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In vitro Anti Oxidant Activity of Buccal film containing Kaempferol

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Abstract: *Kaempferol is a natural flavonoid with potent antioxidant activity, but it's therapeutic use is limited by its low aqueous solubility. Here, a buccal film was formulated to improve its dissolution property and antioxidant activity. The antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and nitric oxide radical scavenging activity. Optimised batch of buccal film was selected for the activity. Result indicated that buccal film of kaempferol exhibited potent antioxidant activity.*

Key-words: *Kaempferol Buccal film, DPPH, Ascorbic acid, Nitric oxide, Sodium nitro prusside etc.*

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I. Introduction

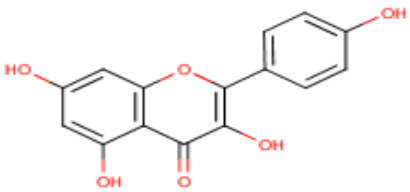
Flavonoids exert a wide range of benefits to human health. They are considered as one of irreplaceable antioxidants in our daily diet because they function as singlet oxygen scavengers, free radical quencher, or chelators of metal ions, which catalyze the oxidative reactions. It was suggested that free radicals and lipid peroxidation were involved in several human diseases and age-related pathologies. Flavonoids can directly scavenge superoxide and peroxy nitrite in an effective way [Deng et. al. 2019].

Flavonoids, plant polyphenols are a group of plant secondary metabolites characterized by a diphenyl propane structure. They are widely distributed in the plant kingdom and are common constituents of fruits, vegetables and some beverages [Montaño et. al. 2011]. Many of them have been used as traditional medicine in India and other Asian countries for more than thousands of years. These plant polyphenols are with strong antioxidant capacities and thus largely contribute to the pharmaceutical and dietary properties of plant derived food [Csepregi et.al.2016].

Free radicals are naturally occurring by-products of our own metabolism. Free radicals are electrically charged molecules that attack various cells, tearing through impermeable cellular membranes to react with the nucleic acids, proteins and enzymes present in the body. Free radicals can also be cause the lipid peroxidation in foods which leads to their deterioration [Naphade et.al.2009]. Oxidation is known as to be the major cause of foods and materials degradation [Mohammad et.al.2009]. Oxidation is a chemical process that allows transfer of electron from a substance to an oxidizing agent. Oxidation reaction can be produce various free radicals which are act as start chain reactions that damage cells [Aher et.al.2011]. The free radicals are species which having very short half life, high reactivity and damaging activity for macromolecules like as proteins, DNA and lipids. Free radicals may defined as the molecular sharks which damage the molecules in cell membranes, mitochondria, DNA and they are very unstable, tend to rob electrons from the molecules in the immediate surroundings in order to replace their own losses. The most commonly reactive oxygen species are includes superoxide anion (O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH). The nitrogen derived free radicals are like as nitric oxide (NO), peroxy nitrite anion (ONOO), Nitrogen dioxide (NO_2) and Dinitrogen trioxide (N_2O_3) [Rathore et.al.2010].

Antioxidants are compounds which have ability to bring either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are substances used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals. Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals [Aurelia et. al. 2011].

The Kaempferol (3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), a yellow compound with a low molecular weight (MW: 286.2 g/mol) and molecular formula ($C_{15}H_{10}O_{6)}$ is a common natural flavonoid which representative of the subcategory of flavonol that commonly found in many plant-derived foods and in plants used in traditional medicine [Montaño et. al. 2011].



Kaempferol

It is widely distributed in the plant kingdom such as onion, apples, citrus fruits, grapes, kale, endive and tea, saffron along with formed as secondary metabolites through the phenyl propenoid biosynthetic pathway [Telange et.al. 2014]. Although it has broad spectrum importance, researcher have been isolated it from different plants like *Crocus sativus* [Hosseinzadeh et.al.2007], *Carica papaya* [Nugroho et. al. 2017] , *Pedalium murex* [Sharma and Sarin 2012], *Ficus amplissima* [Arunachalam et.al. 2013] and also reported its various pharmacotherapeutic effects like anticancer, antioxidant, anti-inflammatory and hepatoprotective etc. [Montaño et. al. 2011]. However, the oral bioavailability of kaempferol is relatively low because of its low lipid solubility and its limited membrane permeability. [Zhaoxiang et.al. 2016]. Kaempferol has been identified in many botanical families and has been found in Pteridophyta, Pinophyta and Magnoliophyta [Montaño et. al. 2011]. Kaempferol revealed low to moderate absorption, which results poor bioavailability ~2%.It is hydrophobic in nature and freely soluble in methanol, 1, 4 – dioxane, Ethanol and dimethyl formamide, ethyl acetate with melting point 276–278 °C [Telange et.al. 2014].

II. Materials and Methods

2.1 Kaempferol Collection-

Kaempferol sample was purchased from Yucca Laboratories Pvt. Ltd, Mumbai.

2.2 Principle-

DPPH [1, 1-Diphenyl-2-picryl hydrazyl] is a stable free radical, which shows absorbance at 517 nm. The antioxidant reacts with DPPH and converts it to 1, 1-Diphenyl-2-picryl hydrazine which do not absorb at 517 nm. When sodium nitroprusside was mixed with aqueous solution at physiological pH, suddenly it generates nitric oxide, which reacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Nitric oxide scavengers compete with oxygen leading to reduced production of nitrite ions.

2.3 Reagent-

Ethanolic solution of DPPH, sample/s stock, Ascorbic acid, Sodium nitroprusside, Sulphanilamide, Potassium ferricyanide, Trichloroacetic acid, Ferric chloride, N-(1- naphthyl) ethylenediamine dihydrochloride. All other reagents were of analytical grade.

2.4 Instrument-

Shimadzu UV – visible spectrophotometer

2.5 DPPH Radical Scavenging Activity -

The antioxidant activity of the buccal film of kaempferol was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH carried out by using the method of Molyneux (2004). To 1 ml of DPPH solution, equal amount of test compound at various concentrations (20-100 ug/ml) were added in a final volume of 2.0 ml. After incubation for 20 minutes at room temperature, absorbance due to changes in color from deep violet to light yellow were recorded at 517 nm. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The different concentrations of ascorbic acid were used as reference compound. Lower absorbance of the reaction mixture indicated higher free radical activity. The experiment was performed in triplicate.

Calculation:

Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$$\text{Percentage Inhibition: -} \quad \frac{\text{Absorbance of Control - Absorbance of test}}{\text{Absorbance Control}} \times 100$$

2.6 Nitric oxide Radical Scavenging Activity-

Nitric oxide radical scavenging activity was measured spectrophotometrically according to the method described by Govindharajan (2003). About 1 ml of Sodium nitroprusside (5 mM) in phosphate buffer (pH 7.4, 0.1 M) was mixed with different concentrations of the samples in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 25°C for 2 h. After incubation 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent [1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride]. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-(1- naphthyl) ethylenediamine dihydrochloride) was measured spectrophotometrically at 546 nm. Control tube was maintained with all chemicals excluding sample. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$$\text{Percentage Inhibition: -} \quad \frac{\text{Absorbance of Control - Absorbance of test}}{\text{Absorbance Control}} \times 100$$

III. Results

The DPPH assay is purely based on the assumption that an antioxidant serves as a hydrogen donor and thus reduces the DPPH free radicals (the color turns from deep violet to light yellow). This assay is known as a basic and quick tool to carry out evaluation of antioxidant activity of plant samples. In this study, results showed that all kaempferol samples had significant levels of radical scavenging activity in a dose dependent manner (Table3.1and Figure 3.1). Nitric oxide is an unstable free radical which involved in many biological processes and associated with several diseases. In this study, results showed that all samples had significant levels of radical scavenging activity in a dose dependent manner (Table 3.2 and Figure 3.2).

DPPH Radical Scavenging Activity -

Concentration (ug/ml)	**Percentage Inhibition (Mean ± SEM) (n=3)**	
	Sample	**Standard**
20	29.17 ± 0.03	33.36 ± 0.08
40	41.6 ± 0.08	49.22 ± 0.06
60	52.2 ± 0.03	63.18 ± 0.08
80	61.27 ± 0.03	71.34 ± 0.03
100	68.77 ± 0.05	79.54 ± 0.08
IC50	49.22	49.08

Table 3.1 DPPH free radical scavenging activity of optimised buccal film of kaempferol

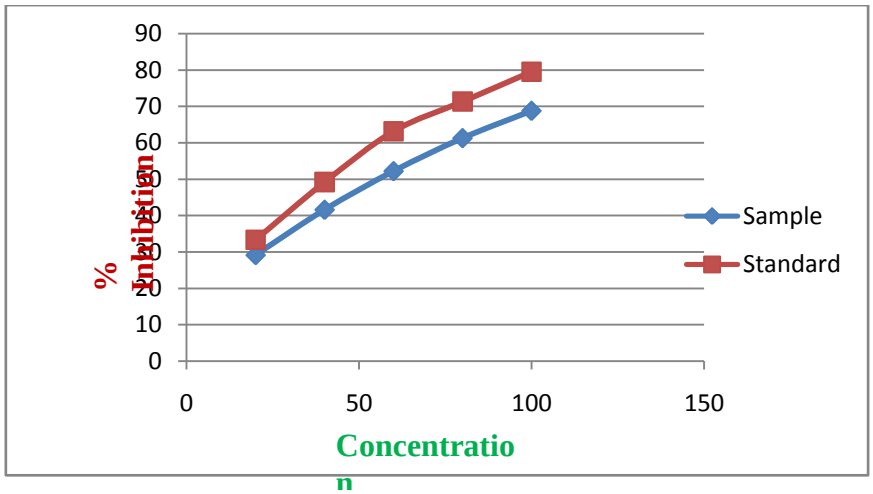


Fig. 3.1 DPPH free radical scavenging activity of optimised buccal film of kaempferol

Nitric oxide Radical Scavenging Activity-

Concentration (ug/ml)	**Percentage Inhibition (Mean ± SEM) (n=3)**	
	Sample	**Standard**
20	30.54 ± 0.07	36.69 ± 0.33
40	35.64 ± 0.06	41.76 ± 0.06
60	46.32 ± 0.14	53.05 ± 0.11
80	50.95 ± 0.07	59.21 ± 0.03
100	54.48 ± 0.10	60.80 ± 0.06
IC50	49.34	49.25

Table 3.2 Nitric oxide radical scavenging activity of optimised buccal film of kaempferol

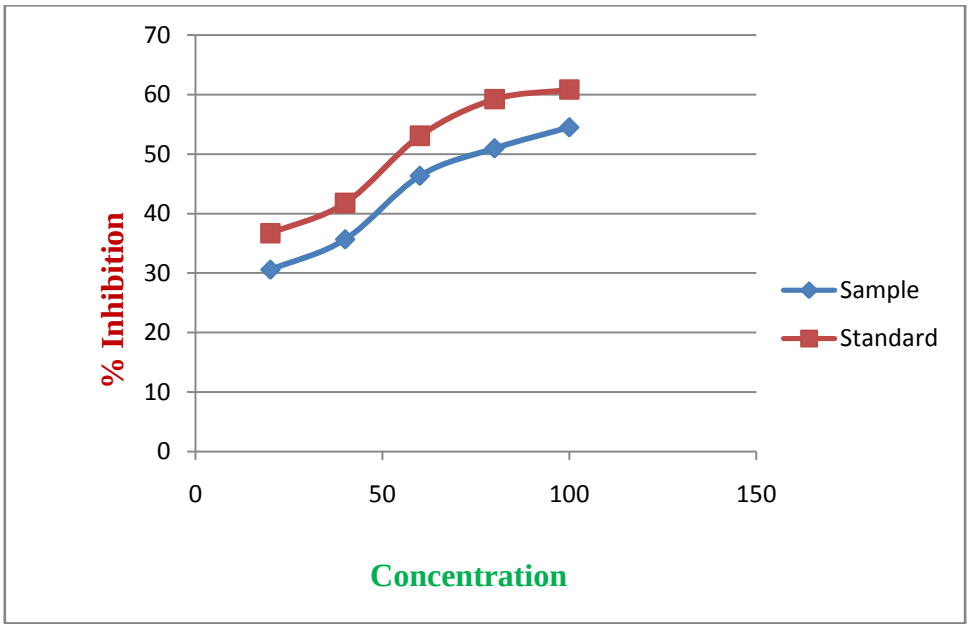


Fig. 3.2 Nitric oxide radical scavenging activity of optimised buccal film of kaempferol

IV. Discussion

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as antioxidant, anti-inflammatory, hepatoprotective, antiulcer, antiallergic, antiviral and anticancer activities [Umamaheswari., 2008]. Flavonoids are capable of effectively scavenging the reactive O_2 species because of their phenolic hydroxyl groups and so they are potent antioxidants [Cao.et.al. 1997]. In the present study, DPPH free radical scavenging activity and nitric oxide radical scavenging activity of samples of kaempferol were evaluated. All the samples of kaempferol when added to the reaction mixture scavenge hydroxyl radicals in a concentration dependent manner. The reducing capacity of a sample may act as a significant indicator of its potential antioxidant activity.

V. Conclusion:

Higher plants are act as a source of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. Present study shows that kaempferol a polyphenolic compound exhibits strong antioxidant activity compared to that of the reference compound. The results would help to determine the potency of the kaempferol formulation as a potential source of natural antioxidants.

References

[1]. Naphade S.S., S.S. Khadabadi, S.L. Deore, N.S. Jagtap, and S.P. Hadke, Antioxidant Activity of different extracts of plant *Tricholepsi Glaberrima* DC (Asteraceae), International Journal of Pharm Tech Research, 2009, 1(3), 502-505.

[2]. Mohammad hassanzadeh-khayyat, Mehdi mojarab, Seyyed ahmad emami, Antioxidant activity of methanolic extracts of different species of Artemisia from Iran, Pharmacology online, 2009, 2, 797-807.

[3]. Aher V. D, Wahi Arunkumar, Pandey Abhijit M., Antioxidants as Immunomodulator: an expanding research avenue, International journal of current pharmaceutical research, 2011, 3(1), 8-10.

[4]. Rathore K.S, Sisodia S.S., Panchawat S. A review on herbal antioxidant, International Journal of Pharma Tech Research, 2010, 2(1), 232-239.

[5]. Molyneux P., The Use of Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity, J Sci Technol, 2004, 26, 211-219.

[6]. Govindharajan R., S Rastogi, M Vijayakumar, AKS Rawat, A Shirwaikar, S Mehrotra, P. Pushpangadan, Studies on the antioxidant activities of Desmodium gangeticum, Biol Pharm Bull, 2003, 26, 1424-27.

[7]. Nataraj Loganayaki , Perumal Siddhuraju , Sellamuthu Manian, Antioxidant activity and free radical scavenging capacity of phenolic extracts from Helicteres isora L. and Ceiba pentandra L., Journal of Food Sci. Technology, 2013, 50(4), 687–695.

[8]. Deng Sui-Ping, Yang Yi-Li, Cheng Xing-Xing, Synthesis, Spectroscopic Study and Radical Scavenging Activity of Kaempferol Derivatives: Enhanced Water Solubility and Antioxidant Activity, International Journal of Molecular Sciences, 2019, 20,(975), 1-15.

[9]. Bigoniya P., Singh C. S., Shrivastava B. In Vivo and In Vitro Hepatoprotective Potential Of Kaempferol, A Flavone Glycoside From Capparis Spinosa, Int. J. Pharm. Bio Sci, 2013, 3(4),139-152.

[10]. Csepregi Kristóf , Susanne Neugart , Monika Schreiner, Éva Hideg, Comparative Evaluation of Total Antioxidant Capacities of Plant Polyphenols, Molecules, 2016, 21, 208-216.

[11]. Gupta Ramesh C., Nutraceuticals: Efficacy, Safety and Toxicity, A Book by Elsevier, 1-1020.

[12]. Montaño J.M. Calderón, E. Burgos-Morón, C. Pérez-Guerrero, M. López-Lázaro, A Review on the Dietary Flavonoid Kaempferol**,** Mini-Reviews in Medicinal Chemistry, 2011, 11, 298-344.

[13]. Telang Darshan R., Arun T. Patil, Anil M. Pethe, Amol A. Tatode, Sridhar Anand, Vivek S. Dave, Kaempferol-phospholipid complex: formulation, and evaluation of improved solubility, in vivo bioavailability, and antioxidant potential of Kaempferol, J. Excipients and Food Chem., 2016,7 (4), 89-116.

[14]. Telange Darshan R., Arun T. Patil, Amol Tatode, Bhushan Bhoyar, Development and Validation of UV Spectrophotometric Method for the Estimation of Kaempferol in Kaempferol: Hydrogenated Soy Phosphatidyl Choline (HSPC) Complex, Pharmaceutical Methods, 2014, 5(1), 34-38.

[15]. Zhaoxiang zhou, Meng wang, Zengjun guo, Xiaoying zhang, Pharmacokinetic evaluation of the interaction between oral kaempferol and ethanol in rats, Acta Pharm., 2016, 66, 563–568.

[16]. Vellosa José Carlos Rebuglio, Luis O. Regasini, Najeh Maissar Khalil, Vanderlan da Silva, Bolzani, Omar A. K. Khalil, Francine Alessandra Manente, Harli Pasquini Netto, Olga M. M., de Faria Oliveira, Antioxidant and cytotoxic studies for kaempferol, quercetin and isoquercitrin, Ecletica Química São Paulo, 2011, 36 (2), 7-20.

[17]. Linrong Cao and Jieming Li, Plant-Originated Kaempferol and Luteolin as Allelopathic Algaecides Inhibit Aquatic Microcystis Growth Through Affecting Cell Damage, Photosynthetic and Antioxidant Responses, Journal of Bioremediation & Biodegradation, 2018,9 (2) , 1-6.

[18]. Hosseinzadeh Hossein ,Vahidehsadat Motamedshariaty, Farzin Hadizadeh, Antidepressant effect of kaempferol, a constituent of saffron (*crocus sativus*) petal, in mice and rats, Pharmacology Online, 2007, 2, 367-370.

[19]. Jirge Supriya S, Pratima A. Tatke, Satish Y. Gabhe, Simultaneous Estimation of Kaempferol, Rutin, and Quercetin in Various Plant Products and Different Dosage Forms of Bhuiamla and Amla, Journal of Planar Chromatography, 2014, 27(4), 267–273.

[20]. Sharma Priyanka and Sarin Renu, Isolation and characterization of quercetin and Kaempferol in vivo and in vitro from *Pedalium murex*, International Research Journal of Pharmacy,2012, 3(6), 184-187.

[21]. Nugroho Agung , Hesty Heryani, Jae Sue Choi, Hee-Juhn Park, Identification and quantification of flavonoids in Carica papaya leaf and peroxynitrite scavenging activity, Asian Pacific Journal of Tropical Biomedicine, 2017, 7(3), 208–213.

[22]. Huang Xiaojian, Qiyong Pan, Zekai Mao, Pengcheng Wang, Rui Zhang, Xiaohu Ma, Jingyuan Chen And Hongbo You, Kaempferol inhibits interleukin-1β stimulated matrix metalloproteinases by suppressing the MAPK-associated ERK and P38 signaling pathways, Molecular Medicine Reports, 2018, 18, 2697-2704.

[23]. Arunachalam Karuppusamy, Murugaiyan Iniyavan, Thangaraj Parimelazhagan, A HPTLC Method for the Identification of Potential Therapeutic Compound of Kaempferol from *Ficus amplissima* Smith, International Journal of Pharmaceutical Sciences Review and Research, 2013,22(1), 166-171.

[24]. Ghareeb M. A., Saad A. M., Allia M. A., A New Kaempferol Glycoside with Antioxidant Activity from *Chenopodium ambrosioides* Growing in Egypt, Oriental Journal of Chemistry, 2016, 32(6), 3053-3061.
[25]. Qian Y. S., Srinivasan R., Mayuren C., Shadab M., Venkata S. M., Ravindran H.K., Production, Characterization and Evaluation of Kaempferol Nanosuspension for Improving Oral Bioavailability, Current Pharmaceutical Biotechnology, 2016, 17(5),1-7.
[26]. Qiang X.,Yanbin Y., Qiang L., Zejun H., Zhixia L., Preparation and characterization of monodisperse molecularly imprinted polymer microspheres by precipitation polymerization for kaempferol, Designed Monomers and Polymers, 2017, 20(1), 201–209.
[27]. Lee J., Jae H. K., Kaempferol Inhibits Pancreatic Cancer Cell Growth and Migration through the Blockade of EGFR-Related Pathway In Vitro, Plos One, 2016, 11(5), 1-14.
[28]. Tatsimo S. J. N., Tamokou J. D., Havyarimana L., Dezső C., Antimicrobial and antioxidant activity of kaempferol rhamnoside derivatives from Bryophyllum pinnatum, BMC Research Notes, 2012, 5(158),1-6.
[29]. Guo Peng, Yun-Yun Feng, Anti-inflammatory effects of kaempferol, myricetin, fisetin and ibuprofen in neonatal rats, Tropical Journal of Pharmaceutical Research, 2017, 16 (8), 1819-1826.
[30]. Akroum S., Dalila B., Dalila S., Korrichi L., Antibacterial, antioxidant and acute toxicity tests on flavonoids extracted from some medicinal plants, International Journal of Green Pharmacy, 2010, 165-169.
[31]. Behbahani M., Sayedipour S., Pourazar A., Shanehsazzadeh M., In vitro anti-HIV-1 activities of kaempferol and kaempferol-7-*O*glucoside isolated from *Securigera securidaca*, Research in Pharmaceutical Sciences, 2014, 9(6), 463-469.
[32]. Kelly S. d. L. M., Guilherme E. N. D., Meri E. F. P., Flavonoids with Gastroprotective Activity, Molecules, 2009, 14, 979-1012.
[33]. Yuva B., Laïd B., Hasan A., Alzahrani., Balkees A. B., Molecular Mechanism Underlying Anti-Inflammatory and Anti-Allergic Activities of Phytochemicals: An Update, Molecules, 2013, 18, 322-353.
[34]. Tanvir E. M.,Md. Sakib Hossen,Md. Fuad Hossain, Antioxidant Properties of Popular Turmeric *(Curcuma longa)* Varieties from Bangladesh, Journal of Food Quality, 2017, 1- 8.
[35]. Aurelia Magdalena Pisoschi1 and Gheorghe Petre Negulescu, Methods for Total Antioxidant Activity Determination: A Review, Biochemistry & Analytical Biochemistry, 2011, 1(1), 1-10.
[36]. Umamaheswari and Chatterjee TK., In vitro antioxidant activities of the fractions of *Coccinia grandis* L. leaf extract, Afr. J. Traditional, complementary and Alternative Medicines, 2008, 5(1), 61-73.
[37]. Cao G., Sofic E. and Prior RL. Antioxidant and pro-oxidative behavior of flavonoids: Structure activity relationships, Free Radical.Biol. Med., 1997, 22, 749-760.

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