ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF PARTIALLY PURIFIED PROTEIN FROM HOT WATER EXTRACT OF TURMERIC (*CURCUMA LONGA L*)

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Summary

Turmeric (*Curcuma longa Linn*) – also called as Indian Saffron of Zingiberaceae family, widely used mainly as food additive, colourant and much more as medicine mainly in South – East Asian countries including India. Curcumin, the yellow colored phenolic compound present in turmeric exhibits many biological activities like antioxidant, antibacterial, anti-inflammatory. A water soluble antioxidant protein isolated from hot water extract of Turmeric. Sephadex G-25 gel filtration chromatography profile of the aqueous extract showed 3 different peaks. Peak -1 was examined for its antioxidant and antimicrobial activity. The protein at 10µg concentration showed about 85 and 78 % inhibition in Hydroxyl radical scavenging assay and lipid peroxidation assay respectively. Curcumin, BHA, α-tocopherol. Disc diffusion method was used to identify the anti microbial activity of the protein. The protein was checked for its antibacterial activity against human pathogenic bacteria's. MIC value of antioxidant protein ranged from 100-121 µg/ml. The protein was also found to be effective against fungi - *Candida albicans,* MIC was about 140 µg/ml. Streptomycin and Amphotericin was used as a standard antibacterial and antifungal agents. These results show that, the antioxidant protein from Turmeric is an effective antioxidant as well as antimicrobial agent.

Keywords: Turmeric; proteins; antioxidant; antimicrobial, free radicals, Minimum inhibition concentration

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Introduction

Antioxidants play a very important role in the defense system of body and act as radical scavenger, hydrogen donors, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, metal chelating agents. Antioxidants are capable of slowing or preventing the oxidation of other molecules in the body[1,2,3]. They also prevent these oxidation chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being self oxidation[4]. Standard antioxidants like Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toulene (BHT) are effective antioxidants, but they are toxic at higher concentration and cause side effects which rules out their prolong human use[5].

Microbial infection is the concern for human. Antimicrobials kill or inhibit the growth of microbes such as bacteria, fungi, protozoa's and viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). The toxicity to humans and other animals from antibiotics is generally considered to be low where as prolonged use of antibiotics shows a negative impact on health[6]. Some times, antibiotics are associated with adverse effects including hypersensitivity, immune-suppression and allergic reactions[7] and also emergence of antibiotic resistant microbial strain had been a concern. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases form various natural sources such as medicinal plants with lesser side effects. Indigenous herbal remedies are widely used against many infectious diseases, but only few of them have been studied chemically and biologically in order to identify their active constituents[8]. Many antimicrobial proteins have been isolated from plants and mechanism of action demonstrated[9,10,11]. However, very few proteins with antibacterial activity has been reported till date, such as antibacterial proteins isolated from radish seeds[12,13].

Turmeric (*Curcuma longa Linn*) – also called as Indian Saffron of Zingiberaceae family, widely used mainly as food additive, colourant and much more as medicine mainly in South –East Asian countries including India. Earlier a novel water soluble antioxidant protein was isolated from aqueous extract of Turmeric called Turmerin (~14kDa)[14]. Turmerin comes in the second peak in gel permeation profile.

In the present study, we have checked for the antioxidant and antimicrobial activity of peak- 1 fraction of gel permeation chromatography.

Materials and methods

Turmeric powder was obtained from the local market of Mysore, Karnataka, India, BHA, α-tocopherol, 2-deoxy ribose were purchased from Sigma Aldrich Co., (St. Louis, USA), Sephadex G-25 from Pharmacia co., Sweden. Other chemicals unless otherwise mentioned were procured from Merck India Co., Mumbai.

Pure clinical isolates of human pathogenic bacteria, *Escherichia Coli, Vibrio Cholerae, Staphylococcus aureus, Streptococcus sps, Bacillus subtilis, Klebsiella pneumoniae, Salmonella paratyphi, Salmonella typhimurium, Pseudomonas*, *Proteus vulgarigus* and fungi *Candida albicans, Fusarium sps, Aspergillus flavus and T. Mentagranophytes* were obtained from Microbiology department, Adichunchanagiri Institute of Medical Sciences (AIMS), B.G. Nagara, Karnataka, India.

Isolation and partial purification of protein

Ten grams of Turmeric powder was added to 300 ml boiling double distilled water and again boiled for 10 minutes, vortexed at ambient temperature for two hours. It was centrifuged at 10,000 rpm at 4^{o}C for 20 min. The supernatant was lyophilized, filtered through Whatmann No. 1, followed by 0.22 micron filter, and stored at –20^{o}C for further use.

3 mg Protein was loaded on to Sephadex G-25 (Vt = 140 ml, Vo = 47ml, flow rate 12 mL/hr, mobile phase 100 mM Tris buffer saline, pH 7.4) and fractions were monitored at 280nm using Shimadzu spectrophotometer (1601A).

Proximate analysis

The protein content was estimated by Bradford's method[15] for both crude and Peak I of the Turmeric boiling water extract. The total sugar was estimated by the phenol–sulphuric acid method[16], total phenolic content was done according to Folin–Ciocalteau method[17], total flavonoids content was estimated was done according to Woisky and Salatino method[18] and the Curcuminoids concentration was estimated by Vijayalakshmi et al method [19].

Lipid peroxidation inhibition activity by TBARs:

The spectrophotometric assay for evaluating antioxidant activity was based on the inhibition of peroxidation in RBC ghost[20]. An assessment of oxidation was achieved by measurement of thiobarbituric acid reactive substances[21]. The human erythrocyte ghost was isolated according to the method of Dodge et al.[22]. 100 µ1 of ghost suspension (300 µg membrane protein equivalent) was subjected to peroxidation by 10:100 µmol of ferrous sulphate and ascorbic acid[23,24] in final volume of 1 ml of Tris buffered saline (20 mM, pH 7.4, 150 mM NaCl). The reaction mixture was treated with or without Peak I (10µg). Butylated hydroxy anisole (BHA) was used as positive control. The contents were incubated for 1 hour at 37°C. The reaction was terminated by the addition of 10 µl of 5% phenol and 1 ml of 1% trichloroacetic acid (TCA). To each system 1 ml of 1% thiobarbituric acid (TBA) was added, the contents were kept in a boiling water bath for 15 min, cooled and centrifuged at 6000 rpm for 10 min. The absorbance of supernatants was measured colorimetrically at 535 nm. Appropriate blanks were included for each measurement. The negative control without any test sample was considered as 100% peroxidation. The % inhibition of lipid peroxidation was determined accordingly by comparing the absorbance of the test samples with negative control.

Hydroxyl radical scavenging activity

Deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium[25]. The reaction mixture containing $FeCl_3$ (100 μM), EDTA (104 μM), H_2O_2 (1 mM) and 2-deoxy- D-ribose (2.8 mM), were mixed with or without Peak I at 10μg concentration in 1 ml of final reaction volume made with potassium phosphate buffer (20 mM pH 7.4) and incubated for 1 h at 37°C. The mixture was heated at 95°C in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally the reaction mixture was cooled on ice and centrifuged at 5,000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm. All readings were corrected for any interference from brown color of the extract or antioxidant by including appropriate controls. The negative control was without any antioxidant was considered as 100% deoxyribose oxidation. The % hydroxyl radical scavenging activity of test sample was determined accordingly in comparison with negative control.

Antimicrobial activity and minimum inhibition concentration of Peak I

Antimicrobial activity was evaluated by using Disc diffusion method on nutrient agar medium for Bacterial culture and Sabouraud's dextrose agar medium for yeast strains[26]. For *Streptococcus sps* Blood Nutrient agar was used as media. Streptomycin and Amphotericin were used as positive controls, for bacterial and yeast strains and sterile water as negative control.

The minimum inhibitory concentration (MIC) was determined by serial dilution of Peak-I protein of Turmeric in the broth medium, with different concentrations. Plates were incubated for 24 hours at 37 ^{0}C for bacteria and 48 hours at 24^{0}C for yeast cultures. MIC was recorded as lowest extract concentration demonstrating no visible growth in the broth. All the plates were done in triplicates (Karou *et al.,* 2005).

Results and discussion

G-25 Gel filtration gave 3 main peaks, in which the first peak (Peak I) was chosen for further studies **(Fig-1).** Peak I contains water soluble higher molecular weight proteins and Native gel confirms its acidic nature. The fractions of peak I was pooled, lyphilized and dialyzed against double distilled water for 48 hours and stored at -10°C for further studies. The proximate analysis showed the dialyzed peak I did not contains reducing and non reducing sugars and also no Curcumin **(Table-1)**. It showed about 78 and 85% inhibition against lipid peroxidation and hydroxyl radical scavenging activity at 10μg concentration respectively **(Table -2)**.

Fig.1. Sephadex G-25 Fractionation of Turmeric extract

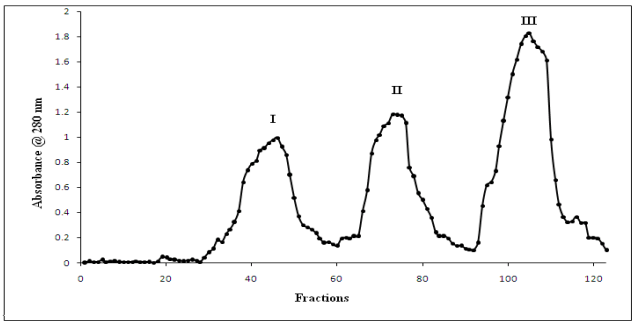


Fig. 1 Fractionation of crude protein of Turmeric extract through Sephadex G-25 column (1.2 cm diameter and 120 cm height), bed volume was 140 ml. The column is equilibrated with tris buffer pH 7.4. The flow rate was adjusted to 12ml/hr.

Table -1. Biochemical characterization of Peak I

Tests	Crude (g%)	Peak I (dialyzed) (g%)
Protein	**0.235**	**0.112**
Curcuminoids	0.182	Nil
Sugars	7.4	0.002
Polyphenols	0.6	Nil
Flavinoids	0.89	0.003

The results represents mean ± S.D (n = 3)

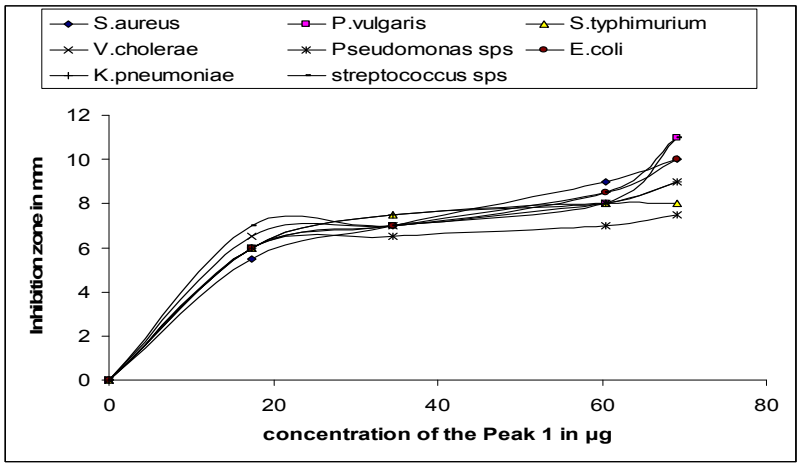
Table -2: Antioxidant activity of Peak I of Turmeric extract in comparison with Standard antioxidant

Sl. No	Antioxidants	Concentration	Inhibition (%) Hydroxy radical	Inhibition (%) TBARS
1	**α tocopherol**	400 μM	85 %	92 %
2	**Curcumin**	400 μM	74 %	80 %
3	**BHA**	400μM	85%	78%
3	**Crude extract of Turmeric**	25 μg	72 %	75 %
4	**Peak I**	10μg	85 %	78 %

The results are mean ±S.D (n = 6)

Peak I proteins were tested against many human pathogenic bacterial strains, among all, it is found to be more effective against a *Klebsiella pneumoniae, Vibrio cholerae, Bacillus subtilis and pseudomonas sps*. MIC values of Peak I against the bacterial strains ranged from 100 µg/ml to 121 µg/ml concentration. Steptomycin was taken as positive control, where its MIC value varied from 17 µg/ml – 24 µg/ml concentration (Table-3). Dose dependent antibacterial activity was done for peak I (Fig-2). *Salmonella paratyphi and Bacillus subtilis* did not show any inhibition

Fig. 2. Dose dependent Antibacterial activity of Peak I of Turmeric extract



Dose dependent antibacterial activity of Peak I in Agar diffusion assay against human Pathogenic bacterial strains

Table-3: Minimal inhibitory concentration (MIC) of Peak I against different microbial strains in serial dilution method

Microorganisms	MIC of Peak I (µg/ml)	Positive control Streptomycin/Amphotericin (µg/ml)
Bacterial strains:		
Escherichia coli	116 ± 0.5	16 ± 0.5
Klebsiella pneumoniae	121 ± 0.5	14 ± 0.5
Proteus vulgaris	100 ± 1	13 ± 1
Pseudomonas	100 ± 0.5	13 ± 1
Salmonella typhimurium	121 ± 2	15 ± 0.5
Staphylococcus aureus	103 ± 1	14 ± 1
Streptococcus sps	102 ± 0.5	17 ± 0.5
Vibrio cholerae	110 ± 0.5	14 ± 1
Salmonella paratyphi	Nil	15 ± 0.5
Bacillus subtilis	NIl	16 ± 0.5

Yeast strain		
Candida albicans	140 ± 0.5	22 ± 1
Fusarium sps	Nil	19 ± 0.5
Aspergillus flavus	Nil	23 ± 1
T. Mentagranophytes	Nil	22 ± 0.5

Inoculum was prepared from fresh overnight broth culture in nutrient broth. Plates were incubated for 24 hrs at 370C. Values are expressed as mean ± SD (n=3).Streptomycin is used as positive control for bacterial strain and Amphotericin for Fungal and yeast strains. Peak I protein was tested against *Candida albicans*, for which MIC values of 140 µg/ml concentration. Whereas, Amphotericin showed an MIC value of about 22 µg/ml concentration. *Fusarium sps, Aspergillus flavus* and *T. Mentagranophytes* did show any inhibition against Peak I

The above results indicates that, peak I is a good antioxidant and also anti microbial agent. It is effective at lower concentrations and also non toxic. It shows a broad spectrum of anti microbial activity.

Conclusion

Peak I fractions of Aqueous extract of turmeric can be effectively used as antioxidant as well as antimicrobial agent. Further study need to be done in purifying the peak I and characterizing it.

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