Evaluation of free radical scavenging and immunostimulant activities of water soluble polymeric substances from *Cinnamomum zeylanicum* bark

A thesis submitted in the partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

By

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CERTIFICATE

This is to certify that the thesis entitled **"Evaluation of free radical scavenging and immunostimulant activities of water soluble polymeric substances from *Cinnamomum zeylanicum* bark"** submitted by Navdeep Kaur in partial fulfillment of the requirement for the award of Degree of Master of Science in Biotechnology to Thapar University, Patiala, is a record of the student's own work carried out by her. The report has not been submitted for the award of any other degree or certificate in this or any other University or Institute.

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CANDIDATE'S DECLARATION

I, hereby declare that the work presented in the thesis entitled **"Evaluation of radical scavenging and immunostimulant activities of water soluble polymeric substances from *Cinnamomum zeylanicum*"** and its free radical scavenging activities of *Cinnamomum zeylanicum* bark" in the partial fulfillment of the requirement for the award of the degree of Master of Science in Biotechnology, Department of Biotechnology, Thapar University, Patiala, is an authentic record of my work during the period of one year from July 2015 to June 2016, under the guidance of Dr. Manoj Baranwal, Assistant Professor, Thapar University, Patiala. I have not submitted the matter embodied in this thesis for the award of any other degree or diploma.

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(301401009)

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ABSTRACT

Cinnamomum zeylanicum is a commonly used spice with many medicinal properties but not much scientific data is available on bioactive properties of water soluble polymeric substances of *Cinnamomum.* Hence, in the present study, free radical scavenging and immunostimulating effects of water soluble polymeric substances evaluated. Chemical analysis of polymeric substances confirm the presence of protein and the polysaccharide contents was found to be 250 µg/mg taking glucose as standard. DPPH assay results shows that free radical scavenging activity of polymeric extract increased with concentration and Further MTT assay were carried out to study the effect of extract on growth of unstimulated and concanavalin A stimulated peripheral blood mononuclear cells (PBMCs). It has been observed that 500 and 1000 µg/ml of polymeric substances induce the proliferation of both unstimulated and stimulated PBMCs which suggest the immune stimulating activity. Present study suggest that *Cinnamomum zeylanicum* possess bioactive water soluble polymeric substances which has the potential to be used for medicinal purpose.

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CHAPTER 1: INTRODUCTION

The plants having medicinal properties play a very important role in the treatment of a range of diseases. These plants derived medicines are called as traditional or Herbal medicines. In India, plants based medicines or drugs are being used for treatment of various diseases from ancient times, because of have therapeutic values (Narayanaswamy *et. al*., 2011). Plants or herbs are known to be as a part of "Nature's Pharmacy". Their ways of action similar to modern drugs and the medicines made by plants are generally safer than the modern drugs. To formulate the medicines or drugs by plants, one can uses the whole plant or a part of plant like the flowers, the roots, the bark, or the fruits, (Vangalapti *et. al*., 2012) and the materials such as essential oils, gums, resins or the preparation of herbal medicines can be done by fractionation, concentration, extraction etc (WHO, 1997).

In medical systems, herbal medicines act as a major remedy to maintain human health. In developing countries, majority of world's population depends upon the herbal medicines (WHO, 2002). In the traditional and medieval world spices are being used for the therapeutic purposes. Spices like Garlic, Curcumin, Ginger, Black pepper, Cinnamon and Coriander have different natural phytochemicals and used as home remedies (Vasanthi *et. al.,* 2010).

Cinnamon is a common spice which is used around the world for several centuries. It belongs to the family Lauraceae. The inner bark of cinnamon used for the preparation of medicines in Ayurveda system. Powder of cinnamon bark used as the good mouth freshner. The two major bioactive compounds present in cinnamon are Cinnamaldehyde and Eugenol (Manosi *et. al.,* 2013). There are many other compounds which are present in different parts of cinnamon (root bark, stem bark, leaf, flower and fruit) but in fewer amounts. These compounds are cinnamate, cinnamic acid, trans cinnamaldehyde, L-borneol, caryophyllene, caryophyllene oxide, E-nerolidol, L-bornyl acetate, eugenol andeseential oils present in cinnamon (Jakhetia *et al.,* 2010). Cinnamon bark has been extensively studied and its essential oils and water based extracts have been seen to possess pharmacological properties- such as anti-microbial (Gende *et. al.,* 2008), anti-inflammatory (Manosi *et. al.*,2013), anti-oxidant (Mazimba *et. al.,* 2015), anti-diabetic (Desoky *et. al.,* 2008*),* immune modulating activity (Balekar *et. al.,* 2014) and anti-ulcer (Jakhetia *e.t al.,* 2012). It is used in production of toothpaste, mouthwash, lotion, pharmaceuticals, cosmetics, chocolates, coffee, candies, tea, alcoholic beverages, and stimulating appetite.

Although medicinal value of *Cinnamomum zeylanicum* is well established, but limited report on the bioactive properties of water soluble polymeric substances obtained from it. Hence present study is oriented towards the assessment of free radical scavenging and immunostimulant activities of water soluble polymeric obtained from *Cinnamomum zeylanicum.*

CHAPTER 2: REVIEW OF LITERATURE

2.1 Spices and Herbs

Spices and herbs are widely used for its preservation, flavouring and medicinal properties. Earlier 50,000 B.C., it was determined that the parts of plants like leaves are used by the humans for meat flavouring and making wine (Kaefer *et. al.,* 2007). The medicines made using herbs and spices are widely used by the Egyptian and Chinese for the treatment of various diseases and in Rome and Greece they were used them as flavouring agents. Scientific study suggested that spices and herbs have antioxidant property and contains phenolic compounds (Vasanthi *et. al.,* 2010).

2.2 Polyphenols present in Herbs and Spices

Polyphenols belongs to a family of large compounds where the hydroxyl groups attached to aromatic rings. There are four major group of dietary polyphenols which are flavanoids (anthocyanidins, flavonones, flavonols, isoflavonones), phenolic acids (hydroxylcinnamic acids, hydoxybenzoic acids), stilbenes and lignans (Opara *et. al.,* 2014). Polyphenols show different properties like antioxidant, anti-inflammatory, neuroprotective and anti-cancer. The diseases and the formation of harmf ul compounds due to metabolic disorders are blocked by these phenolic substances (Vasanth *et. al,* 2010).

2.3 Herbal medicines

It is a preparation made by plants having therapeutic effects or human health welfare. It includes the active ingredients or a part of a plant materials like leaves, bark, root, fruit, seed, stems, gums, resins, wood, essential oils, dry powdered etc (WHO, 2000).It has been determined that over 60% of the drugs are made by herbal preparations for anticancer and anti-hypersensitive. Therefore medicinal plants have become essential source for discovery of new drugs. (Kigen *et. al.,* 2013).

Herbal medicines exhibit marked differences when compared with the modern or synthetic drugs as follows:

- The active ingredients present in the herbal medicines are not known, whereas in modern medicines, it is well defined or identified.
- The quality and availability of raw material used for herbal preparations are frequently problematic.
- Herbal medicines are more easily accepted by the people because of their wide therapeutic use as compared to modern medicines.

- In case of cost, modern medicines/drugs have much higher cost than the herbal medicines.
- In case of herbal medicines, the steps involved (quality control, stabilization and stability) possible but not easy.
- Herbal medicines having low or negligible side effects whereas in synthetic drugs it is much higher.
- The clinical or scientific data usually not available for herbal medicines but in case of modern medicines it is easily available.
- Herbal preparations containing mixture of compounds whereas in modern medicines a particular compound is present.

Table 1. Examples of herbal drugs and their active ingredient present in it (Martins Ekor, 2000).

HERB	Active ingredients	Properties
Ephedra sinica	alkaloid ephedrine and pseudoephedrine	Relieves common cold and asthma
Allium sativum (Garlic)	Alliin	Lower cholesterol level
Tussillago farfara	Pyrollizidine alkaloids (PAs)	Relieves common cold
Aconitum sp.		Relieve pain
Ginkgobiloba	Ginsenosides	Relieves asthma
Eucalyptus globules (Eucalyptus)	Eucalyptol	Anti-inflammatory
Papaver rhoeas (Poppy)	Rhoeadine	Narcotic
Zingiber officinale (Ginger)	Gingerol, shogaol, paradol	Anti-tumour, anti-bacterial, anti-obesity, anti-diabetic (Rahmani *et.al.*, 2014)

Ocimum sanctum (Tulsi)	Eugenol and ursolic acid	Relieves common cold and fever (Mohan *et. al.,* 2011)
Azadirachta indica (Neem)	Nimbidin, azadirachtin, gallic acid, mahmoodin	Anti-inflammatory, anti-fungal, anti-malarial, anti-tumour
Aloe vera	Anthraquinones	Anti-oxidant, anti-microbial, anti-inflammatory, immunomodulatory (Kumar *et. al.*, 2014)

2.3.1 Herbal medicines used as traditional/ alternative medicines

Herbal medicines used as traditional medicines continually expand worldwide. According to Ayurveda, Siddha and Unani, – India is recognized for using herbs in traditional systems, which are well documented in the ancient scriptures. The word Ayurveda means "science of life" and also called as "science of longevity" because it helps us to live a long and healthy life (Pandey *et. al,* 2013). The terms traditional, alternative, complementary and conventional are used interchangeably (WHO 2000).The products obtained from them are now used for treatment of various health problems in different healthcare systems (WHO, 2004). It is determined that 80% of the world population primarily depends upon the herbal medicines. The use of herbal medicines as traditional medicines in different region is shown in figure 1. Like modern medicines, the herbal medicines which have a license also hold a license for safety, efficacy, quality of products (Ekor, 2014).

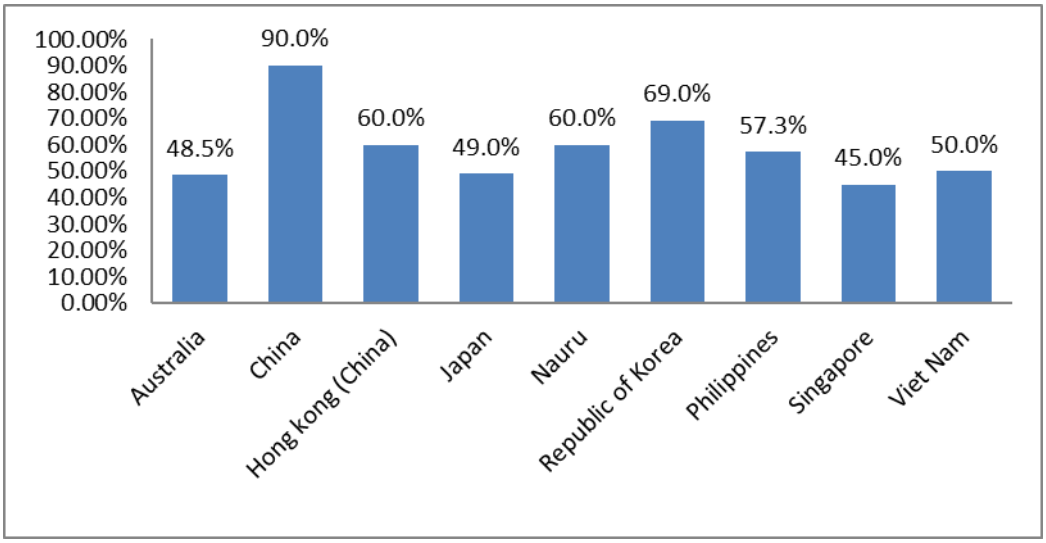


Figure 1. Percentage of population using traditional medicines (WHO, 2002)

Table 2. Some examples of common medicinal plants and their uses as traditional medicine (Pandey *et. al.*, 2013)

Name of plant	Common name	Uses
Asparagus racemosus	Shatavari	Provide female hormones, immunomodulation, purify the blood
Commiphora mukul	Guggul	Relieves common cold, immunomodulation
Momordica charantia	Karela, Bitter melon	Having active ingredient, Gurmarin that have strong sugar regulating effect
Piper longum	Pippali,IndianLongPepper	Stimulate the digestive and respiratory system
Withania somnifera	Ashwagandha	Health tonic
Terminalia chebula	Haritaki	Anti-mutagenic activity
Garcinia cambogia	Garcinia	Heart tonic
Glycyrrhiza glabra	Yashtimadhu,Licorice	Anti-mutagen, anti-oxidant, relieves muscle spasms

Gymnema sylvestre	Gurmarar	regulate sugar metabolism
Moringa pterygosperma Gaertn	Shigru, Horseradishtree	Used as Antibiotic
Terminalia chebula	Haritaki	Used as detoxifying agent
Tinospora cordifolia Miers	Guduchi	Anti-bacterial
Zingiber officinale	Sunthi, Ginger	Act as an adjuvant

India has a vast repository and the largest producer of medicinal plants. About 20,000 medicinal plants have been registered in which 7,000 used as traditional medicines. More than 7800 units are involved in the manufacturing of plant based formulations (Pandey *et. al.,* 2013).

2.3.2 Spices and their antioxidant activity

Nowadays, spices were become the extremely useful items of trade in medieval and ancient world and the trade route is called as the "Silk Road" which connects the East and the West (Kaefer *et. al.,* 2007). Several spices were incorporated in the "approved" monographs, like sage, garlic, ginger, onion, turmeric, rosemary ,cardamom, cinnamon, coriander, cloves, pepper, thyme (Vasanthi *et. al,* 2010). Spices were used to treat several diseases because they contain active phytochemical components and also possess physiological properties like anti-biotic, anti-cancer and different pharmaceutical activity (Beidokhti, 2013). Many spices contain phenolic compounds which protect the cell against Reactive Oxygen species (ROS) and thus possess the antioxidant activity.

Antioxidants may be defined as the radical scavengers, they are the substances which protects human body against free radicals such as hydroxyl radical, hydrogen peroxide and superoxide anion which leads to diseases like asthma, arthritis, inflammation, neuro degeneration, parkinson's diseases, mongolism, ageing process and perhaps dementia and carcinoma. These free radicals are generated in the body through aerobic respiration or exogenous sources which in turn stimulates the glycation of lipids and proteins inside body and lead to an imbalance between oxidants and antioxidants due to the inactivation of enzymes and thus cause number of disorders (Mathangi *et al.,* 2013).

Due to the presence of phenolic compounds in spices, they stop the formation of free radicals and prevents the body from oxidative injury and thus they called as radical scavengers (Molyneux, 2004).

Example of some spices and their bioactive component (Kaefer, *et. al.*, 2007)

1) Cardamom

Bioactive component: Limonene, caffeic acid.

2) Cinnamon

Bioactive component: Cinnamic aldehyde, 2hydroxycinnamaldehyde, eugenol.

3) Cloves

Bioactive component: Eugenol, isoeugenol, gallic acid.

4) Coriander

Bioactive component: Quercetin, caffeic acid, cineole, geraniol, borneol, 1,8-cineole, α-terpinene, β-carotene, β-pinene, β-sitosterol, cinnamic acid, ferrulic acid, γ-terpinene, kaempferol, limonene, myrcene, p-coumaric acid, p-cymene, quercetin, rutin, vanillic acid.

5) Fennel

Bioactive component: α-Pinene, β-carotene, limonene, quercetin, benzoic acid, β-sitosterol, caffeic acid, cinnamic acid, ferulicacid,fumaric acid, kaempferol, myristicin, 1,8-cineole, p-coumaric acid, quercetin, rutin, vanillic acid, vanillin.

6) Ginger

Bioactive component: Zingiberone, zingiberene, ingerol, paradol, curcumin, shagoal.

7) Saffron

Bioactive component: Crocetin, crocin, β-carotene, safranal, all trans retinoic acid.

8) Turmeric

Bioactive component: Curcumin, curcuminoids.

9) Oregano

Bioactive component: Apigenin, luteolin, myricetin, quercetin, caffeic acid, p-coumaric acid, rosmarinic acid, carvacrol, thymol.

10) Pepper, black

Bioactive component: Piperidine, piperine, limonene, α-pinene, β- pinene.

11) Rosemary

Bioactive component: Carnasol, carnosic acid, cineole, geraniol, α-pinene, β-carotene, apigenin, limonene, naringin, luteolin, caffeic acid, rosmarinic acid, rosmanol, vanillic acid.

12) Pepper

Bioactive component: Capsaicin, α-tocopherol, lutein, β-carotene, ascorbic acid, Vitamin E.

13) Thyme

Bioactive component: Thymol, carvacrol, cineole, α-pinene; apigenin, β-carotene, eugenol, limonene, ursolic acid, luteolin, gallic acid, caffeic acid, rosmarinic, acid, carnosic acid, hispidulin, cismaritin.

14) Dill

Bioactive component: Carvone, limonene, isorhamnetin, kaempferol, myricetin, quercetin, catechin.

15) Sage

Bioactive component: α-pinene, β-sitosterol, citral, farnesol, ferulic acid, gallicacid, geraniol, limonene, cineole, perillyl alcohol, β-carotene, catechin, apigenin, luteolin, saponin, ursolic acid, rosemarinic acid, carnosic acid, vanillic acid, caffeic acid, thymol, eugenol.

2.3.3 Spices and cancer

Cancer is one of the most deadful disease. It originates from a single cell in which normal cell multiply continuously and become abnormal cell. It is also caused by some internal factors (genetic changes) and external factors (environmental). According to WHO (World Health Organization), it is the disease that is the major causes of death worldwide (13% of the death population).

In South Africa, Cervical cancer is mostly found and caused by the HPV (Human Papilloma Virus). For the treatment of it, people in South Africa use "*Combretum caffrum*" plant as traditional medicine which stops the tumour formation (Berrington *et al,* 2012). Thus natural plant derived agents are being used for the treatment of cancer as they possess lower side effects risks. Spices have been consumed since past many centuries primarily because of their taste and aroma. Recent studies have proved their biological activities and efficacy. Many

plant based phytochemicals have been identified to possess anti cancer and therapeutic properties.

Table 3. Some examples of natural products that possess anti-cancer and other properties (Beidokhti, 2013)

Common name	Properties
Beetroot	anti-cancer, anti-viral, anti-inflammatory, antioxidant and detoxifying
Tomato	anti-cancer, anti-viral and antioxidant
Rhubarb	anti-bacterial, anti-cancer, antioxidant and healthy heart
Ginger	antiseptic and detoxifying
Green tea	anti-allergenic, anti-cancer, anti-inflammatory, antioxidant and healthy heart
Cranberry	antibacterial and antioxidant
Raspberry	anti-bacterial, anti-cancer, antioxidant, detoxifying and healthy heart
Cherry	anti-cancer, anti-inflammatory, anti-oxidant and detoxifying
Blueberry	anti-bacterial, anti-cancer, anti-inflammatory, antioxidant and healthy heart
Mango	anti-viral, anti-inflammatory, antioxidant, detoxifying and healthy heart
Apple	anti-allergenic, anti-inflammatory, detoxifying and healthy heart
Guava	anti-cancer, anti-viral, antioxidant and detoxifying
Apricot	anti-cancer, antioxidant and detoxifying
Papaya	anti-cancer, anti-inflammatory, antioxidant and healthy heart
Pineapple	anti-cancer, anti-inflammatory, antioxidant and healthy heart
Avocado	anti-allergenic, anticancer, anti-inflammatory, antioxidant and healthy heart
Chilli pepper	anti-bacterial, anti-viral, antioxidant and antiseptic
Pumpkin	anti-cancer and antioxidant
Black cumin	anti-allergenic, anti-bacterial, anti-inflammatory and healthy heart
Turmeric	anti-allergenic, anti-cancer, anti-inflammatory, antioxidant and healthy heart

2.3.4 Spices and Diabetes Mellitus

Diabetes Mellitus (metabolic disorder) is a chronic disease characterized by high blood sugar (glucose) level as a result release of glucose in urine termed as "sweet urine". It is mainly caused due to dysfunction of the pancreatic beta cells which secretes insulin hormone. It is generally referred to as diabetes. It is of two types: insulin dependent (type 1) and non insulin dependent (type 2) (Desoky *et al,* 2011). According to (WHO, 2008) disease caused 3rd largest death in countries.

Approximately 90% of the diabetic patients are non insulin dependent. This disease causes long term effects and sometimes leads to damage of multiple organs and no cures (WHO, 1999). This disease rise tremendously and it is estimated that diabetic patient around the world will be approx.366 million in 2030 year (Desoky *et al.,* 2011).

Earlier studies show that diabetes mellitus can be cured by the herbal treatment. There are some examples as shown in Table 4 that have anti-diabetic activities. (Gupta *et. al.,* 2012).

Table 4. Herbal products having anti-diabetic effect

Names	Biological source	Chemical compounds
Bitter melon	*Momordica charantia*	Momordicin 1 and 2, cucurbitacin B,Terpenoid, Glycosides,
Fiery costus	*Costusigneus*	Flavonoids, Beta-carotene, deoxyribose, phenol, insulin precursors
Dandelion	*Taraxacum officinale*	Triterpenoids and sterols (beta-sitosterol, taraxasterol, cycloartenol, Vitamin A and C, alkaloids, tannins, starch, pectin, caffeic acid, beta-carotene
French Lilac	*Galega officinalis*	Triterpinoids (sophoradiol, soyasapogenol b, , peganine, ,vasicinone, Sophorediol, galactogil, galegine and alkaloids (saponines, lutein, luteoline 5 glucosides, pentahydroxyflavone 5 glucoside, flavanoids
Gulvel	*Tinospora cordifolia*	tinosporone, cordifolisides A to E, berberine, tinosporic acid, alkaloid, arabinogalactan polysaccharide, Picrotene

Turmeric	*Curcuma longa*	Curcumin
Amla	*Emblica officinalis*	vitamin C, phyllemblin, tannin, calcium, phosphorus, pectin
Bael	*Aegle marmelos*	Furocoumarin
Gurmar	*Gymnema sylvestre*	gymnemic acid, tartaric acid, calcium oxalate, anthraquinone
Indian Kino Tree	*Pterocarpus marsupium*	kinotannic acid
Ginseng	*Panax ginseng*	Ginsenosides (aglyconedammarol), panaxosides (oleanolic acid)
Nayantara	*Catharanthus roseus*	Alkaloid (vincamine), tannins
Cinnamon	*Cinnamomum zeylanicum*	Starch, mannitol, tannins, mucilage, calcium oxalate
Saptrang	*Salacia oblonga*	alpha- glycosidase inhibitors (kotalanol, salicinol, sesquiterpene) and triterpenes
Onion	*Allium cepa*	Amino acid (leucine, methionine, isoleucine, arginine, tryptophan, lysine and phenolic acid (caffeic acid, p-hydroxybenzoic acid, coumaric acids)
Garlic	*Allium sativum*	Carbohydrates, proteins, fat, mucilage, volatile oil, copper, iron, phosphorous
Opuntia	*Opuntia ficus indica*	Candicine, tyramine, methoxytyramine, hordinine, N-methyltyramine
Blueberry	*Vaccinium myrtillus*	Anthocyanosides (delphinidin, myrtillin, cyanidin) and flavanoids (astragaline, hyperoside, quercitrin, isoquercitrin)

2.4 Cinnamon: An Introduction

It is mentioned in the Chinese texts that Cinnamon is one of the common spice having medicinal properties. In West Africa, *Cinnamomum zeylanicum* was first introduced in 1970 (university of Kumasi) by National herbarium of Ghana (Boniface *et al.*, 2012). This spice is widely used in preparation of several foods as Egyptian used it for traditional medicines

(Hamidpour *et. al.*, 2014). It is known as the oldest herbal medicine and it originates from Sri Lanka. (Wong *et. al.*, 2014).

Figure 2. Cinnamon quills and its powdered form

It belongs to:

Domain – Eukarya

Kingdom – Plantae

Phylum – Magnoliophyta

Class – Magnoliopsida

Order - Laurales

Genus – Cinnamomum

Species – *Cinnamomum zeylanicum*

The Cinnamomum genus consists of several species that occur in Asia and Australia. Most of Cinnamomum species are aromatic and contains volatile oil (Jayaprakasha *et. al.*, 2002).

2.4.1 Botanical Elucidation

The trees of *Cinnamomum zeylanicum* are evergreen and mainly propagated by seeds and their seeds are of different length and width size as shown in Table 5. It has silky yellow flowers as shown in figure 3 and ovular leaves with 7-20cm in length and fruits of *Cinnamomum zeylanicum* are pulpy and purple in colour as shown in figure 4. In India, it

comes from south India but it is mainly obtained from the Sri Lanka. Cinnamon word comes from the Greek word “Kinnamon” (Vangalapati *et al.*, 2012).

Table 5. Cinnamon seeds with different length and with size.

Category of different seeds	Length (mm)	Width (mm)
Small seeds	<8.5	<6.1
Medium seeds	8.5 to 12.5	6.1 to 9.9
Large seeds	>12.5	>9.9

Figure 3. Flowers of cinnamon tree

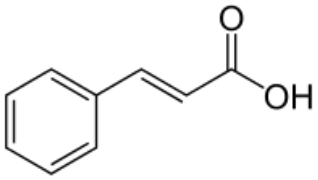
Figure 4. Fruits of cinnamon tree

Cinnamon zeylanicum and their different types contain different compounds which have pharmacological activities and these compounds are used in a herbal medicines for treating metabolic disorders such as type II diabetes mellitus (El-Desoky *et al.*,2011), and other diseases like inflammation, heart disease (cardiovascular disease), respiratory infections, urinary tract infections, gyanecological disorders etc. (Vangalapati *et al.*, 2012).

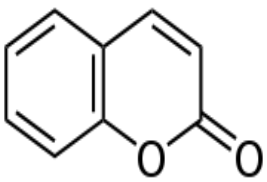
Table 6. Different varieties of cinnamon and their scientific names

S. no	Names	Scientific names
1.	Ceylon cinnamon, True cinnamon, Mexican cinnamon	*Cinnamomum zeylanicum, Cinnamomum verum*
2.	Indonesian cinnamon, Korintje cinnamon, Padang cassia	*Cinnamomum burmanni*
3.	Saigon cinnamon, Vietnamese cassia, Vietnamese cinnamon	*Cinnamomum loureiroi*
4.	Cassia cinnamon, Chinese cinnamon	*Cinnamomum aromaticum*

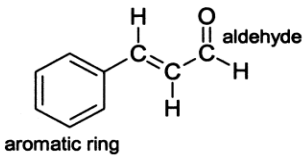
Structures of important chemical compounds in cinnamon are as follows



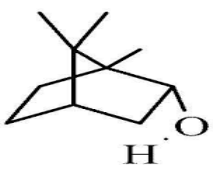
A: Cinnamic acid



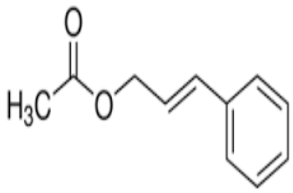
B: Coumarin



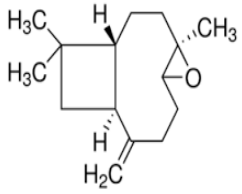
C: Cinnamaldehyde



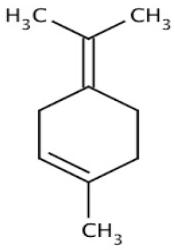
D: L-borneol

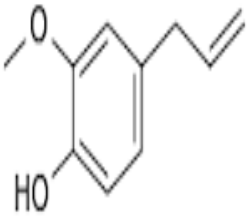


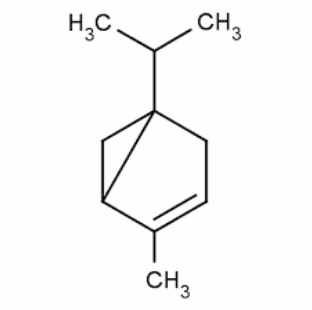
E: Cinnamyl acetate



F: Caryophyllene oxide







G: Terpinolene **H: Eugenol** **I: alpha-thujene**

Some nutrition substances that present in cinnamon are as follows

Proteins

Carbohydrates

Minerals like Calcium, iron, zinc, sodium, choline, magnesium, manganese, Phosphorous

Vitamins (A, C, K, B_3) (Vangalapati *et al.*, 2012).

Chemical compound are present in different parts (bark, root bark, leaves, buds and flowers) of cinnamon as shown in Table: 7 (Vangalapati *et al.,* 2012).

Table 7. Chemical compound present in different parts of cinnamon

Parts of cinnamon	Different compounds
Bark	Cinnmaldehyde (65 to 80%) and eugenol (5 to 10%)
Leaves	Cinnamaldehyde (1 to 5%) and eugenol (70 to 95%)
Root bark	Camphor (60%)
Fruit	β – caryophyllene and Trans – cinnamyl acetate
Buds	Terpene hydrocarbons (78%), Alpha Bergamotene (27.38%), Alpha – Copaene (23.05%), Oxygenated terpenoids (9%)
Flowers	Cinnamyl acetate (41.98%), Trans-alphabergamotene (7.97%), Caryophylleneoxide(7.2%)

Around 300 volatiles have been reported in the essential oil of *C. zeylanicum*. The essential oil is rich in trans-cinnamaldehyde which is a pale yellow, viscous organic compound present in major quantities and gives cinnamon its flavour and odour. Eugenol are also found in large amounts in the essential oil and are found to possess antibacterial, antiviral and antifungal properties. It has been reported that both cinamaldehyde and eugenol having the activity against gram positive and gram negative bacteria. Cinnamaldehyde is effective against *Staphylococcus aureus*, *Clostridium botulinum*, *Salmonella enteric* and eugenol effective against *Escherichia coli* (Gill *et al.,* 2004). There are some other compounds have been reported but they are present in lesser amount. These are coumarin, cinnamyl acetate, cinnamyl alcohol, cinnamyl acetate, hydroxyl cinnamaldehyde (Vangalapati *et al.,* 2012).

Coumarin is a substance (benzo-α-pyrone) naturally found in various plants and used as flavouring agents and mainly it is found in *Cinnamomum zeylanicum*. The use of coumarin in making synthetic food is stopped by US Food and Drug Agency in 1954, because of carcinogenc and hepatotoxic effects on rats and mice respectively has been observed (Blahov *et al.,* 2012). Federal Institute of Risk Management (FIRM) in Germany determined that consuming high level of coumarin causes kidney and liver damage. FDA (Food and Drug Administration) in Germany recognized a "Tolerable Daily Intake" (TDI) according to weight of the body that is (0.1 mg coumarin/ kg body weight) (Maheshwari *et al.,* 2013).

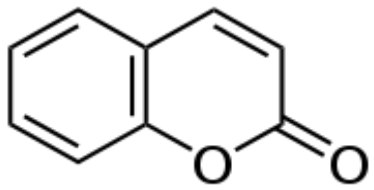


Figure 5. Structure of Coumarin

2.5 Biological Activities of *Cinnamomum zeylanicum*

Table 8. Reported activities of *Cinnamomum zeylanicum*

Activities	Reference
Anti-microbial	Gende *et al.,* 2008
Anti-inflammatory	Manosi *et al.,* 2013
Anti-oxidant	Mazimba *et al.,* 2015
Anti-diabetic	El-Desoky *et al.,* 2008
Immune modulating	Balekar *et al.,* 2014
Anti-ulcer	Jakhetia *et al.,* 2012
Anti-bacterial	Luo *et al.,* 2013
Anti-tumour	Wargovich *et al.,* 2001
Anti-fungal and Anti-obesity	Vangalapati *et al.,* 2012

The extract of *Cinnamomum zeylanicum* was found that it was not cytotoxic and it acts as anti-diabetic. It was determined when rat hepatoma cells were treated with CE (cinnamon extract) and it inhibits the hepatic glucose production using different concentrations (1–25 µg/ml) and major inhibition effect was observed at 25 µg/ml (Chenga *et al.,* 2012).

In vivo studies conducted on male albino rats mice showed cinnamon aqueous extract (CAE) has polyphenolic compound which show antioxidant activity (Morgan *et al.*,2014).

In vivo studies conducted on mice induced with melanoma have shown that aqueous extract of *C. Zeylanicum* potentiates $CD8^+$ T cell activity (Kwon *et al.,* 2009). Also, $CD8^+$ T cells were found to express IFN-γ and TNF-α and granzymes.

It was found that the water soluble polymeric extract of *Cinnamomum zeylanicum* bark contained sugars unit and showed radical scavenging activity. It was found to have arabinogalactan sugar unit in it and which showed the highest scavenging activity at the concentration of 750µg/ml and the glucan sugar unit in it showed the least scavenging activity (Ghosh *et al.,* 2015).

CHAPTER: 3 OBJECTIVES

1) Isolation of water soluble polymeric substances from *Cinnamomum zeylanicum.*
2) Estimation of polysaccharide content in isolated polymeric substances.
3) Evaluation of free radical scavenging and immunostimulation activity of polymeric substances.

CHAPTER: 4 MATERIALS

The chemicals and reagents used during the project are listed as follows

Chemicals/Reagents	Company
Hexane	EMPARTA® Merck
Methanol	EMPARTA® Merck
Chloroform	EMPARTA® Merck
Dichloromethane	EMPARTA® Merck
FBS	Gibco®Life technologies
Histopaque – 1077	Sigma-Aldrich
MTT	Sigma-Aldrich
Trypan Blue	Himedia
Ascorbic acid	LOBA chemic
Concanavalin A (Con A)	Sigma-Aldrich
Glucose	Himedia
EDTA	LOBA chemic
Phenol	Himedia
Sulphuric acid	LOBA chemic
Liquid nitrogen	
Sodium hydroxide	Himedia
Sodium Bicarbonate	Himedia
Copper sulphate	Himedia
Sodium potassium tartarate	Himedia
BSA (Bovine serum Albumin)	Himedia
DMSO (Dimethyl Sulfoxide)	SRL
RPMI 1640	Himedia
Pencillin	Himedia
Streptomycin	Himedia
Glutamine	Himedia

CHAPTER: 5 METHODOLOGY

5.1 Preparation of *Cinnamomum zeylanicum* extract

Barks of *Cinnamomum zeylanicum* (family: Lauraceae) were collected or taken and weighed**.** These barks of *Cinnamomum zeylanicum* made them free from impurities by washing thoroughly with tap water and then with distilled water twice. After washing, these barks were dried at 37°C or sun dried for two days. The dried barks were transferred to a clean, dry porcelain pestle and mortar and powdered with the help of liquid nitrogen. Now**,** to isolate the water soluble polymeric substances, powdered form of the barks were subjected to soxhlet extraction using four different solvents based on their increasing polarity index (Hexane, Dichloromethane, Chloroform, Methanol).

Table 9. Solvents with different polarity index

Solvents	Polarity
Hexane	0.1
Dichloromethane	3.1
Chloroform	4.1
Methanol	5.1

5.2 Soxhlet extractor: It is a versatile tool, invented by Franz Ritter von Soxhlet in 1879. Soxhlet extractor originally designed for the extraction of a lipid from a solid material. But it is not limited to the extraction of lipids. Actually, this device is only required when the impurities is insoluble in solvent and the compound having limited solubility in that solvent.

Different parts of soxhlet apparatus: There are three main different parts of soxhlet apparatus and each performs different functions i.e Condenser, Porous container, Distillation pot.

Condenser: The function of condenser is to cool down the solvent vapours and allow it to turn back into a liquid.

Porous container: It holds the thimble where the desired sample filled. Thimble retains the insoluble components and allows the component to pass which one dissolved. Thus the porous container holds the desired sample and allows the condensed solvent to saturate and pass through the thimble to extract the active compound.

Distilling pot: The desired solvents which we used are filled in distilling pot. This distilling pot placed on a heating mantle. It acts as a reservoir for the concentrated material.

Basic set up: The desired sample is put inside the thimble and placed into the porous container (soxhlet extractor) and then set the apparatus as shown in figure 6. Now the flask containing solvent is attached to the soxhlet extractor. Finally from upside attach the condenser.

Basic operation: The solvent is heated to reflux. Due to this, the vapours of the solvent goes upside from the distillation arm, and the thimble holding desired sample flooded with that warm solvent. When the vapour of the solvent goes upside, the condenser cool down the vapours and drops into a thimble containing sample. When it fills with the warm solvent, it is automatically emptied by the siphon arm and comes back to the distilling pot. This cycle repeats many time, hours or days, thus it is a continuous process. A part of non volatile compound dissolves in each cycle and the desired compound is concentrated in the distillation flask.

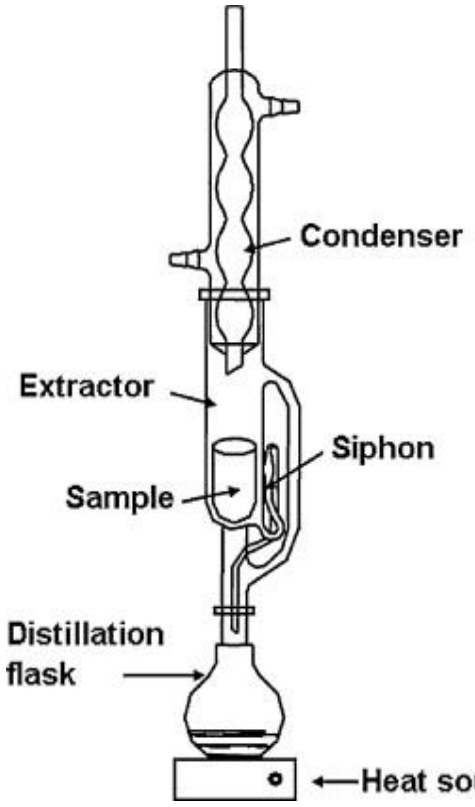


Figure 6. Soxhlet apparatus showing different parts

Soxhlet extraction: - For extraction of desired sample, the powdered form of *Cinnamomum zeylanicum* was placed into the extraction thimble which is made by whatmann filter paper and the thimble was placed in soxhlet extractor. The 150 ml of desired solvent was filled into the distilling pot and the extractor was connected to condenser. There was two inlet on condenser, one for water inlet and other for water outlet as shown in figure 7. Condenser was used for continuous water flow to cool down the solvent. This whole assembly was placed on a heating mantle and adjust its temperature 100°C below the boiling point of solvent. For each solvent, twenty cycles of extractions were carried out at fixed temperature. The time of each cycle completion was noted. After the end of twenty cycles, the solvent was to cool down and then the apparatus was dismantled. The extraction was carried out in the order of increasing polarity as shown in table 9 so that the highly non polar compounds drawn out at first. After extraction with soxhlet, theresidue remained after soxhlet extraction, allowed to dry and was used for isolation of water soluble polymeric substances.

Table 10. Solvents with different boiling point and extraction temperature

Solvent	Boiling Point	Extraction temperature
Hexane	68.5 to 69.1°C	58°C
Dichloromethane	39.8 to 40.0°C	29°C
Chloroform	61.5°C	51°C
Methanol	64.7°C	54°C

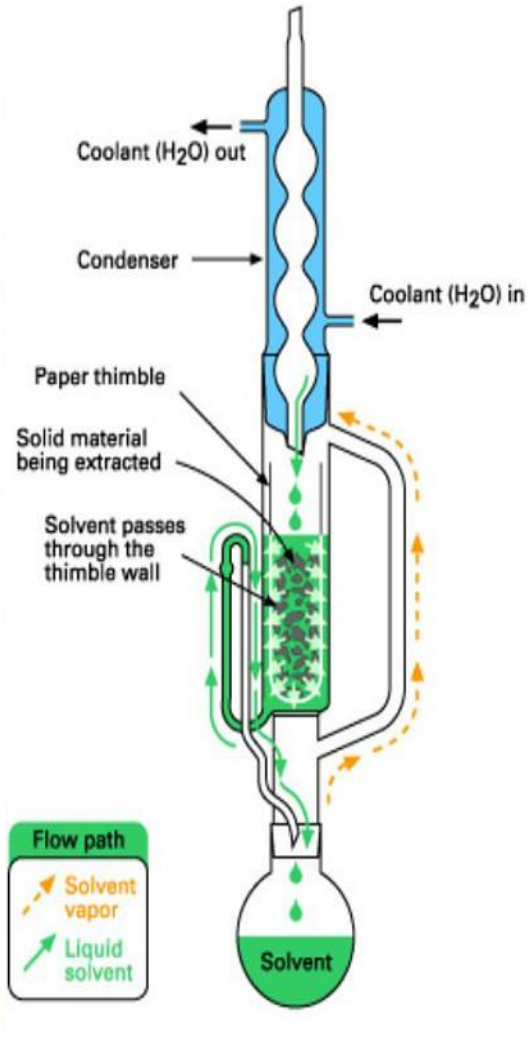


Figure 7. Soxhlet extractor showing flow of solvent during extraction

5.3 Extraction of Water-soluble polymeric substances

For extraction of water soluble polymeric substances, the remained dried residue was taken and stirring was done by adding the autoclave sterile water (pH: 6.0) to it and put the magnetic bead into it. The cap of bottle was sealed with parafilm or clean film to avoid contamination. All steps were carried out under laminar air flow. Stirring was done at normal temperature for 12 h magnetic stirrer. After 12 hr. the residual material was separated from the liquid extract with the help of whatman filter paper under laminar air flow. The liquid extract was stored at 4˚C. After separation, again stirring was done by adding the autoclave sterile water to the left residual material for 12 h. The residual material was again separated from the liquid extract and combined it to above liquid extract to maximize polymeric substances.

After getting liquid extract, the dialysis was done. The dialysis of the liquid extract was done with the help of dialysis membrane (semi-permeable) against autoclave sterile water to separate the colloidal particles from the dissolved substances (ions or molecules) by the process of diffusion. The liquid extract was kept in the dialysis membrane, whose one end of the dialysis tube is already tied with thread and after filling the liquid extract to it, tied its another end and placed into a flask containing autoclave sterile water in such a way that half of the dialysis tube dipped into the water as shown in figure 8.

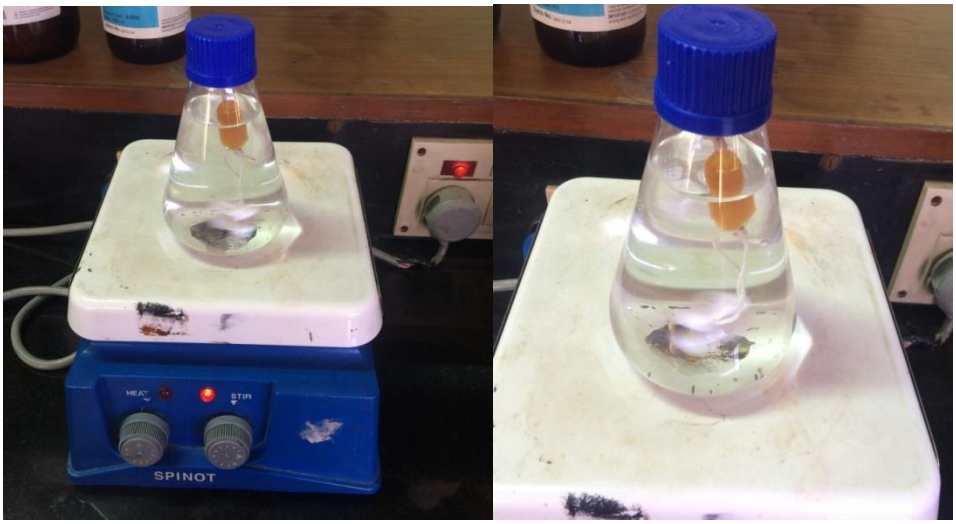


Figure 8. Dialysis process

After the dialysis process was completed, the liquid extract from the dialysis tube was drawn out and lyophilized it to get fine powdered. The powdered material after lyophilized was dissolved in autoclave sterile water and added ethanol (double the volume of water) under laminar air flow and stored at 4˚C overnight for precipitation. After precipitation, centrifuged it at 10,000 rpm for 20 min. The pellet was taken and discarded the supernatant. The pellet was dissolved in autoclave sterile water by repipetting under laminar air flow. Sonicator was also used for well mixing. After dissolving the pellet, lyophilized the sample to get yield of water soluble polymeric substances.

5.3.1 Dialysis membrane

It is a semi-permeable membrane used to separate the colloidal particles from the dissolved substances by the process of diffusion.

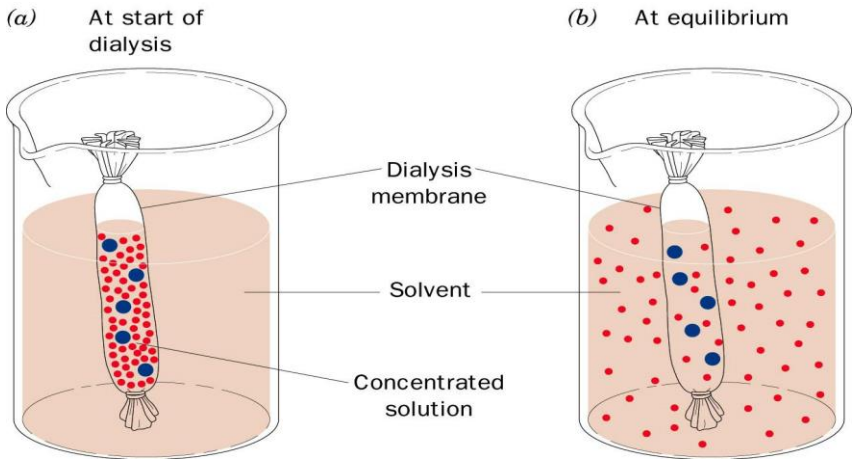


Figure 9. Dialysis process showing images

Before using dialysis membrane for experiment, it was activated by following steps:

1) The dialysis membrane (tubing) was cut to desired length and immersed into 1litre of 2% $NaHCO_3$/1mM EDTA in 2litre glass beaker.
2) After that, this was boiled for 10 minutes and rinsed it by adding double distilled water for 10 min
3) After 10 min, the water was decanted and 50% ethanol was added in sufficient amount so that the membrane was submerged completely in it and stored at 4˚C.

5.3.2 Lyophilisation

Lyophilisation is a process of freeze drying where water is removed from the sample to get a stable (dry) product. The principle involved in this process is sublimation (Nireesha *et. al.*, 2013). The lyophilization term coined by Rey. The process involves following advantages (Girish *et. al*., 2014).

- Increase product stability in dry state.
- Without heat, solvent will be remove.
- Storage and handling is easy.

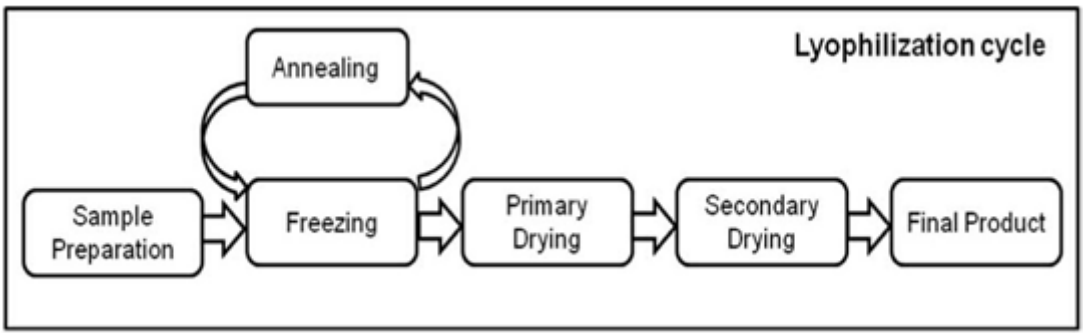


Figure 10. Steps of Lyophilization Process (Nireesha *et. al.*, 2013)

5.4 Phenol Sulphuric acid test

Phenol sulphuric acid method is the easiest and the best method for the determination of carbohydrate analysis. Other methods used resorcinol, anthrone, or orcinol which are not convenient to use and also these methods required large amount of monosachharides, thus they are unable to use for precious samples (Masuko *et al*., 2004).

For sugar analysis in microtitre 96 well plate, 50µl of standards and samples is prepared and 150µl sulphuric was added immediately and 30µl phenol was added. After this, incubating it for 5 minutes in water bath and after that cool down it for 5 minutes and OD was taken at 490nm.

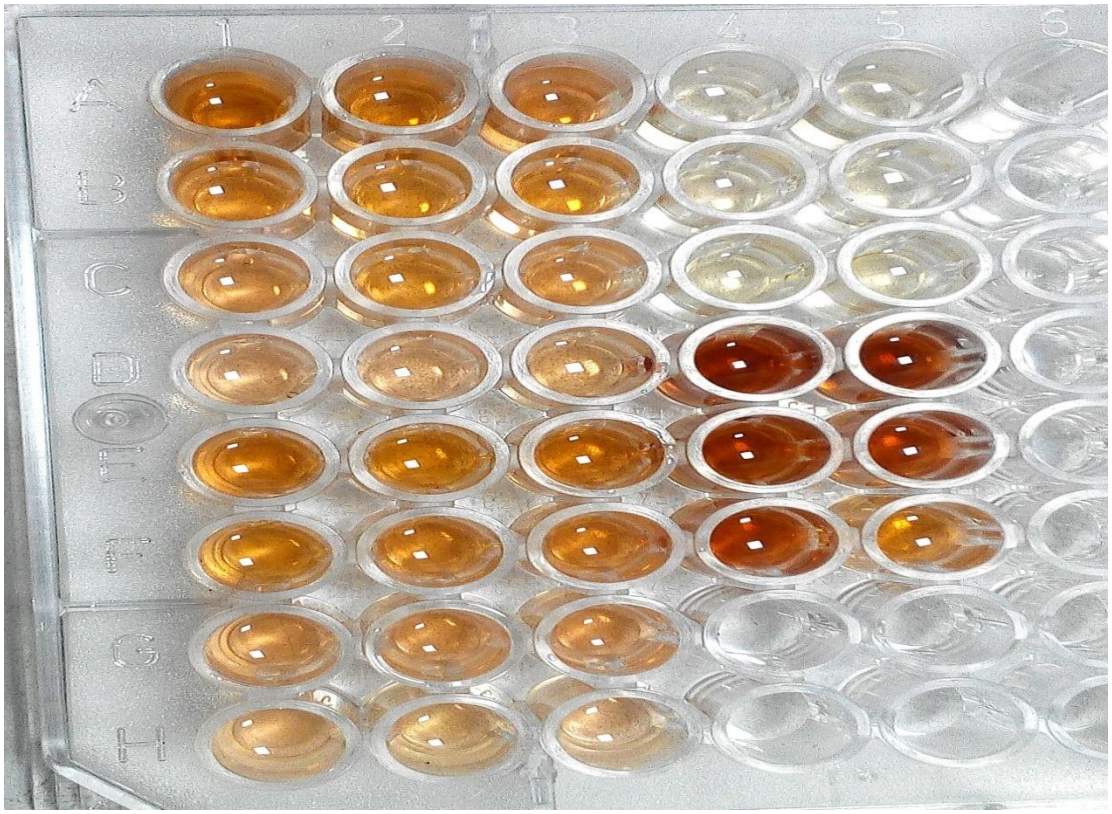


Figure 11.Phenol sulphuric acid test performed on microtitre plate

5.4.1 Determination of carbohydrate content in water soluble polymeric extract of *Cinnamomum zeylanicum*

For the determination of carbohydrate in water soluble polymeric extract of *Cinnamomum zeylanicum*, following preparations were done.

Preparation of 5% phenol: 5g of phenol crystal was dissolved in 100ml distilled water.

For determination of sugar analysis in water soluble polymeric extract of *Cinnamomum zeylanicum*, the glucose stocks (500ug/ml and 1000ug/ml) were prepared. From this different concentrations of glucose standards 10µl, 20µl, 30µl and 40µl were taken make the volume up to 50µl with distilled water. From the extract 15µl, 10µl and 5µl concentrations and make up the volume up to 50µl were taken in microtitre 96 well plate and in each well 150µl sulphuric acid was added immediately and mixed it well. After mixing, 30µl phenol was added and the plate was incubated in water bath at 25˚C for 5min. After 5min allowed it to cool down and absorbance was taken in ELISA plate reader at 490nm.

5.5 Preparation of PBS (Phosphate Buffer Saline)

One litre of 1X PBS was prepared by adding 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, 0.24g of KH_2PO_4 was added in 800ml of double distilled water. pH was adjusted to 7.4 using HCL or NaOH. Volume was made up to 1 litre using double distilled water. PBS was autoclaved for 20 minutes at 121°C. After autoclaving, PBS was filter sterilized and stored at 4°C.

5.5.1 RPMI MEDIA

Media is prepared by dissolving powdered RPMI media in sterile water. Sodium bicarbonate, glutamine and then antibiotics viz. Penicillin (100 IU/ml) and Streptomycin (100µg/ml) are added to it. The media was then filtered through 0.22µm filter and stored in -20˚C. Media was then supplemented with FBS (10%) and HEPES buffer (10mM) are added just before use.

5.5.2 Cell counting

Hemocytometer or Neubauer chamber is used to count cells growth. The counting grid in Neubauer chamber is 3x3mm in size and the grid has sub division of 9 square having width is 1mm and it holds the volume up to 0.0001 ml as shown in figure 12.

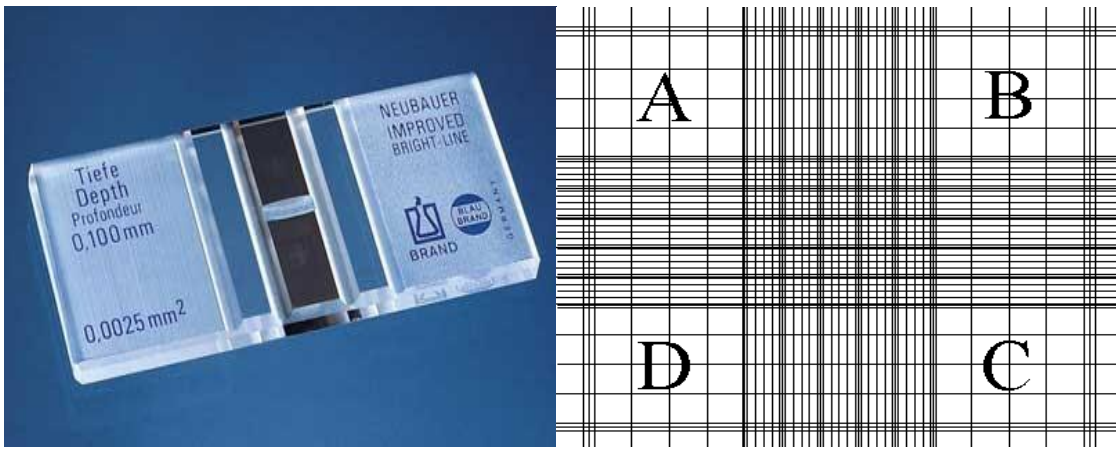


Figure 12. Neubauer Chamber or Hemocytometer

Before using the hemocytometer to count cells, cover slip and hemocytometer were cleaned. After that the extract (sample) was loaded to the edge of the counting chamber. The sample was drawn into the chamber by capillary action. Then, hemocytometer was placed under microscope and set the magnification at 40X. Cells were counted in each section A, B, C and D as shown in figure: 11.

Cell count is calculated by the number of average cells in every chamber dilution factor.

Cell count = {(A+B+C+D)/4} x dilution factor x 10^{-4} Where A, B, C and D are the cell counts in chamber A, B, C and D, respectively.

5.5.3 Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

PBMC isolation was carried out using Histopaque-1077 as per manufacturer's instruction. To a 15 ml conical centrifuge tube, 5mlof Histopaque-1077(room temperature) was added. 5ml whole blood from a healthy donor was carefully layered onto the Histopaque-1077 and centrifuged at 400 x g for exactly 30 minutes at room temperature. After centrifugation, the top plasma layer (yellow coloured) was carefully removed with a pipette and opaque interface was collected containing mononuclear cells (PBMCs) in clean conical centrifuge tube. The cells were washed by adding 10 ml of sterile 1X PBS buffer and centrifuged at 250 x g for 10 minutes. The supernatant was discarded and the cell pellet was resuspended with 5 ml of sterile 1X PBS buffer. Again centrifugation was done at 250 x g for 10 minutes. After discarding the supernatant, cell pellet was resuspended in 1 ml of complete cell culture medium. Cells were counted using hemocytometer as discussed in section 5.5.2.

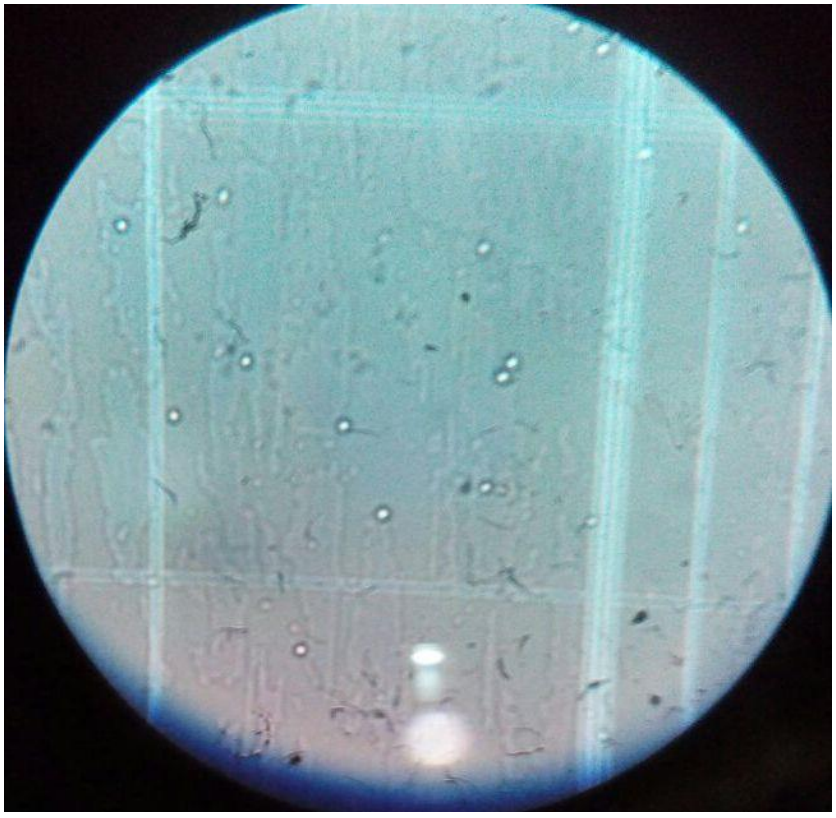


Figure 13. Cells under microscope

5.5.4 Effect of extract on Peripheral Blood Mononuclear Cells (PBMCs)

To assess the effect of the water soluble polymeric extracts of *Cinnamomum zeylanicum* on primary cells (PBMC's) the MTT assay was used.

Most commonly used assay for cell viability, proliferation and cytotoxicity is MTT assay. It's a colorimetric assay of tetrazolium salt thiazolyl blue or methyl-thiazolyl-tetrazolium (MTT). MTT appears yellowish in color (aqueous solution). It undergoes for reduction by the enzyme called dehydrogenases present in cells which are metabolically active. It was reported that succinate dehydrogenase enzyme present in mitochondria of viable cells reduced MTT into its formazan product. This on reaction yields formazan product of blue violet colour. Formazan is known for its lipid solubility. This can be extracted with the help of organic solvents. Estimation is done spectro photometerically. It has been reported by the researchers that MTT formazan is directly proportional to the number of living cells in the sample (Juan *et. al.,* 2012).

Seeding of freshly isolated PBMCs was done at a density of 2×10^5 cells $well^{-1}$ of 96-well micro titre plate and Concanavalin A mitogen (2 µl) was added after 2 h. Complete media in required volume was then added to each well for cell culture. The water soluble polymeric extract taken in varying concentration were prepared (1000µg/ml, 500µg/ml, 250µg/ml and 200µg/ml) and added to each well in duplicates. Total volume of each well was made 200µl (cells, media, sample) along with 2µl mitogen. Positive control (cells and media) were added in triplicates. After 48 h of incubation in CO_2 incubator, 20 µl of MTT reagent was added to each well and again incubation was provided for 4 hours. Microtitre plate was centrifuged at 200 rpm for 10min.160µl of media was removed from each well and purple formazan crystals formed after 4 hours of incubation were dissolved in 100µl DMSO in each well and absorbance was observed at 570nm taking 620nm as reference wavelength on ELISA plate reader. Then difference between the two wavelengths was retrieved by the ELISA microplate reader.

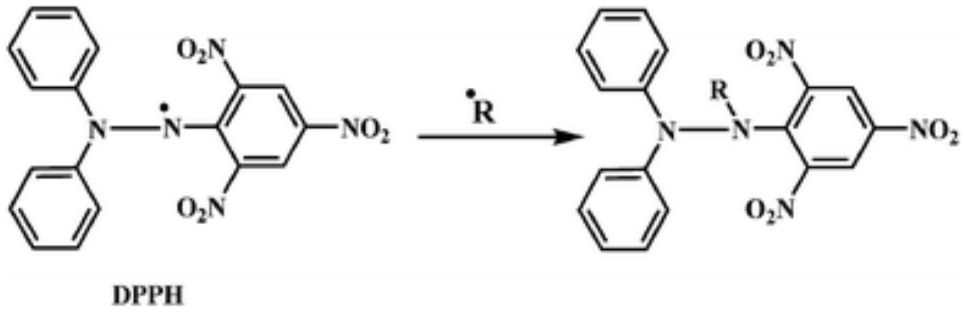
Figure 14. MTT reduced to formazan product

Figure 15. Development of Formazan crystal in 96-well plate

5.6 Free radical scavenging activity (Antioxidant Assay)

Antioxidants are the substances that prevent the body against free radicals which may cause deleterious effects or may cause metabolic and genetic disorders like carcinogenesis, mutagenesis and ageing (Molyneux, 2004). In order to know the antioxidant capacity of the cinnamon water extracts, a well known antioxidant assay known as the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed.

When DPPH is mixed with a substance that can donate a hydrogen atom (free radical) then this gives rise to the reduced form and violet colour is reduced to pale yellow giving rise to a non radical form, 2,2-diphenyl-1picrylhydrazyl is reduced to 2,2-diphenyl-1-picrylhydrazine (non radical) (Mathangi *et al*.,2013).



Diphenylpicrylhydrazyl (free radical) **Diphenylpicrylhydrazyl (non radical)**

Figure 16. Free radical reduced to non free radical

6 mg/ml of water soluble polymeric extract of *Cinnamomum zeylanicum* were prepared as stock solution. Different concentrations 500µg/ml, 250µg/ml, 100µg/ml and 50µg/ml were added to the 200µl in 96 well plate in triplicates. Ascorbic acid (100µM) in distilled water was taken as standard. 150µl of DPPH (100µM) was prepared in methanol and then added in to the each well. Plate was incubated in dark for 45 minutes, after which the absorbance was measured at 517nm in Elisa plate reader. It was observed that the violet colour changes to pale yellow colour as shown in figure 17.

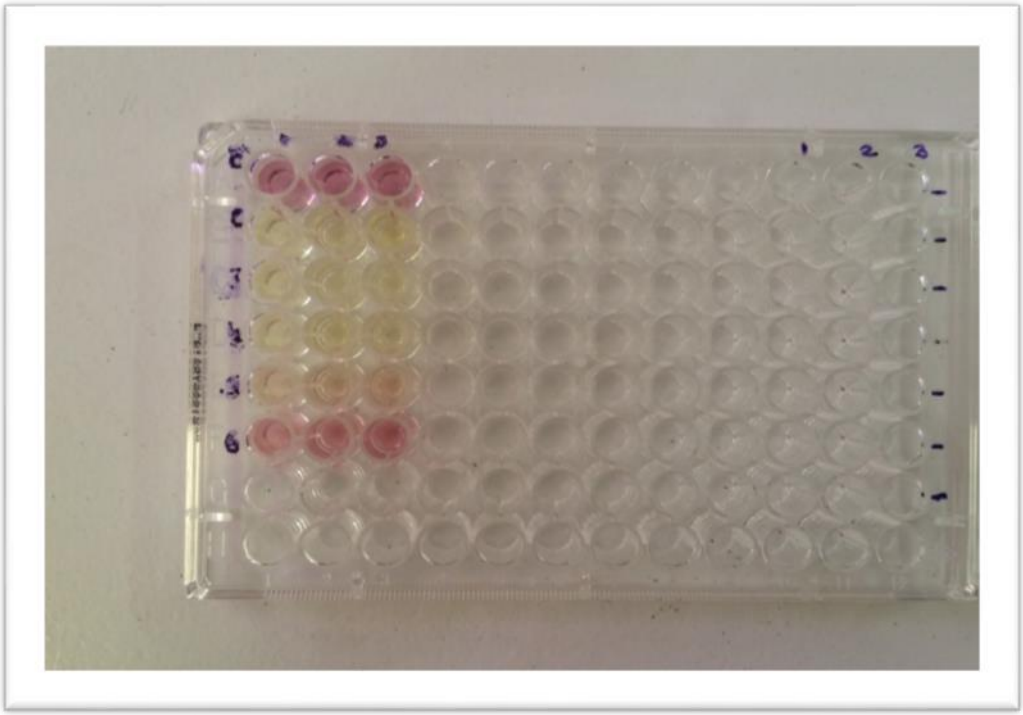


Figure 17. Violet colour (free radical) is reduced to pale yellow (non radical form).

Scavenging activity was expressed as:

$$\text{Radical Scavenging Activity (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

5.7 PROTEIN DETERMINATION ASSAY

There are many different assays for the protein determination. The most common types are Bradford and Lowry's method. Both are colorimetric assays. Bradford assay only use over a short range (0µg/ml - 2000µg/ml). The total protein content in water soluble polymeric

extract of *Cinnamomum zeylanicum* was determined by the Lowry method. The reaction involved in Lowry method is the peptide bond of the protein reacts with cupric copper and reduction of phosphomolybedic acid by residues of protein.

5.7.1 Determination of Protein content in water soluble polymeric extract of *Cinnamomum zeylanicum*

For determination of protein in extract, BSA was used as standard and other preparations were done as follows:

Reagent A

4g NaOH was dissolved in 800ml distilled water and added 20g Na_2CO_3in it and make up the volume up to 1000ml with distilled water.

Reagent B

2g $CuSO_4$was dissolved in 100ml distilled water.

Reagent C

2g sodium potassium tartarate was dissolved in 100ml distilled water.

Reagent D

Reagent D was freshly prepared by mixing 0.5ml of reagent B and 0.5ml of reagent C. after that 99ml reagent A was added.

Reagent E

Reagent E was Folin-Ciocalteau 1N was used.

Different concentrations of BSA (2mg/ml) and sample (20 to 100µl) were taken in test tubes and volume was made up to 1ml with distilled water. The 5ml freshly preapared reagent D was added to each test tubes and incubated it for 10min at room temperature. After incubation 1500µl folin reagent was added and incubated it for 60min. The absorbance was taken after 1h at 750nm.

CHAPTER 6 RESULTS AND DISCUSSION

6.1 Yield of water soluble polymeric extract of *Cinnamomum zeylanicum* bark

Initially, 20g of bark powder was taken and run the sequential Soxhlet extraction with four different solvents, i.e., Hexane, Dichloromethane, Chloroform and Methanol respectively, according to increasing polarity. Each solvent was run for 18-20 cycles and the extraction temperature was set to ten times below the boiling point of temperature. After sequential extraction, the residue remained was subjected for the extraction water soluble polymeric substances. The polymeric substances were lyophilized to get powder form. Finally, the yield obtained was 12.6mg from 20g which was reconstituted in autoclave sterile water.

6.2 Determination of the protein contents presence in polymeric extract

The protein content was determined by Folin-lowry's method. From the standard (BSA) different concentrations (10, 20, 30, 40 and 50 µg/ml) and (80 and 100 µl) samples of crude extract were taken to check the presence of protein contents. After comparing from the absorbance value of standard, it is found that the samples absorbance is lie in between 40 to 50µg/ml which confirms the presence of protein content in polymeric extract figure: 18.

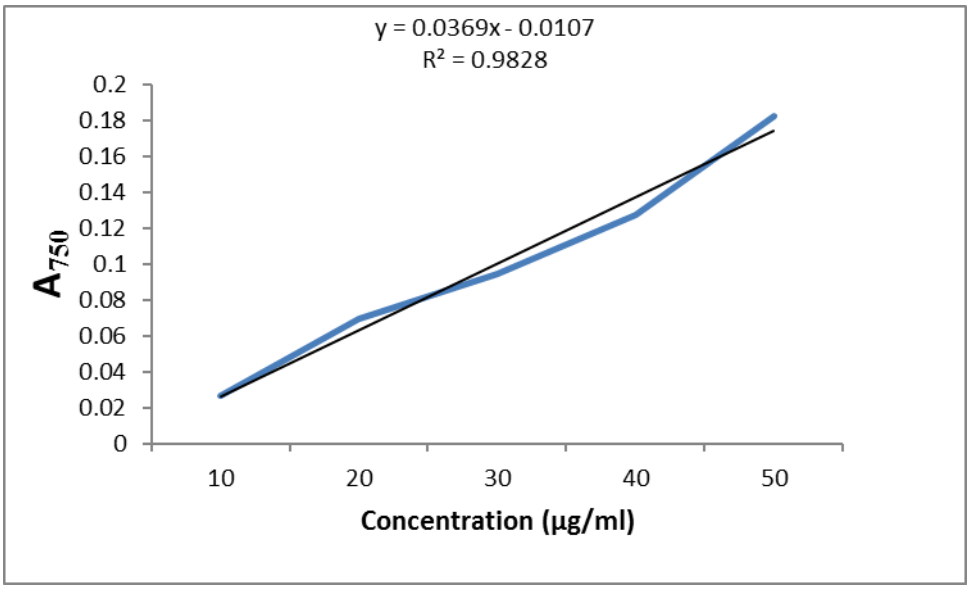


Figure 18. Standard curve of BSA

From the standard curve, it was calculated that sample (80µl) contain 44.6µg/ml protein content and sample (100µl) contain 49.7µg/ml protein content.

6.3 Estimation of Carbohydrate contents in polymeric substances of *Cinnamomum zeylanicum*

These polymeric substances are supposed to be rich in polysaccharides content. Hence, the carbohydrate analysis in water soluble polymeric substrate was determined by phenol sulphuric acid method. Glucose was taken as standard and a standard graph was plotted with different concentrations of glucose (Figure 19). The glucose concentration was calculated from the equation obtained in standard graph and is given in Table: 11.

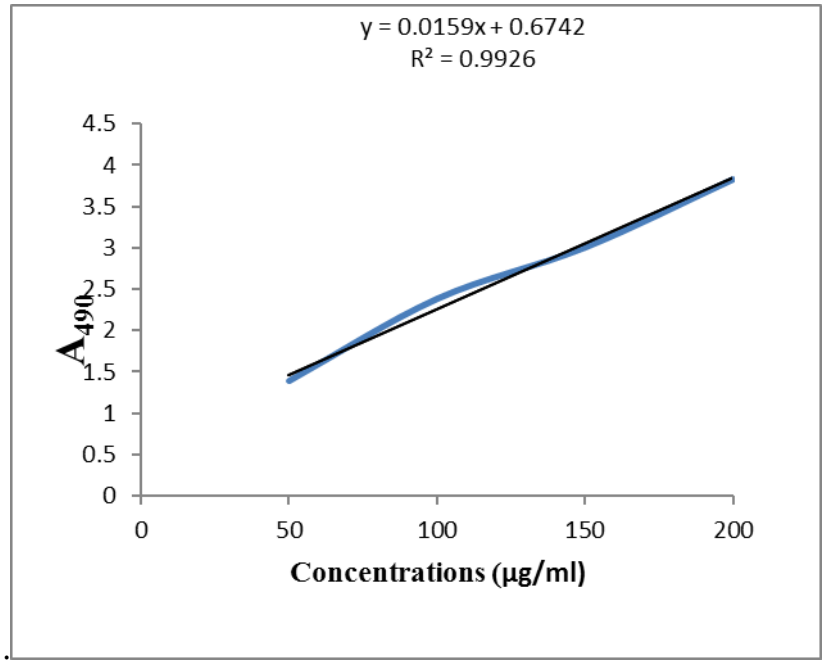


Figure 19. Standard curve of glucose

Table: 11 Carbohydrate content present in *Cinnamomum polymeric* substances

Sample concentration	OD	Glucose concentration in sample	Glucose concentration per mg
450 µg/ml	2.36	112.57 µg in 450 µg	250µg

6.4 Free radical scavenging activity

Free radical scavenging activity of water soluble polymeric extract of *Cinnamomum zeylanicum* was detected through DPPH assay. The experiments were performed in triplicates for different concentrations (50, 100, 250 and 500µg/ml) of the polymeric extract. Ascorbic acid was taken as positive control. It has been found that the scavenging effect is increased

with concentrations (Table 12 and Figure 20). The most pronounced effect was seen in the 500 µg/ml concentration which showed up to 80% scavenging activity.

Table: 12 Free radical scavenging activity of *Cinnamomum* polymeric substances

Different concentrations	Experiment 1	Experiment 2	Mean±SD	Scavenging activity (%)
Ascorbic acid	0.1079	0.1076	0.1077±0.0002	84.3
50 µg/ml	0.348	0.347	0.3479±0.00069	-9
100 µg/ml	0.159	0.16	0.156±0.0064	52.14
250 µg/ml	0.06	0.202	0.131±0.100	74.6
500 µg/ml	0.08	0.161	0.120±0.057	80.9

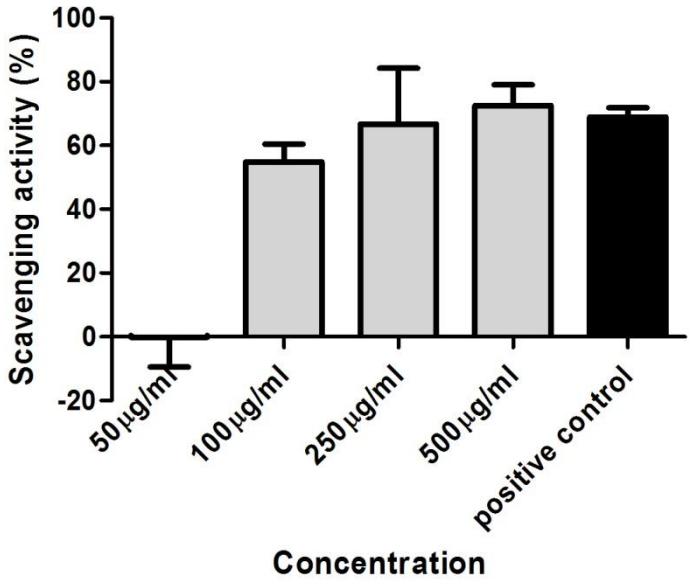


Figure 20. Free radical scavenging activity of polymeric extract of *Cinnamomum zeylanicum*

6.5 Effect of *Cinnamomum zeylanicum* polymeric extract on the growth of peripheral blood mononuclear cells

Different concentrations (125, 200, 250, 500 and 1000 µg/ml) of polymeric extracts was taken to study the effect on peripheral blood mononuclear cells (PBMCs). MTT assay was

performed for estimating PBMC proliferation. Experiments were carried out with unstimulated PBMCs and ConcanavillinA (con A) stimulated PBMCs. 200and 250 µg/ml concentrations appears to have less effect on the growth of unstimulated and stimulated PBMCs as compared to control (media + cells only) as shown in figure 21 and control (M+C+Con A) as shown in figure 22. Interestingly, 500 and 1000 µg/ml appears to exert the proliferative effect on both unstimulated and stimulated PBMCs which shows the immunostimulating potential of *Cinnamomum* polymeric substances.

Table 13. Effect of polymeric extract of *Cinnamomum zeylanicum* on unstimulated PBMCs

Concentrations (µg/ml)	Experiment 1	Experiment 2	Mean±SD
Control (M+C)	0.07863	0.0867	0.083±0.0028
Control (M+C+Con A)	0.132433	0.1453	0.139±0.009
125	0.14955	0.1968	0.17±0.0334
250	0.11835	0.193633	0.16±0.053
500	0.2714	0.19333	0.23±0.055
1000	0.2764	0.17823	0.28±0.07

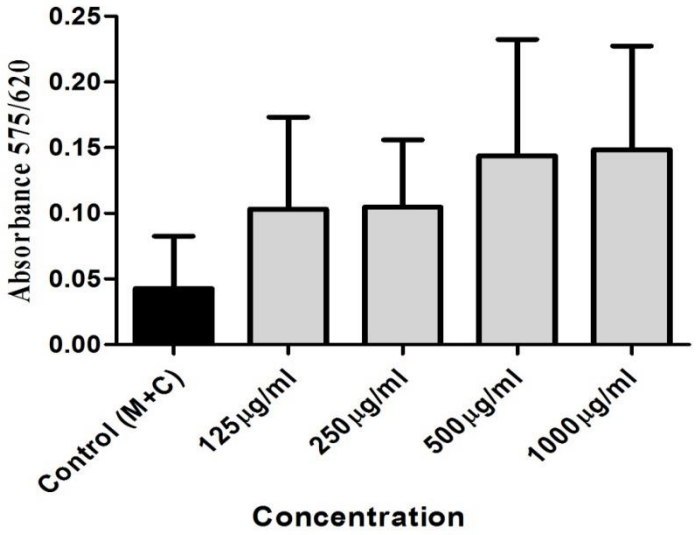


Figure 21. Effect of polymeric extract of *Cinnamomum zeylanicum* on unstimulated PBMCs

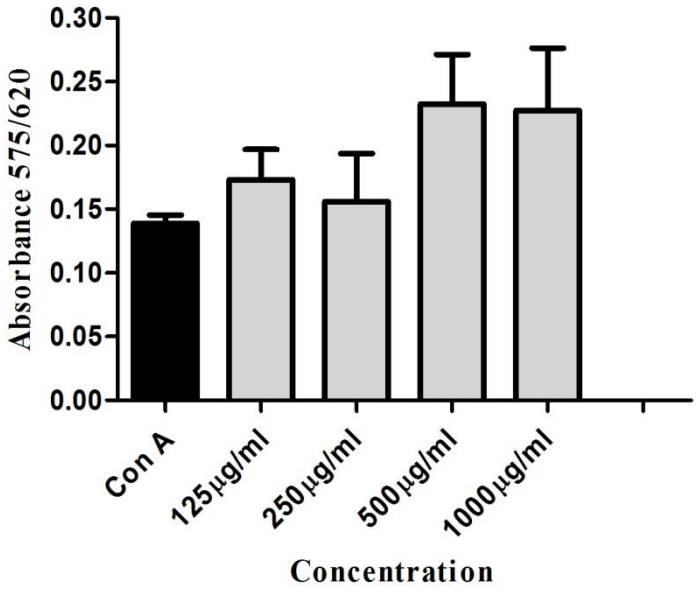


Figure 22. Effect of polymeric extract of *Cinnamomum zeylanicum* on concanavalin stimulated PBMCs

SUMMARY AND CONCLUSION

Spices are being used since ages in Ayurveda to cure illness. They are known to possess certain phytochemicals which show anticancer, analgesic, antimicrobial, antifungal, antioxidant properties. Also, they are found to have fewer/negligible side effects as compared to their modern medicine counterparts. They come under the category of traditional Medicines and are being researched and studies due to their many advantages over pharmaceuticals. *Cinnamomum zeylanicum* is one of the oldest medicinal herb known and is even mentioned in the Chinese literature since 4000 years ago. It is also used for medicinal purposes due to its unique properties. It is found to have antimicrobial, anti-inflammatory, immune-suppressant and immune-stimulant.

The present study involved the extraction of water soluble polymeric substances from *Cinnamomum zeylanicum*. The extract was analysed by means of various biochemical assays to detect its protein and carbohydrate content (Phenol sulphuric acid method and Folin-Lowry method respectively) and free radical scavenging activity (DPPH assay). Further, the effect of this extract was tested on PBMCs.

Biochemical analysis of polymeric substances confirms the presence of protein as well as carbohydrate in it. The highest free radical scavenging activity (antioxidant activity) observed at the concentration of 500µg/ml (80.9%). The highest proliferation activity observed at the concentration of 1000µg/ml on the unstimulated and stimulated PBMCs. Thus, the results indicates that the water soluble compounds obtained from *Cinnamomum zeylanicum* are good source of antioxidant and immunomodulatory compounds

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