IN-VITRO ANTIOXIDANT ACTIVITY OF EXTRACTS OF AVIPATTIKAR CHURNA

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Summary

Free radicals are highly reactive chemical species indicated in various diseases and ailments. In order to study the antioxidant ability of Avipattikar churna**,** the aqueous and methanolic extracts of Avipattikar churna was evaluated for *in vitro* antioxidant potential. The free radical scavenging activity was carried out using the reactivity of extracts towards different radicals such as 2,2 – Azino bis (3-ethyl Benzo Thiazole – 6 – Sulphonic acid (ABTS), 1,1-Diphenyl-2- Picryl Hydrazine (DPPH) and 1:10- Phenanthroline hydrate (O-phenenthroline). The superoxide scavenging activity was measured by spectrophotometric method using (Nitroblue tetrazolium) NBT. The total antioxidant potential of aqueous and methanolic extracts of Avipattikar churna was also evaluated.

Keywords: Antioxidant, Avipattikar churna, Free radical scavenging

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Introduction

"Avipattikar churna" is a polyherbal Ayurvedic medicine used as remedy for hyperacidity, indigestion, anorexia, urinary retention, constipation and piles[1]. Recent studies have implicated the role played by free radicals and lipid peroxidation in the development of ulcers[2]. The present study reports the antioxidant potential of aqueous and methanolic extract of Avipattikar churna. Free radical scavenging activity was evaluated by using ABTS, O-phenanthroline, DPPH, superoxide radical scavenging and total antioxidant capacity assays.

Materials and Methods

Plant material

Avipattikar churna consists of fourteen ingredients viz., *Zingiber officinale, Piper nigrum, Piper longum, Terminalia chebula, Terminalia bellirica, Embelica officinalis, Cyperus rotundus, salt (vida lavana), Embelia ribes, Elettaria cardamomum, Cinnamomum tamala, Syzgium aromaticum, Operculina terpethum, and Saccharum officinarum*[3]. All these ingredients were procured from the local market of udupi, Karnataka, India and were authenticated by botanist V. Aravinda Hebbar, Professor and Head of the department of botany, M.G.M College, Udupi, Karnataka. A voucher specimen of the same has been deposited in the museum of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal for future reference.

Preparation of In-house Avipattikar churna

The churna was prepared as per the procedure given in Ayurvedic Formulary of India[1]. All the ingredients viz., *Zingiber officinale, Piper nigrum, Piper longum, Terminalia chebula, Terminalia bellirica, Embelica officinalis, Cyperus rotundus,* salt (vida lavana), *Embelia ribes, Elettaria cardamomum, Cinnamomum tamala, Syzgium aromaticum, Operculina terpethum,* and *Saccharum officinarum* were powdered separately, passed through 80 # sieve and then mixed together in specified proportions to get uniformly blended churna.

Preparation of the extracts

For preparing aqueous extract of Avipattikar churna, 100 g churna was extracted with 300 ml of chloroform water by maceration. The extract evaporated under vacuum and was stored in desiccator until further use. For preparing methanolic extract of Avipattikar churna, 100 g churna was extracted by maceration in closed flask for about two days with methanol (in ratio 100:300). The extract was filtered and evaporated to dryness and the yield was noted.

In vitro antioxidant studies

In vitro free radical scavenging activity was determined by using ABTS, O-phenanthroline, DPPH, superoxide radical scavenging and total antioxidant capacity assay methods.

ABTS radical cation decolourisation assay[4]

ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS solution (7mM) with 2.45 mM ammonium persulfate. The mixture was allowed to stand in dark temperature for 12-16 hr before use. Different concentrations (2- 512 µg/ml) of aqueous extract (0.5 ml) were added to 0.3 ml of ABTS solution and final volume was made upto 1 ml with methanol. Absorbance was read at 745 nm and the percentage inhibition was calculated by using the formula given below.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{test}}{\text{Control}} \times 100$$

O-phenanthroline assay[4]

The reaction mixture consisted of 1ml O-phenanthroline (0.005g in 10 ml methanol), 2 ml ferric chloride 200 µM (3.24 mg in 100 ml distilled water) & 2 ml of various concentrations of the extract (2-512µg/ml). The mixture was incubated at ambient temperature for 10 min, then the absorbance of the same was measured at 510 nm and the percentage inhibition was calculated.

DPPH radical scavenging activity

DPPH scavenging activity was measured by spectrophotometric method[5]. To methanolic solution of DPPH (20 µM), 0.05 ml of aqueous extract dissolved in methanol was added at different concentrations (2- 512 µg/ml). An equal amount of methanol was added to control. After 20 min the decrease in absorbance of test mixtures (due to quenching of DPPH free radicals) was read at 517 nm and percentage inhibition was calculated.

Superoxide scavenging assay

Superoxide scavenging activity was measured by spectrophotometric method[6]. To 0.5 ml of different concentration of extract (2-512 µg/ml), 1 ml of alkaline DMSO and 0.2 ml NBT 20 mM (50 mg in 10ml phosphate buffer pH 7.4) were added. The absorbance was measured at 560 nm.

Total antioxidant capacity[7]

0.1 ml of extract (10 mg/ml) dissolved in water was added to 1 ml of the reagent prepared by dissolving 0.6 M sulphuric acid (3.14 ml in 100 ml), 28 mM sodium phosphate (397.49 mg in 100 ml) and 4 mM ammonium molybdate (494.36 mg in 100 ml), incubated at 95^{o}C for 90 min, and then cooled to room temperature. The absorbance was measured at 695 nm. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.

Results

In this study the free radical scavenging activity of Avipattikar churna in terms of ascorbic acid equivalents was estimated. In *in vitro* antioxidant studies, the extracts of Avipattikar churna exhibited ABTS (Figure 1), DPPH (Figure 2), O-phenanthroline (Figure 3) and superoxide scavenging activity (Figure 4) in a dose dependent manner. DPPH is relatively stable free radical. The assay is based on measurement of scavenging ability of antioxidants towards the stable free radical DPPH. The DPPH radical reacts with the suitable reducing agent, the electrons become paired off and the solution looses colour stoichiometrically depending on the number of electrons taken up[8]. From the present result it may be postulated that aqueous and methanolic extract of Avipattikar churna reduces the radical to corresponding hydrazine when it reacts with the hydrogen donor in the antioxidant principles. The IC_{50} value of aqueous extract and methanolic extract of Avipattikar churna was estimated (Table 1). The total antioxidant capacity of aqueous extract and methanolic extract of Avipattikar churna was estimated at 1000 µg/ml concentration. The methanolic extract (1000 µg/ml) of Avipattikar churna was found to be equivalent to 95.66 µg/ml of ascorbic acid and 1000 µg/ml aqueous extract of Avipattikar churna was found to be equivalent to 17.888 µg/ml of ascorbic acid (Figure 5). The studies were of significance as demand for herbal products as antioxidants is increasing constantly.

Table 1: IC_{50} value of different antioxidant assay models

Models	Methanolic extract (µg/ml)	Aqueous extract (µg/ml)	Ascorbic acid (µg/ml)
ABTS scavenging assay	46.339	19.155	12.255
DPPH scavenging assay	30.599	108.853	9.994
O-Phenanthroline assay	435.7	63.198	21.889
Superoxide scavenging assay	96.59	4.767	2.097

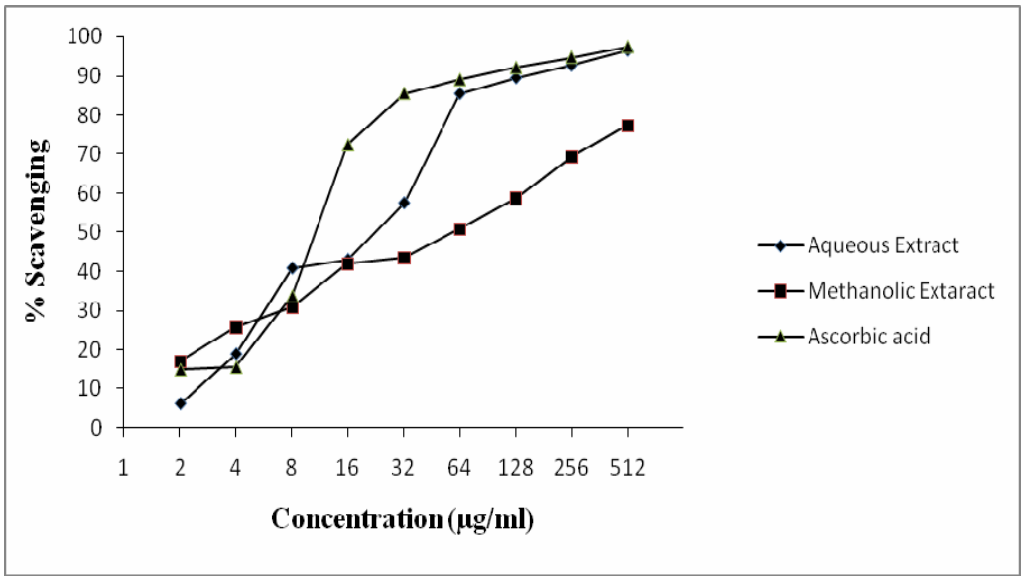

Figure 1: Effect of aqueous and methanolic extracts of In-house Avipattikar churna on ABTS radical scavenging assay.

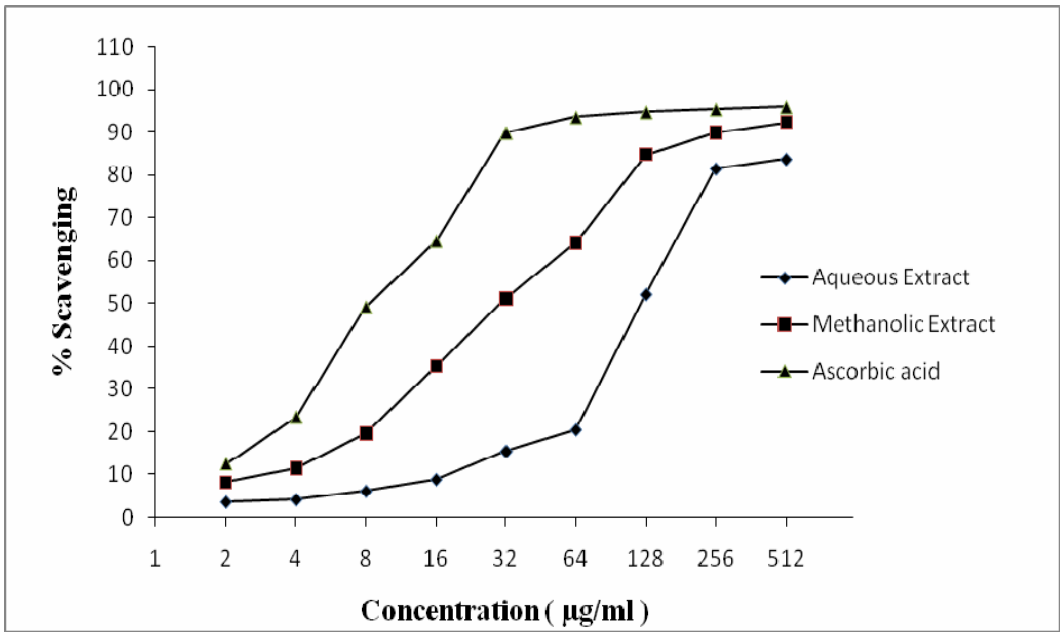

Figure 2: Effect of aqueous and methanolic extracts of In-house Avipattikar churna on DPPH radical scavenging assay.

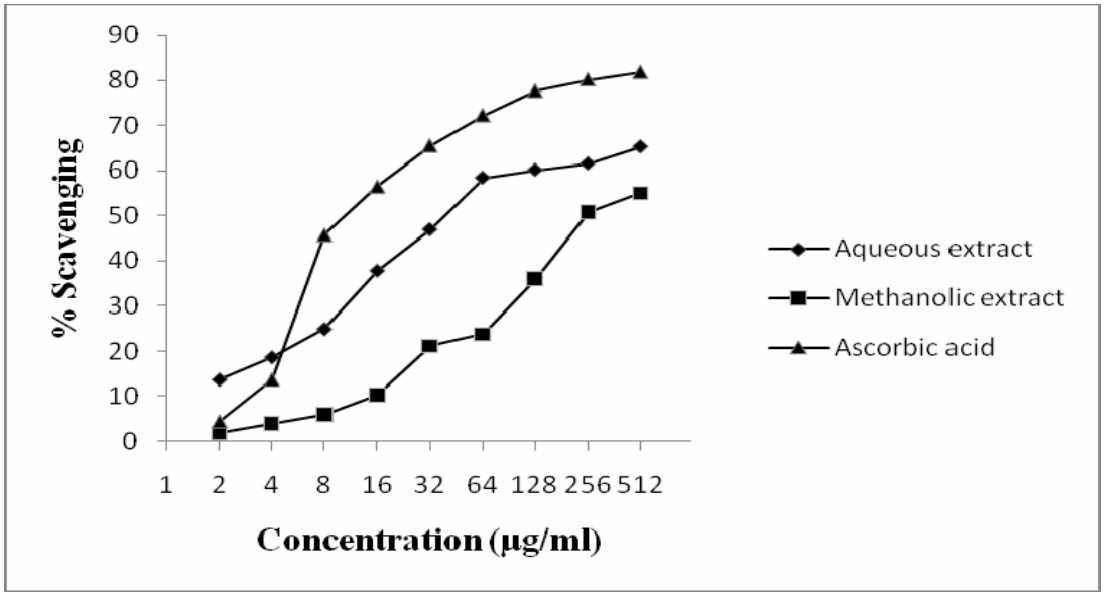

Figure 3: Effect of aqueous and methanolic extracts of In-house Avipattikar churna on O-phenanthroline assay.

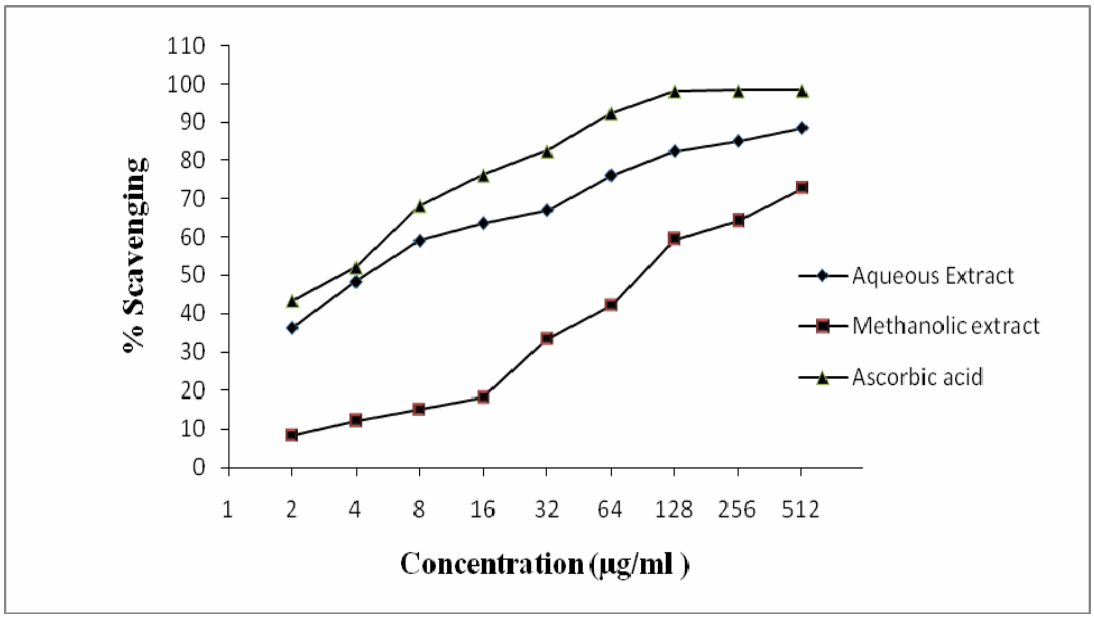

Figure 4: Effect of aqueous and methanolic extracts of In-house Avipattikar churna on Superoxide scavenging assay.

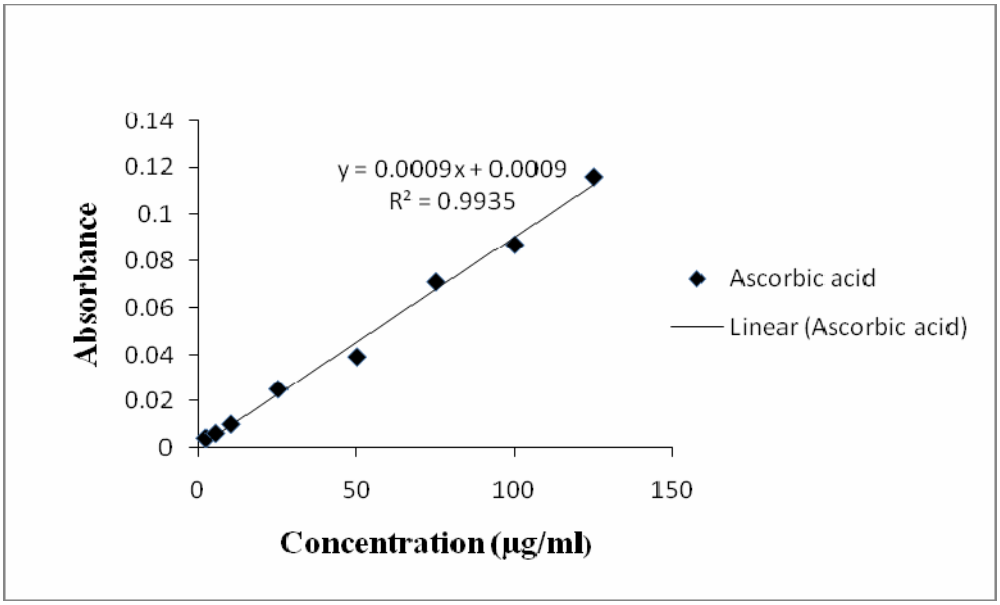

Figure 5: Ascorbic acid standard plot in Total antioxidant capacity assay.

Discussion

Oxidation processes are very important for living organism. The uncontrolled production of reactive Oxygen species (ROS) and the unbalanced mechanism of antioxidant protection results in the onset of many diseases and accelerates aging. ROS include free radicals such as hydroxyl radicals (OH-), Superoxide (O_2-) and non-free radical species such as H_2O_2 and singlet oxygen (O_2)[9,10,11]. These species are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signalling and immune function. Over production of reactive species, induced by exposure to external oxidant substances or a failure in the defence mechanisms, cause damage to cell strctures, DNA, lipids and proteins[12] occur which increase risk of more than 30 different disease processes[13]. Interestingly the body possess defence mechanism against free radical induced oxidative stress, which involves preventive mechanisms, repair mechanisms, physical defences and antioxidant defences. Enzymatic and Non-enzymatic antioxidants like ascorbic acid (Vitamin C), α- tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, etc. act by one or more of the mechanisms like reducing activity, fee radical scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. It is possible to reduce the risks of chronic diseases and progression by either enhancing the body's natural antioxidant defences by supplementing with proven antioxidants[14]. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in processed foods have side effects and are carcinogenic[15, 16]. This is one of the reasons why discovery and synthesis of novel antioxidants is a major active area. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by many other mechanisms and thus prevent disease[17]. Natural antioxidants were closely related to their biofunctionalities, such as the reduction of chronic diseases like DNA damage, mutagenesis, carcinogenesis and inhibition of pathogenic bacteria growth which is often associated with the termination of free radical

propogation in biological systems[18]. Antioxidant capacity is widely used as a parameter for medicinal bioactive components. The present study reveals the *in Vitro* antioxidant potential of aqueous and methanolic extracts of Avipattikar churna. The ingredients present in Avipattikar churna have been shown in different studies to possess biological properties related to antioxidant mechanisms eg. The five phenolic amides of Piper nigrum are documented to possess antioxidant activity, more significant than α-tocopherol[19]. Zerumbone, a sesquiterpene and gingerol, a plant phenol both active constituents of another ingredient of Avipattikar churna, *Zingiber officinale* are reported antioxidants[20.] Agarwal et al.[20] have reported the ability of aqueous extract of Piper longum, Zingiber officinalis to augment mucin secretion and to decrease cell shedding in the stomach of the rat. Hence, the significant antioxidant activity of extracts of Avipattikar churna in the present study may be attributed to these aforementioned potent antioxidant ingredients of Avipattikar churna.

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