)
GVI - CKME (20 mg/kg bw)	1.7±0.05	2.8±0.01	3.15±0.01 (17.10%)	3.18±0.12 (17.61%)	3.15±0.12[a] (17.32%)	2.65±0.06[a] (31.34%)
GVII - CKME (40 mg/kg bw)	1.75±0.01	2.76±0.12	3.1±0.01 (18.42%)	3.12±0.15 (19.17%)	3.0±0.01[a] (21.25%)	2.3±0.01[a] (40.41)

Outcomes were expressed as mean increase in paw diameter ± SD and analyzed by One-Way ANOVA. [a]p<0.05 significant compared to carrageenan treated group. SD: Standard deviation, h: Hours, min: Minutes, DMSO: Dimethyl sulfoxide, CKME: Citrus karna methanolic extract

Hydrogen peroxide selectively inhibits thiol-containing enzymes by oxidizing the thiol (-SH) group. As soon as H_2O_2 encounters the cell membrane, it crosses and reacts with Fe^{2+} and Cu^{2+} to produce hydroxyl radicals. The prominent concentration-dependent hydrogen peroxide scavenging effect of CKME was similar to that of ascorbic acid. Hydroxyl radicals are the most reactive and damaging oxygen radicals. This strong effect may be due to the presence of several electron-donating compounds that neutralize H_2O_2 to water, including alkaloids, flavonoids, and etc[33,34].

Vascular tissue activates a complex biological defense mechanism called inflammation against different biological stress factors such as pathogens, irritants, or damage. Literature studies on many polyphenols, triterpenoids, and saponins have demonstrated significant anti-inflammatory activity through various mechanisms[35]. Paw swelling or footpad edema is considered to be a useful model for evaluating the anti-inflammatory effects of phytochemicals and synthetic chemicals[36]. The ability to reduce or halt swelling induced by carrageenan was observed during the screening of test compounds for acute anti-inflammatory activity.

CKME exhibited inhibition of carrageenan-induced paw edema similar to that of standard drugs such as diclofenac and indomethacin. Because of the presence of phytochemicals that inhibit cyclooxygenase, which in turn inhibits prostaglandin synthesis, CKME may inhibit the inflammation generated by carrageenan[37]. This effect may be due to the presence of flavonoids because they can mitigate the inflammatory process through various mechanisms. In addition to its antioxidant qualities, linalool (Table 3, 4) inhibits the production of the proinflammatory enzyme cyclooxygenase-2[38]. The anti-

Table 3. Prediction of antioxidant activity of *Citrus karna* Raf. fruit peel by PASS software.

Sr. No.	Phytoconstituents	Pa	Pi	Activity
1.	Ascorbic acid	0.951	0.002	Antioxidant
		0.564	0.007	Free radical scavenger
		0.889	0.001	Oxygen scavenger
2.	Beta-carotene	0.823	0.003	Antioxidant
		0.887	0.001	Oxidizing agent
		0.873	0.003	Oxidoreductase inhibitor
3.	Beta-phellandrene	0.548	0.005	Antioxidant
		0.569	0.030	Oxygen scavenger
4.	Caffeic acid	0.670	0.004	Free radical scavenger
		0.611	0.004	Antioxidant
		0.672	0.010	Oxygen scavenger
		0.654	0.023	Oxidoreductase inhibitor
5.	Citric acid	0.662	0.011	Oxygen scavenger
		0.670	0.020	Oxidoreductase inhibitor
		0.664	0.016	Peroxidase inhibitor
		0.532	0.006	Oxidizing agent
6.	Chlorrogenic acid	0.651	0.005	Free radical scavenger
		0.849	0.004	Oxidoreductase inhibitor
7.	Cis-osmin	0.620	0.030	Oxidoreductase inhibitor
8.	Citral	0.565	0.004	Oxidizing agent
9.	Geranial	0.565	0.004	Oxidizing agent
10.	Geraniol	0.548	0.005	Antioxidant
		0.569	0.030	Oxygen scavenger
11.	Limonene	0.511	0.006	Antioxidant
12.	Linalool	0.552	0.054	Oxidoreductase inhibitor
13.	Naringin	0.955	0.001	Free radical scavenger
		0.800	0.003	Antioxidant

PASS: Prediction of activity spectra for substance

Table 4. Prediction of anti-inflammatory activity of *Citrus karna* Raf. fruit peel by PASS software.				
Sr. No.	**Phytoconstituents**	**Pa**	**Pi**	**Activity**
1.	Ascorbic acid	0.773	0.009	Anti-inflammatory
2.	Beta-bisabolene	0.720	0.013	Anti-inflammatory
3.	Beta-carotene	0.688	0.017	Anti-inflammatory
4.	Beta-phellandrene	0.641	0.024	Anti-inflammatory
5.	Beta-pinene	0.624	0.027	Anti-inflammatory
6.	Caffeic acid	0.648	0.003	Anti-inflammatory
		0.661	0.021	Anti-inflammatory
7.	Chlorrogenic acid	0.556	0.042	Anti-inflammatory
8.	Geraniol	0.641	0.024	Anti-inflammatory
9.	Limonoids	0.606	0.030	Anti-inflammatory
10.	Linalool	0.786	0.008	Anti-inflammatory
11.	Naringin	0.701	0.016	Anti-inflammatory
12.	Terpineol	0.646	0.023	Anti-inflammatory
PASS: Prediction of activity spectra for substance				

edematous activity significantly increased after 3 h. This effect of CKME is mediated by various means such as inhibiting signaling of histamine-stabilizing mast cells, through receptor disruption of histamine, inhibition of histidine decarboxylase gene transcription, and probable inhibition of the release and/or action of kinin and prostaglandin[39,40].

CONCLUSION

In conclusion, the obtained results suggest the usefulness of *Citrus karna* Raf. fruit peel extract CKME for the treatment of inflammation and associated complications. It also possesses strong antioxidant and anti-inflammatory properties. The richness of plants in polyphenolics such as flavonoids might be a contributing factor for this potential effect. These results provide *Citrus karna* Raf. with new pharmacological knowledge, suggesting a potential medicinal use for the herb in certain traditional treatments. However, further detailed evaluation of the mechanism of action and phytochemicals present in CKME is highly needed.

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Ethics

Ethics Committee Approval: Experimental procedures and protocols were performed to complete the present work and were sanctioned by the Institutional Animal Ethics Committee of the Sudhakarrao Naik Institute of Pharmacy, Pusad (Ref. No. SNIOP/IAEC/2021-22/22, date: 21.05.2022).

Informed Consent: The study does not require patient consent.

Author Contributions

Concept: P.J., M.G., Design: M.G., Data Collection and/or Processing: P.J., R.K., Analysis and/or Interpretation: P.J., M.G., R.K., Literature Search: P.J., M.G., Writing: P.J., R.K.

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