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In Vitro Antioxidant Activity of Kaempferol Microemulsion

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ABSTRACT

Flavonoids are promising antioxidants. Kaempferol is a natural flavonoid with potent antioxidant activity, but its use is limited because of its low aqueous solubility. The present study sought to investigate the capacity of single herbal formulations of Kaempferol to act as nitric oxide radical (NO), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical antagonists using in vitro models. Observed Results indicate that an optimized batch of microemulsion exhibited potent antioxidant activity.

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1. INTRODUCTION

Nature is always a source of medicinal agents for thousands of years and an impressive number of modern drugs are isolated from natural sources, many based on their use in traditional medicine. Antioxidants are compounds that can bring either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. Antioxidants are involved in the defense mechanism of the organism against the pathologies associated with the attack of free radicals [1].

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 [2]. Many of them have been used as a 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 [3]. Antioxidant compounds have been receiving great attention from natural products consumers and researchers due to several pharmacological properties. Antioxidants played an important role in lowering oxidative stresses caused by reactive oxygen species [4]. Antioxidants are substances that employ various mechanisms to scavenge free radicals by inhibiting their formation or interrupting their propagation. Thus, through various mechanisms antioxidants can inhibit the adverse effects of oxidative stress [5].

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 plants used in traditional medicine also reported its various therapeutic effects like anticancer, antioxidant, anti-inflammatory and hepatoprotective, etc. However, the oral bioavailability of Kaempferol is relatively low because of its low lipid solubility and its limited membrane permeability. [6]. Kaempferol has been identified in many botanical families and has been found in Pteridophyta, Pinophyta and Magnoliophyta. Kaempferol revealed low to moderate absorption, which results in poor bioavailability ~2%. It is hydrophobic in nature and freely

soluble in methanol, 1, 4 – dioxane, Ethanol, and dimethylformamide, ethyl acetate with melting point 276–278 °C [7].

2. MATERIALS AND METHODS

Chemicals and reagents-

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

Kaempferol Collection-

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

DPPH Radical Scavenging Activity -

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 does not absorb at 517 nm. The antioxidant activity of the microemulsion 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, an equal amount of test compound at various concentrations (20-100 ug/ml) was added in a final volume of 2.0 ml. After incubation for 20 minutes at room temperature, absorbance due to changes in colour from deep violet to light yellow was 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 a reference compound. Lower absorbance of the reaction mixture indicated higher free radical activity.

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

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

Nitric oxide Radical Scavenging Activity-

When sodium nitroprusside was mixed with an aqueous solution at physiological pH, suddenly it generates nitric oxide, which reacts with oxygen to produce nitrite ions. Nitric oxide scavengers compete with oxygen leading to reduced production of nitrite ions.

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 the 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 was measured spectrophotometrically at 546 nm. The control tube was maintained with all chemicals excluding the sample. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

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

3. RESULTS AND DISCUSSION

Results of the present study showed that all kaempferol samples had significant levels of radical scavenging activity in a dose-dependent manner (Table3.1and Figure 3.1). 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). In this study of Nitric oxide radical scavenging activity 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 –

Table No. 3.1: DPPH free radical scavenging activity of optimized batch of microemulsion containing Kaempferol

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)	
	Sample	Standard
20	11.60 ± 0.033	21.14±0.066
40	18.43 ±0.147	24.62±0.066
60	22.45±0.140	29.17±0.033
80	28.27±0.066	33.69±0.033
100	32.59±0.091	39.39±0.066
IC50	49.64	49.55

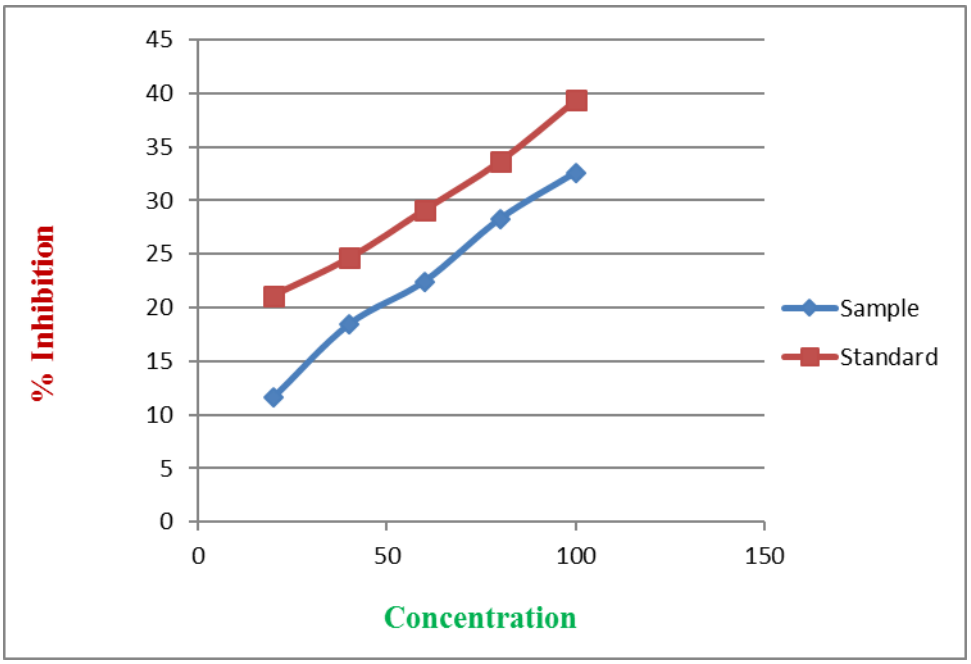

Figure No. 3.1: DPPH free radical scavenging activity of optimized batch of microemulsion containing Kaempferol

Nitric oxide Radical Scavenging Activity-

Table No. 3.2: Nitric oxide radical scavenging activity of optimized batch of microemulsion containing Kaempferol

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)	
	Sample	Standard
20	20.43±0.036	30.54±0.070
40	26.52±0.118	38.97±0.033
60	33.73±0.066	42.68±0.170
80	37.61±0.066	47.75±0.115
100	42.18±0.066	50.68±0.170
IC50	49.51	49.38

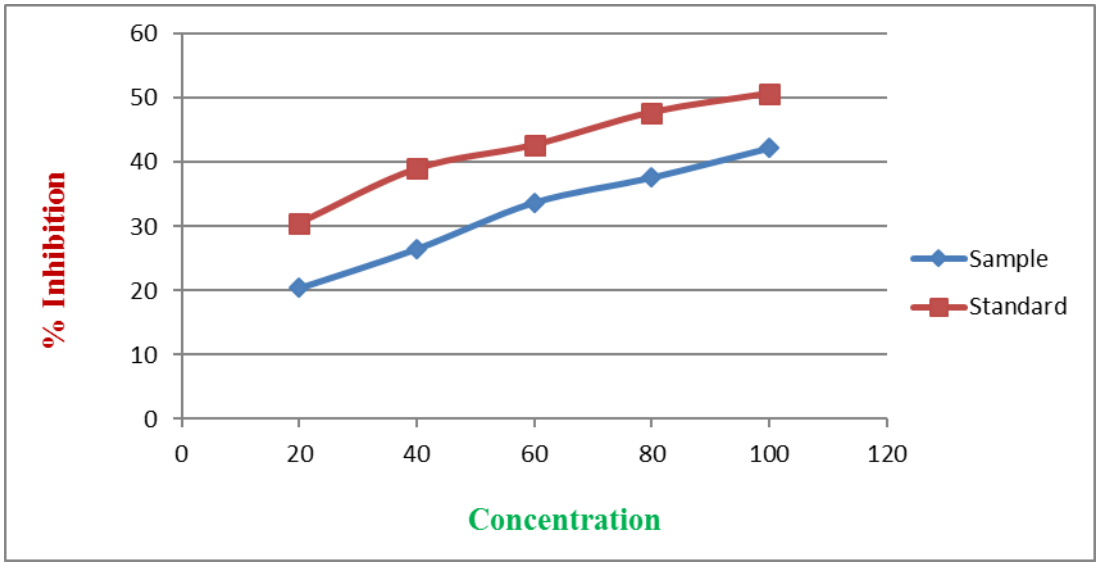

Figure No. 3.2 Nitric oxide radical scavenging activity of optimized batch of microemulsion containing kaempferol

4. CONCLUSION

The results obtained from the study confirmed the benefits of the medicinal plants. Some flavonoids present in them demonstrated high antioxidant activities and also a low acute toxicity effect. These data show that the optimized batch of microemulsion had an antioxidant activity more than 40% at100 µg/ml in Nitric oxide radical scavenging activity and more than 30% at100 µg/ml in DPPH free radical scavenging activity. This confirms that flavonoids are nonnegligible compounds.

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