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Comparative study of Antioxidant Activity of Herbal Drugs and their Formulations using *Asparagus racemosus* and *Centella asiatica*

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ABSTRACT

Asparagus racemosus and *Centella asiatica* are two very common and well known medicinal plant available in India. Both the plants are traditionally used as antiamoebic drugs. Not only are those, both the drugs used as the herbal treatment of several diseases of human beings. In between them some are really critical disease. Several important phytoconstituents have been already isolated and characterized from both the plants and in between them some are the major active marker for the treatment of above said critical diseases. Instead of all those characters, these two plants have very potent antioxidant property. Here, we have taken an attempt to prepare a formulation of the mixture (50:50) of the aqueous extracts of both the plants and compare the antioxidant property of that formulation and extracts. In this study calcium chloride cross linked alginate microsphere and chitosan coated microsphere have been prepared by using the above said plant extracts and the anti-oxidant property of those formulations were compared with the extracts and also standard drugs. The result therefore reflects that the antioxidant potency of the combined extract entrapped in formulations was almost equivalent to the original extract combinations. This entails despite of the different process control stresses in the manufacturing steps the potency of the polymer entrapped drug substances does not changed. So researchers may look forward in further purification of the extract and then an effective drug loading to get an equivalent potent action from the same quantity of a standard antioxidant.

Keywords: Antioxidant activity**,** *Asparagus racemosus*, *Centella asiatica*, microsphere

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INTRODUCTION:

Herbal Medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals and by trial and error[1]. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native people[2]. Many drugs commonly used today are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound. Herbal medicinal products are defined as any medicinal product, exclusively containing one or more active substances. WHO report 80% of the world population relies on the drug from natural origin. A large number of medicinal plants are used in the treatment of diabetes. Diabetes is a metabolic disorder with major complication associated with hyperglycemia, inflammation, foot ulcer, nerve disorders and sexual depression[3]. If treatment means to cure the disease, there is no drug which can cure Diabetes completely and some evidence were found practically and theoretically treatment of diabetes in yoga and Ayurveda. Keeping in view of the importance of the disease and also considering the fact that green medicine are safe. So, two different herbal drugs *Asparagus racemosus* and *Centella asiatica* extracts were selected for preparation of microsphere in this project. *Asparagus racemosus* Willd. (Liliaceae), commonly known as 'Shatavari', is a much-branched, spinous under shrub found growing wild in tropical and sub-tropical parts of India. *Asparagus racemosus* is a well known Ayurvedic rasayana which prevent ageing, increase longevity, impart immunity, improve mental function, vigor and add vitality to the body and it is also used in nervous disorders, dyspepsia, tumors, inflammation, neuropathy, hepatopathy. *Centella asiatica* is a profusely branched prostrate herb consisting of active principles such as Vallarine, Asiaticoside, Sitosterol, Tannin, Oxy –asiaticoside. Asiaticoside is used in the treatment of leprosy. Sitosterol and tannin possess antiprotozoal & spasmolytic property. According to Siddha literature, its leaves are used in the treatment of syphilis. *Centella asiatica* may be useful in the treatment of Anxiety and may be used as a promising anxiolytic agent in the future. These two plant extracts

have a very potential antioxidant property in human body. So an attempt has been taken to compare their antioxidant property when they are in free extract form and also when they are in a formulation called microsphere[3-4].

Figure 1: Leaves of *Centella asiatica*

Figure 2: Roots of *Asparagus racemosus*

MATERIALS AND METHODS

Plant materials and their extraction

Cold maceration extraction method was carried out to extract the chosen drugs with alcohol as a solvent. First, adequate amount of Thankuni leaves (*Centella asiatica*) and Satavari roots (*Asparagus racemosus*) were collected from the Medicinal Plants' Garden, Gupta College of Technological Sciences, Asansol, authenticated from Department of Botany, Netaji Mahavidyalaya, Hoogly (NM/BOT/AUTH/032) and shade dried for two weak. Then the leaves and the roots were cut into small pieces and extracted freshly by using distilled water for seven days. For better extraction the mixtures were occasionally stirred. After 7 days the extracts were filtered and kept it in Rotary flash evaporator for volume reduction. The temperature was maintained under 40^{o}C. After getting a semisolid residue, it was kept in vacuumed desiccators to make it completely dried powder.

Preparation of microsphere [5-8]

Several different compositions were prepared, from which only two compositions were successful to give microsphere and others have given distorted sphere shapes. The compositions and there preparation procedures are given bellow. For these formulations, alginic acid was obtained from LOBA CHEMIE Pvt. Ltd., Mumbai, India and calcium chloride was obtained from MERCK Specialties Pvt. Ltd. and chitosan was obtained from PUREX Laboratories Pvt. Ltd. and at last the quercetin was obtained from S.D. Fine Chemicals.

Sodium alginate-Calcium chloride microsphere

1% w/v of drug solution was prepared in 3% w/v aqueous sodium alginate solution. Then 4%w/v calcium chloride solution was prepared. Then the drop of drug-sodium alginate solution was dropped in 4% calcium chloride with the help of syringe and 18 gauge needle. The drops were kept there in the calcium chloride solution for 15-20 minutes for the cross-linking. Then it was strained with strainer and washed with distilled water for at least three times. After that extra water were strained out with the help of tissue paper and the microspheres were kept in the hot air oven at 45^{o}C to 50^{o}C to dry.

Sodium alginate-Calcium chloride microsphere encoated with 0.3%w/v chitosan Solution

1%w/v of drug solution is prepared in 3% w/v aqueous sodium alginate solution. Then 4%w/v calcium chloride solution is prepared. Next 0.3%w/v chitosan was prepared. Then the drop of drug-sodium alginate solution was dropped in 4% calcium chloride with the help of syringe and needle. The drops were kept there in the calcium chloride solution for 15-20 minutes for the cross-linking. The microspheres were strained and dropped into the 0.3%w/v chitosan solution with continuous stirring in a shaker for 15 minutes so that the chitosan form an uniform layer around the microspheres. After 15 minutes the chitosan coated microspheres were strained with strainer and washed with distilled water for at least three times. After that extra water were strained out with the help of tissue paper and the microspheres were kept in the hot air oven at 45^{o}C to 50^{o}C to dry.

Evaluation of microsphere

Test for checking antioxidant property [9-13]

Various concentrations of test solution and 50 µl of DPPH (0.659mM) solution were incubated at 25°C for 30 min. Following which the absorbance is read at 510 nm. A control reaction was carried out without test sample. Linear graph of concentration vs. percentage inhibition was prepared and IC_{50} values were calculated. The percentage inhibition was calculated according to following equation

$$\% \text{ inhibition} = (A_0 - A_t)/ A_0 \text{ X } 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract.

Standard drug (Quercetin)

Standard curve was plotted using a potent known antioxidant drug (quercitin) by taking a stock solution of 40µg/ml and then a serial dilution was done to get different concentrations.

Extracted drugs (SHATAVARI and THANKUNI)

At first, 10mg of DPPH was dissolved in 10 ml of methanol and 10ml of 1mg/ml solution of DPPH (stock solution) was prepared. Then 1200µl (1.2ml) of the stock solution was taken in a volumetric flask and the volume was made up to 30ml with methanol.

20mg of Thankuni leaves extract and 20mg of Satavari root extract were weighed accurately and dissolved in 40ml of methanol. The absorbance of the solutions of different concentrations were measured and IC_{50} was observed.

C) Microsphere (containing drug extracts)

At first, 10mg of DPPH was dissolved in 10 ml of methanol and 10ml of 1mg/ml solution of DPPH (stock solution) was prepared. Then 1200µl (1.2ml) of the stock solution was taken in a volumetric flask and the volume was made up to 30ml with methanol.

233.86 mg microspheres were kept in 100ml Phosphate buffer (pH 6.8) for 24 hour so that the total drug extract may release from the microsphere. Then it was filtered out through the filter paper. The absorbance of the solutions of different concentrations were measured and IC_{50} was observed.

Total phenolic content [13]

0.01ml (1mg/ml) methanolic solution of extracts (1:1) was taken. 4.5ml distilled water and 0.1ml F.C. reagent were added to it and shaken for 3 minutes. 0.1ml of 2% sodium carbonate solution was added and kept in the dark for 2 hour. After 2 hour the absorbance of the solution was measure at about 760nm. Similarly by both the microspheres, the standard drug (Gallic acid) and blank were carried out.

Particle size determination through high resolution optical microscope [11]

The sizes of the microspheres were determined using optical microscope (Magnus,) fitted with an ocular micrometer. The ocular micrometer was calibrated with a stage micrometer. Fifty randomly chosen microparticles were taken to measure their individual shape and size. Microparticles visualized under 4X magnification and diameter noted. Particle size of the microspheres in first formulation ranges between 10-12µm (Fig.3) and that of the second formulation was between 8-10 µm (Fig.4) which can be actually reasoned due to the presence of both calcium chloride and chitosan in the polymer matrix the microspheres of second formulation are much bigger in size than that of the microspheres of first formulation where only calcium chloride was used for cross linking.

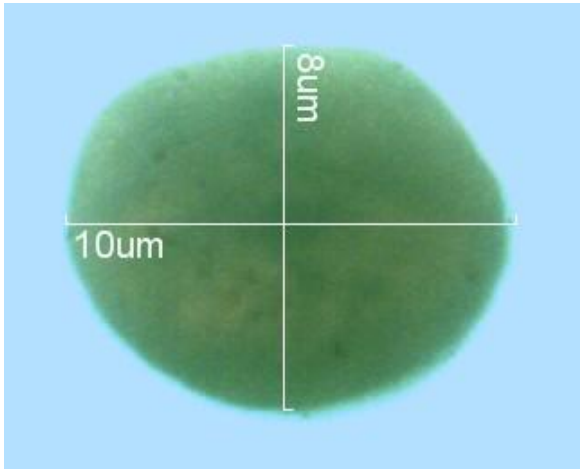

Figure.3: Calcium chloride cross linked alginate microsphere observed under high resolution microscope

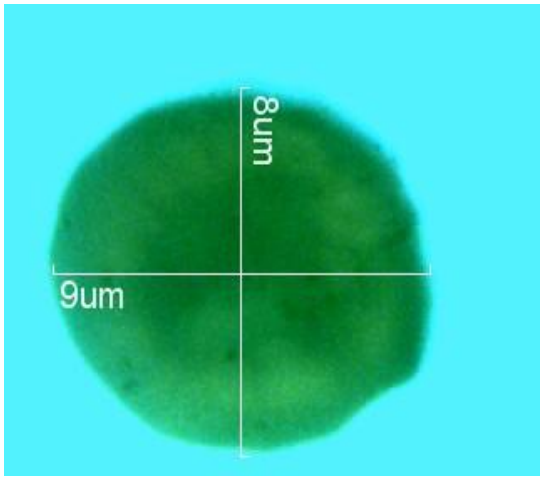

Figure.4: Chitosan coated microsphere observed under high resolution microscope

RESULTS AND DISCUSSION

This study analyzes the antioxidant capacity of herbal drugs and their formulation using *Asparagus racemosus* and *Centella asiatica***.** Radical scavenging activities are important due to the deleterious role of free radicals in foods and in biological systems. DPPH assay evaluates the ability of antioxidants to scavenging free radicals. The method is based on the reduction of alcoholic DPPH solution into non-radical from DPPH-H in the presence of a hydrogen-donating antioxidant.The extract combination and its formulation exhibited a concentration dependent DPPH radical scavenging capacity (Table 1). Total phenolic content of the extract combination and the microspheres were compared (Table2).

Table 1: IC_{50} Value of marker, extract combined and both the formulations

IC_{50} of quercetin	IC_{50} of extract combination	IC_{50} of Chitosan coated microsphere	IC_{50} of calcium chloride coated microsphere
17µg/ml	140µg/ml	70µg/ml	90µg/ml

Table 2: Total phenolic compound determination of extract combined and both the formulations

Total Phenolic Compound of extract combination	Total Phenolic Compound of Chitosan coated microsphere	Total Phenolic Compound of calcium chloride coated microsphere
0.0641 µg/ml	0.0122 µg/ml	0.00136 µg/ml

By comparing the experimental drug with the standard drug it was found that the efficacy and potency of the experimental drug is also high comparison with the standard drug, in short experimental drug is also potent and efficacious. Also by combining *Asparagus racemosus* and

Centella asitica as experimental drug it was found that there is no drug interaction or toxicity and the antioxidant property have been increased with minimal toxic effect.

After performing the DPPH test it has been concluded that although the IC_{50} of both microspheres were less than individual drug mixture (Extract combined) and so this microspheres can be used in the human body as a well accepted antioxidant drug formulation.

After performing the Total Phenolic content it has been concluded that the mixture of extract shows maximum phenolic content and although the chitosan coated microsphere and calcium chloride coated microsphere showed less phenolic content but the difference is not very high to prepare the formulation and with less phenolic content also both the microspheres were showed a better antioxidant property than the extract combined.

After determination of particle size through high resolution microscope it has been concluded that as these formulated particles release the drug in the intestine, from which the drug is absorbed through Peyer's Patch principally, the size range of the microspheres $\leq$ 10 µm is optimal for taking up by the patches.

The surface of chitosan coated particles is smoother than the uncoated alginate particles. Smoother the surface lesser will be the chance of recognition of particles as foreign materials.

CONCLUSION

From the results therefore it has been reflected that the antioxidant potency of the combined extract entrapped in formulations was almost equivalent to the original extract combinations. This entails despite of the different process control stresses in the manufacturing steps the potency of the polymer entrapped drug substances does not changed. So researchers may look forward in further purification of the extract and then an effective drug loading to get an equivalent potent action from the same quantity of a standard antioxidant.

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REFERENCE

1. Dev S. Ethno botanical survey of traditional plants. Current Science 1997; 73: 909-928.

2. Yoganarsimhan, SN. Medicinal Plants of India-Karnataka. Bangalore, India: Interline Publishing (P).Ltd 2000: 25-28.
3. Yoganarsimhan, SN. Medicinal Plants of India-Tamilnadu. Bangalore, India: Interline Publishing (P).Ltd 2000: 56-59.
4. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol 1, Dehradun: Industrial Books Distributer; 1995: 326-328.
5. McGinity JW, O'Donnell PB. Preparation of microspheres by the solvent evaporation technique. Advanced Drug Delivery Review 1997; 28(1): 25-42.
6. Jayaprakash S, Mohamed HS, Mohamed Firthouse PU, Kulaturanpillai K, Abhijith Nagarajan M. Preparation and evaluation of biodegradable microspheres of Methotrexate. Asian J Pharm 009; 3(1): 26-29.
7. Venkatesan P, Muralidharan C, Manavalan R, Valliappan K. Selection of better method for the preparation of microspheres by applying Analytic Hierarchy Process. J Pharm Sci Res 2009; 1(3): 64-78.
8. Gheorghe Fundueanu, Elisabetta E, Mihai D, Carpov A, Desbrieres J, Rinaudo, M, Nastruzzi C. Preparation and characterization of Ca-alginate microspheres by a new emulsification method. Int J Pharm 998; 170: 11–21.
9. Oyaizu M. Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986; 44: 307-315.
10. Buyukokuroglu ME, Gulcin I, Oktay M, Kufrevioglu OI. In vitro antioxidant properties of dantrolene sodium. Pharmacol Res 2001; 44: 491-495.
11. Korycka-Dahl M, Richardson MJ. Photo generation of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acids. Dairy Sci 1978; 61: 400-407.
12. Miller MJ, Sadowska-krowicka H, Chotinaruemol S, Kakkis JL, Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition. The J. Pharmac. And Experi Thera 1993; 264: 11-16.
13. Boyer RF, Clark HM, Laroche AP. Reduction and release of ferritin iron by plant phenolics. J Inorg Biochem 1988; 32: 171-181.