Studies on the Antioxidant, Thrombolytic activity and Laxative effect activity of methanolic extracts of the Stephania Japonica and Trapa Bispinosa Plants

A Project Report Submitted to the Department of Pharmacy, East West University, in Partial Fulfillment of the Requirements for the Degree of Masters of Pharmacy (M.Pharm).

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CHAPTER 01

INTRODUCTION

INTRODUCTION

A medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs. Humans have used them throughout history to either cure or lessen symptoms from an illness. A pharmaceutical drug is a drug that is produced in a laboratory to cure or help an illness.Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease

(Verma et al., 2008). Plants have been a major source of therapeutic agents since time immemorial. The increasing acceptance of traditional herbal systems of medicine in the world has resulted in the revival of ancient traditions of medicine. Medicinal plants and their derivatives are thus looked upon not only as a source of affordable healthcare but also as an important commodity item of international trade and commerce (Kurian *et al*, 2007).

It is estimated that there are 250,000 to 500,000 species of plants on Earth. A relatively small percentage (1 to 10%)of these is used as foods by both humans and other animal species.It is possible that even more are used for medicinal purposes. Hippocrates (in the late fifth century B.C.) mentioned300 to 400 medicinal plants. In the first century A.D.,Dioscorides wrote *De Materia Medica*, a medicinal plant catalogthat became the prototype for modern pharmacopoeias. Indeed,frankincense and myrrh probably enjoyed their status of greatworth due to their medicinal properties. Reported to have antisepticproperties, they were even employed as mouthwashes. The fall ofancient civilizations forestalled Western advances in the understandingof medicinal plants, with much of the documentation of plant pharmaceuticalsbeing destroyed or lost. During the Dark Ages, the Arabworld continued to excavate their own older works and to buildupon them. Of course, Asian cultures were also busy compilingtheir own pharmacopoeia (Ghani, 1998).

Fig.1.1: Theophrastus - father of botany (Andersen, 2014)

Fig.1.2: First of the alkaloid chemists; Caventou, Pelletier and Quinine (Andersen, 2014)

1.1 Use of plants as Medicine

The use of plants as medicines goes back to early man. Certainly the great civilisations of the ancient Chinese, Indians, and North Africans provided written evidence of man's ingenuity in utilising plants for the treatment of a wide variety of diseases. In ancient Greece, for example, scholars classi®ed plants and gave descriptions of them thus aiding the identification process. Theophrastus has been described by some as the father of botany (Fig. 1).

It was not until the 19th century that man began to isolate the active principles of medicinal plants and one particular landmark was the discovery of quinine from Cinchona bark by the French scientists Caventou and Pelletier (Fig. 2).

Much less is known about the isolation of quinine by J.B. Caventou1 and J.B. Pelletier1. Such discoveries led to an interest in plants from the New World and expeditions scoured the almost impenetrable jungles and forests in the quest for new medicines (David, 2001).

As therapeutic use of plants continued with the progress of civilization and development of human knowledge, scientists endeavored to isolate different chemical constituents from plants, put them to biological and pharmacological tests and identify therapeutically active natural compounds, which have eventually used to prepare modern medicines. In course of time synthetic analogues and derivatives of the natural compounds were also prepared. In this way, ancient uses of Datura plants have led to the isolation of hyoscinic, hyoscyamine, atropine and tigloidine, Cinchona barks to quinine and quinidine, *Rauwolfia serpentina* root to reserpine and rescinnamine, *Digitalis purpurea* to digitoxin and digoxin; Opium to morphine and codeine, Ergot to ergotamine and ergametrine, Sena to sennosides, *Catharanthus roseus* to vincristine and vinblastine to mention a few (Ghani, 1998).

In addition to these, there are many other plant-derived chemical substances of known structures that are used as drugs or necessary components of many modern medicinal preparations. These include camphor, capsaicin, eucalyptol, menthol, minor cardiac glycosides, various volatile oils, etc.

Facilitated by the rapid development of technology of isolation and characterization process, particularly chromatography and spectroscopic methods a large number of therapeutically active plant constituents have been isolate during the last two decades.

Simultaneous advancement in the fields of medical, botany, chemistry, biochemistry, pharmacognosy and pharmacology has tremendously helped the discovery, isolation, characterization, structure elucidation and synthesis of new drugs plants, with the development of further newer techniques and methods of plant analysis and with the tremendous increase in man's knowledge of chemistry and pharmacology more and more medicinal compounds are likely to be discovered from plants.

How prominently plant-derived drugs still feature in modern medicine can be accessed from the following facts: (Ghani, 1998).

1. A recent survey by the United Nations Commissions for Trade and Development (UNCTAD) indicated that about 33% of drugs, produced in the development countries, are derived from plants (UNCTAD/GATT 1974) and that if microbes are added 60% of medicinal products are of natural origin (Sofowara, 1982).

2. According to some sources almost 80% of present-day of medicines are directly or indirectly derived from plants (Meyers, 1892).

3. More than 47% of all drugs used in Russia, are obtained from botanical Sources (Ampofo, 1979).

4. From *Stephania cepharantha* and *Stephania sasabi* (JJEM. 1949, 1:69). In the United States, in 1980 alone, the consumer paid 8 billion dollars for prescription drugs in which the active ingredients are still alive from plants (Sofowara, 1982).

5. 47% of some 300 million new prescriptions written by physicians in America in 1961, contained as one as more active ingredients, a drug of natural origin (Famsworth, 1966).

6. In 1960 47% of drugs, prescribed by physicians in the United States, of America, were from natural sources (Bingel *et al*, 1660).

7. In 1967 25% of the products, which appeared in 1.05 billion prescriptions filled in the United States, contained one or more ingredients derived from higher plants (Karolkovans *et al*, 1966).

8. Even today 80% of the rural population of most developing countries of the world depends as herbal medicine for maintaining its health and well being (Ghani, 1987).

9. The consumption of medicinal plants in increasing in many developed countries, where 35% of drugs contain active principles from natural origin (Irvine, 1995).

10. The North America used 170 drugs from different plants, which are as official in the USP or NF. Surprisingly this large quantity of modern drugs comes from less than 15% of the plants, which are known to have been investigated pharmacologically, out of an estimated 250000 to 500000 species of higher plants growing on earth (Farmsworth *et al*, 1985).

Around 100 plant species have contributed significantly to modern drugs.

Table1.1. Drugs derived from plants, with their ethno medical correlations and sources.

Drug	Action or clinical use	Plant source
Acetyldigoxin	Cardiotonic	*Digitalis lanata*
Adoniside	Cardiotonic	*Adonis vernalis* L.
Aescin	Anti-inflammatory	*Aesculus hippocastanum* L.
Aesculetin	Antidysentery	*Fraxinus rhynchophylla*
Agrimophol	Anthelmintic	*Agrimonia eupatoria* L.
Ajmalicine	Circulatory disorders	*Rauvolfia serpentina* (L.)
Allyl Isothiocyanate	Rubefacient	*Brassica nigra* (L.)
Andrographolide	Bacillary dysentery	*Andrographis paniculata*

Drug	Action or clinical use	Plant source
Anisodamine	Anticholinergic	*Anisodus tanguticus*
Anisodine	Anticholinergic	*Anisodus tanguticus*
Arecoline	Anthelmintic	*Areca catechu* L.
Asiaticoside	Vulnerary	*Centella asiatica* (L.)
Atropine	Anticholinergic	*Atropa belladonna* L.
Berberine	Bacillary dysentery	*Berberis vulgaris* L.
Bergenin	Antitussive	*Ardisia japonica* Bl.
Bromelain	Anti-inflammatory;	*Ananas comosus* (L.)
Caffeine	CNS stimulant	*Camellia sinensis* (L.)
(+)-Catechin	Haemostatic	*Potentilla fragaroides* L.
Chymopapain	Proteolytic; mucolytic	*Carica papaya* L.
Cocaine Local	Anaesthetic	*Erythroxylum coca*
Codeine	Analgesic; antitussive	*Papaver somniferum* L.
Colchicine	Antitumor agent; antigout	*Colchicum autumnale* L.
Convallotoxin	Cardiotonic	*Convallaria majalis* L.
Curcumin	Choleretic	*Curcuma longa* L.
Cynarin	Choleretic	*Cynara scolymus* L.
Danthron	Laxative	*Cassia* spp.
Deserpidine	Antihypertensive; tranqulizer	*Rauvolfia canescens* L.
Deslanoside	Cardiotonic	*Digitalis lanata*
Digitalin	Cardiotonic	*Digitalis purpurea* L.
Digitoxin	Cardiotonic	*Digitalis purpurea* L.
Digoxin	Cardiotonic	*Digitalis lanata* Ehrh.
Emetine	Amoebicide; emetic	*Cephaelis ipecacuanha* (Brotero)
Ephedrine	Sympathomimetic	*Ephedra sinica* Stapf.
Etoposide	Antitumour agent	*Podophyllum peltatum* L.
Gitalin	Cardiotonic	*Digitalis purpurea* L.
Glaucaroubin	Amoebicide	*Simarouba glauca* DC.
Glycyrrhizin	Sweetener	*Glycyrrhiza glabra* L.

Drug	Action or clinical use	Plant source
Gossypol	Male contraceptive	*Gossypium* spp.
Hemsleyadin	Bacillary dysentery	*Helmsleya amabilis*
Kainic Acid	Ascaricide	*Digenea simplex* (Wulf.)
Kawain	Tranquilizer	*Piper methysicum* Forst. f.
Khellin	Bronchodilator	*Ammi visnaga* (L.) Lamk.
Lanatosides A, B, C	Cardiotonic	*Digitalis lanata* Ehrh.
Lobeline	deterrent; respiratory stimulant	*Lobelia inflata* L.
Monocrotaline	Antitumor agent	*Crotolaria sessiliflora* L.
Morphine	Analgesic	*Papaver somniferum* L.
Neoandrographolide	Bacillary dysentery	*Andrographis paniculata* Nees
Noscapine	Antitussive	*Papaver somniferum* L.
Ouabain	Cardiotonic	*Strophanthus gratus* Baill.
Papain	Proteolytic; mucolytic	*Carica papaya* L.
Phyllodulcin	Sweetener	*Hydrangea macrophylla*
Physostigmine	Cholinesterase inhibitor	*Physostigma venenosum*
Picrotoxin	Analeptic	*Anamirta cocculus* (L.)
Pilocarpine	Parasympathomimetic	*Pilocarpus jaborandi*
Podophyllotoxin	Condylomata acuminata	*Podophyllum peltatum* L.
Protoveratrines A & B	Antihypertensive	*Veratrum album* L.
Pseudoephedrine	Sympathomimetic	*Ephedra sinica* Stapf.
Pseudoephedrine,	nor- Sympathomimetic	*phedra sinica* Stapf.
Quinine	Antimalarial	*Cinchona ledgeriana*
Quisqualic Acid	Anthelmintic	*Quisqualis indica* L.
Rescinnamine	Antihypertensive;	*Rauvolfia serpentina* (L.)
Reserpine	Antihypertensive;tranqulizer	*Rauvolfia serpentina* (L.)
Rhomitoxin	Antihypertensive	*Rhododendron molle* G. Don
Rorifone	Antitussive	*Rorippa indica* (L.) Hochr.
Drug	**Action or clinical use**	**Plant source**

Rotenone	Piscicide	*Lonchocarpus nicou* A.
Rotundine	Analgesic; sedative	*Stephania sinica* Diels
Salicin	Analgesic	*Salix alba* L.
Santonin	Ascaricide	*Artemisia maritima* L.
Scillarin A	Cardiotonic	*Urginea maritima* (L.) Baker
Scopolamine	Sedative	*Datura metel* L.
Sennosides A & B	Laxative	*Cassia* spp.
Silymarin	Antihepatotoxic	*Silybum marianum* (L.) Gaertn.
Stevioside	Sweetener	*Stevia rebaudiana* Bertoni
Strychnine	CNS stimulant	*Strychnos nux-vomica* L.
Teniposide	Antitumor agent	*Podophyllum peltatum* L.
Tetrahydropalmatine	Analgesic; sedative	*Corydalis ambigua*
Theobromine	Diuretic; bronchodilator	*Theobroma cacao* L.
Theophylline	Diuretic; bronchodilator	*Camellia sinensis* (L.) Kuntze
Trichosanthin	Abortifacient	*Thymus vulgaris* L.
Tubocurarine	keletal muscle relaxant	*Chondodendron tomentosum* R.
Valepotriates	Sedative	*Valeriana officinalis* L.
Vincamine	Cerebral stimulant	*Vinca minor* L.
Xanthotoxin	Leukoderma;	*Ammi majus* L.
Yohimbine	Aphrodisiac	*Pausinystalia yohimbe*
Yuanhuacine	Abortifacient	*Daphne genkwa* Seib. & Zucc.
Yuanhuadine	Abortifacient	*Daphne genkwa*

1.2 Traditional medicine

Traditional medicine came into being long before the development and spread of western medicine that originated in Europe with the advent of modern science and technology. Sometimes traditional medicine involves a sophisticated theory or system, though the knowledge of traditional medicine is often passed on, verbally or otherwise, from generation to generation.

Traditional medicine is generally defined as the sum total of all knowledge and practices, whether explicable or not, used in diagnosing, preventing, and eliminating physical, mental, or societal imbalances. It relies exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing.

Most people in non-industrialised countries receive traditional health care (THC) for their everyday health care needs. The World Health Organisation has consistently estimated that 60–80% of the population of these countries rely on THC for their basic health care needs, either on its own or in conjunction with modern medical care. Studies show that demand for traditional medicine is increasing in many countries.

Since 1977, the WHO has called for recognition of traditional healers, and in 1978 declared at Alma Ata that African traditional healers should be part of the primary health care team (WHO, 1978a). The increased demand for traditional medical services, in both industrialised and developing countries, is also likely due to their increased formalisation, improved quality and safety standards, and their use as a complementary therapy (Hills *et al*, 2006)

1.2.1 Traditional Herbal Medicine

Traditional herbal medicines are naturally occurring; plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices.

1.2.2 History of Use of Traditional Herbal Medicines

By definition, 'traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in the traditional healing systems from which they emerged. An example is the use of ephedra (= Ma huang) for weight loss or athletic performance enhancement (Shaw, 1998).

1.3 Medicinal plants in Bangladesh

The total numbers of plants with medicinal properties in the subcontinent are present stands at about 2000. About 450 to 500 of such medicinal plants names so far has been enlisted as growing or available in Bangladesh.

In traditional systems indigenous knowledge (IK) plays a central role in discuses diagnosis and health care practices. Rapid westernization and introduction of modern medicine in many places has affected not only the traditional system but also associated with it. Especially in areas with rapid urbanizations the traditional medicinal system and IK have become seriously vulnerable. The risk is not so great for well documented Ayurvedic, Hekimi and Unani ones but many undocumented system of folk medicine which have been handed down form one generations to the next by word of mouth are in example of the indigenous knowledge based folk medicine which has been used and appreciated since prehistoric times.

IK based folk medicine constitutes a very diverse steam in Bangladesh and is ecosystem and ethic community specific different localities have different characteristics. The rich heritage if indigenous knowledge associated with herbal medicine is considered as the root of all systems of traditional remedies in Bangladesh. During the course of development in the remote past, a particular remedy discovered become widespread and subsequently listed refined revised improved upon and gradually incorporated in the traditional codified system among the practitioners.(Shengji, 2002)

1.4 Herbal drug research: Bioactivity guided approach

Books on herbal medicinal practice report numerous medicinal plants, which are still not investigated. These plants can be subjected to pharmacologic screening as per their traditional use to evaluate their utility. In case of significant result, chromatographic and spectroscopic methods can be applied to isolate the responsible agent. Bioactivity guided approach has three characteristic phases of investigation:

- Biological activity is detected in crude material, and a bioassay system is set up to permit the identification of active fractions and discarding the inactive ones.

- The crude material is fractionated by the most appropriate chemical procedures, all fractions are tested, and active fractions are further fractionated, and so on, until pure compounds are obtained.
- The chemical structures of pure compounds are determined.(David*et al.*, 1997)

1.5 Approaches to new product discovery

This subject is covered authoritatively in some recent publications and partially elsewhere in this paper. Several stages are involved in the process of prospecting the chemical properties of plants to discover drugs or other novel products. First, unless discoveries are fortuitous, decisions are made about which plants to sample and how to sample them. Sampling may be in the field or from *ex situ* collections, the latter perhaps represented by plants growing in botanical gardens or by dried specimens in herbaria. These decisions are based on published and unpublished information, including sometimes knowledge of local medical uses and about the relative difficulty of undertaking research in different contexts. The next step involves isolation of chemical fractions for automated screening, for example the *in vitro* testing of activity against cell lines. Promising results may lead to further tests, including perhaps clinical trials, and these may result in the development, including licensing, of marketable products. As an alternative to chemical screening, there is growing interest today in screening extract from plants for genetic information, a branch of science set to grow spectacularly. (Kuboki *et. al*, 2000)

Traditional practitioner dispensing his own medicines is being gradually shifted to herbal drug stores which are profit-oriented. As a result, there is no guarantee of the authenticity and quantity of plant material used in the preparations. The qualities of traditional medicines so produced vary widely and may not even be effective. Therefore, there is a need to select proper and appropriate technologies for the industrial production of traditional medicines such that the effectiveness of the preparation is maintained. Traditional methods used have many disadvantages which could be corrected by selecting the suitable technologies. It has to be stated that the traditional methods were dependent on the status of technology that was available at that time. It therefore follows that these can be modified and improved using the technologies available today to make them

more effective, stable, reproducible, controlled and in dosage forms that can easily be transported or taken to office.

Hence the introduction of appropriate, simple and low-cost technologies should be encouraged maintaining as much as possible the labor-intensive nature of such activities, conservation of biodiversity through small-scale production and preservation of cultural knowledge. Use of sophisticated modern technology will alienate the traditional practitioners as he has no control over such production methods. Even in the use of appropriate technologies, the practitioner who produces these drugs has to be educated about the advantages of using such production and quality control methods. One major concern in introducing modern technology for the production of traditional medicines is whether the final preparation will be acceptable to the practitioner who has sole faith in extemporaneous preparations. This problem has to be overcome by a process of education, whereby the disadvantage of the old methods and the advantage of the new methods can be imparted. The value of medicinal plant as a source of foreign exchange for developing countries depends on the use of those plants as raw materials in the pharmaceutical industry. These raw materials are used to:

- Isolate pure active compounds for formulation into drugs (guinini, reserpine, digoxin etc.)
- Isolate intermediates for the production of semi-synthetic drugs.
- Prepare standardized galenicals (abstracts, powders, tinctures etc.) If one is to produce known pure phyto-pharmaceutical used in modern medicine more processing stages and more sophisticated machinery are required.

Furthermore safety and pollution aspects have to be considered. Certain plants are rich sources of intermediates used in the production of drugs. The primary processing of parts of plants containing the intermediate could be carried out in the country of origin thus retaining some value of the resource material. Processed products (galenicals) from plants could be standardized fluid/ solid extract or powders or tinctures. Standardized extract of many plants are widely used in health care. Some of these have to be formulated for incorporation in modern dosage forms. New formulations require some development work, particularly on account of the nature of the processed products. Plant extract are difficult to granulate, sensitive to moisture and prone to microbial contamination. Hence the types of excipients to be used and the processing parameters have to be determined. (King *et al*, 1998)

1.6. The concept of free radical and reactive oxygen species (ROS)

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating.

Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction. Antioxidants are capable of stabilizing, or deactivating, free radicals before they attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. (Samal , 2013)

1.7. Reactive oxygen species (ROS)

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage. ROS are generated by a number of pathways. Most of the oxidants produced by cells occur as:

• A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.

• Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens) are denatured.

• Xenobiotic metabolism, i.e., detoxification of toxic substances. Consequently, things like vigorous exercise, which accelerates cellular metabolism; chronic inflammation, infections, and other illnesses; exposure to allergens and the presence of "leaky gut" syndrome; and exposure to drugs or toxins such as cigarette smoke, pollution, pesticides, and insecticides may all contribute to an increase in the body's oxidant load.

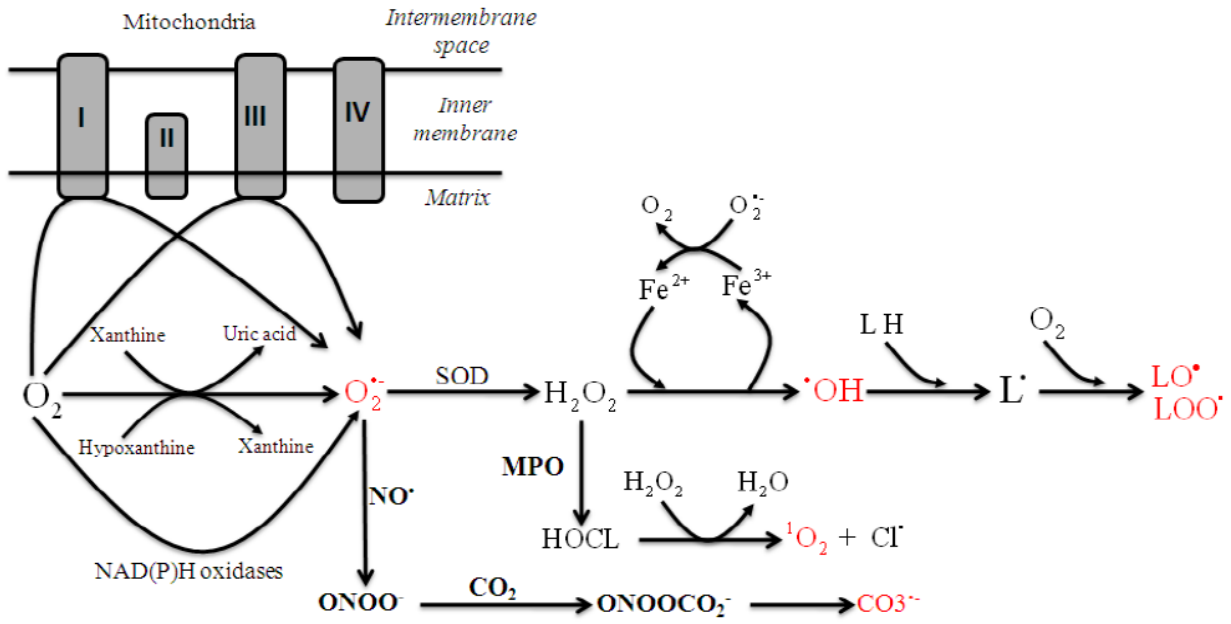


Fig 1.3: Production of free radicals via different routes(Kunwar & Priyadarsini, 2011)

1.8 Oxidative stress and human disease:

Oxidative damage to DNA, proteins, and other macromolecules has been implicated in the pathogenesis of a wide variety of diseases, most notably heart disease and cancer. A growing body of animal and epidemiological studies as well as clinical intervention trials suggest that antioxidants may play a pivotal role in preventing or slowing the progression of both heart disease and some forms of cancer. The conditions that are associated with oxidative damage are:

• Atherosclerosis

• Cancer

• Pulmonary dysfunction

• Cataracts

• Arthritis and inflammatory diseases
• Diabetes
• Shock, trauma, and ischemia
• Renal disease and hemodialysis
• Multiple sclerosis
• Pancreatitis
• Inflammatory bowel disease and colitis
• Parkinson's disease
• Neonatal lipoprotein oxidation
• Drug reactions
• Skin lesions
• Aging(Sies, 1992)

1.9 Antioxidant protection:

Antioxidants are substances that may protect cells from the damage caused by unstable free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage which free radicals might cause. Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, A and other substances (Sies, 1997).

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies 1997).

Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. As oxidative stress might be an important part of

many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline. (Sies, 1997)

1.10 Classification of antioxidants:

Antioxidants are grouped into two namely;

(1) Primary or natural antioxidants.

(2) Secondary or synthetic antioxidants.

1.10.1 Primary or natural antioxidants

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products. Antioxidants of this group are mainly phenolic in structures and include the following (Hurrell, 2003)

(1) Antioxidants minerals - These are co factor of antioxidants enzymes. Their absence will definitely affect metabolism of many macromolecules such as carbohydrates. Examples include selenium, copper, iron, zinc and manganese.

(2) Anti oxidants vitamins – It is needed for most body metabolic functions. They include- vitamin C, vitamin E, vitamin B.

(3) Phytochemicals - These are phenolic compounds that are neither vitamins nor minerals. These include:

Flavonoids: These are phenolic compounds that give vegetables fruits, grains, seeds leaves, flowers and bark their colours. Catechins are the most active antioxidants in green and black tea and sesamol. Carotenoids are fat soluble colour in fruits and vegetables. Beta carotene, which is rich in carrot and converted to vitamin A when the body lacks enough of the vitamin. Lycopene, high in tomatoes and zeaxantin is high in spinach and other dark greens. Herbs and spices-source

include Diterpene, rosmariquinone, thyme, nutmeg, clove, black pepper, ginger, garlic and curcumin and derivatives.

1.10.2 *Secondary or synthetic antioxidants*

These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions, the compound include (Hurrell, 2003):

i. Butylated hydroxyl anisole (BHA).

ii. Butylated hydroxyrotoluene (BHT).

iii. Propyl gallate (PG) and metal chelating agent (EDTA).

iv. Tertiary butyl hydroquinone (TBHQ).

v. Nordihydro guaretic acid (NDGA).

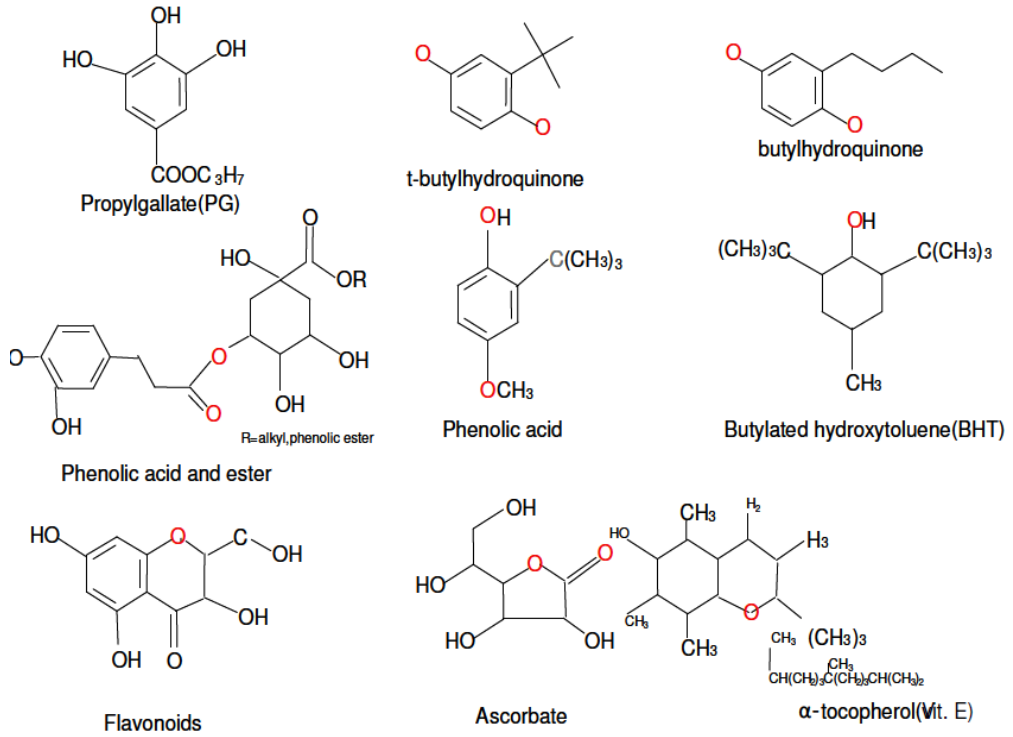


Fig 1.4: Structure of some antioxidants(Sies,1997)

1.11. Characteristics of antioxidants

The major antioxidants currently used in foods are monohydroxy or polyhydroxy phenol compounds with various ring substitutions. These compounds have low activation energy to donate hydrogen. Hence, the resulting antioxidants radical does not initiate another free radical

due to the stabilization of the delocalized radical electron. Propagation and initiation of free radicals chain reaction can be delayed or minimized by the donation of hydrogen from the antioxidants and metal chelating agent. The resulting antioxidant free-radical is not subject to rapid oxidation due to its stability. Antioxidants free-radicals can also react with lipid free radicals to form a stable complex compound thereby preventing some of their damages.

1.12 Formation of free radicals:

Normally, bonds would not split in a way that leaves a molecule with odd, unpaired electrons. However, when weak bond split, free radicals are formed. Free-radicals are very unstable and react quickly with other compound trying to gain stability. Generally, free radicals attack the nearest stable molecules abstracting its electron to attain stability. When the attacked molecule loses its electron, it becomes a free-radical itself, these formations of free-radicals continue on and on and finally result in the disruption of the substance especially in fatty foods. Environmental factors such as pollution, radiation, cigarette smoking and herbicides can also spawn free-radicals in the body but, if antioxidants are not available to check the free-radical production it becomes excessive and cause damage to body and any substance in which oxidation occurs. Of particular interest is the free radical damage in the body system, fatty foods and other substance like polymer and antioxidants mechanism of action in inhibiting these damages (Borek, 1991).

Chemically, a substance is oxidized when electrons are removed and reduced when electrons are added. All chemical reactions involve the transfer of electrons. The body generates energy by gradually oxidizing its food in a controlled manner and storing it in the form of chemical potential energy called ATP (Adenosinetriphosphate). Free radicals are generated largely during the production of ATP in the mitochondria. During this process, radicals coming out from the mitochondria from reactive oxygen species such as superioxide anion (O^{2-}) and hydroxyl radicals (HO.) and other reactive oxygen species such as singlet oxygen (O^2), destroy the body system especially the site where the free radicals is been generated. The ultraviolet light that penetrate the skin and the air pollutant that is high in smog which we inhale generates free radicals too. Food, like lipid in the presence of (Fe^{3+}, Fe^{2+}) lead to the production of hydrogen peroxide from which further hydroxyl radicals are generated in a reaction that appear to depend

on the presence of iron ions. The acceleration of hydroperoxide decomposition to form peroxyl radicals and alkoxyl radical.

$Fe^{3+} + ROOH = Fe^{2+} + ROO^{\cdot} + H^{+}$

$Fe^{2+} + ROOH = Fe^{3+} + RO^{\cdot} + OH^{-}$

Formations of alkyl free radicals by direct reaction with fats and oils.

$Fe^{3+} + RH = Fe^{2+} + R^{\cdot} + H^{-}$

Activation of molecular oxygen for singlet oxygen formation

$Fe^{2+} + O^{2} = Fe^{3+} + O^{2-}$

(Ingold, 1968).

1.13. The chemistry of antioxidants:

It involves the mechanism of action of antioxidant. Two principle mechanisms of action have been proposed for antioxidants. The first is a chain-breaking mechanism by which the primary antioxidants donate electrons to the free radicals present in the system, example lipid radicals. The second mechanism involves removal of ROS (reactive oxygen species) and RNS (reactive nitrogen species) initiator by quenching chain initiator catalyst.

Chain reactions of free radicals

Initiation stage

(1) $RH \rightarrow R^{\cdot} + H^{\cdot}$

(2) $R^{\cdot} \rightarrow R^{\cdot} + O^{2} \rightarrow ROO^{\cdot}$

(3) $2ROOH \rightarrow ROO^{\cdot} + RO^{\cdot} + H2O$

Propagation stage

(1) $R^{\cdot} + O^{2} \rightarrow ROO^{\cdot}$

(2) $ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$

(3) $RO^{\cdot} + RH \rightarrow ROH + R^{\cdot}$

Termination stage

(1) $R^{\cdot} + R^{\cdot} \rightarrow R - R$

(2) $R^{\cdot} + ROO^{\cdot} \rightarrow ROOR$

(3) $ROO^{\cdot} + ROO^{\cdot} \rightarrow ROOR + O^2$

(4) Antioxidants + O2 _ oxidized antioxidants (Borek, 1991).

Further, in free radical chain reactions, when fats are in contact with oxygen, it forms unsaturated fatty acids which give rise to free radicals in equation (i). Also hydroperoxide which exist in trace quantities prior to oxidation reaction, break down to yield radicals in equation (iv) which abstract an hydrogen atom from another molecule and become a hydroperoxide producing further radicals. The antioxidants added to it, will neutralize the free radicals by donating one of their own electrons ending the reactions in equation (vii) and (ix). These occur generally in the body.

1.14 Sources and origin of antioxidants:

Antioxidants are abundant in fruits and vegetables, as well as in other foods including nuts, grains and some meats, poultry and fish. The list below describes food sources of common antioxidants. Beta-carotene is found in many foods that are orange in color, including sweet potatoes, carrots, cantaloupe, squash, apricots, pumpkin and mangoes. Some green, leafy vegetables, including collard greens, spinach and kale, are also rich in betacarotene (Borek, 1991). Lutein, best known for its association with healthy eyes, is abundant in green, leafy vegetables such as collard greens, spinach, and kale. Lycopene is a potent antioxidant found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, blood oranges and other foods. Estimates suggest 85% of American dietary intake of lycopene comes from
tomatoes and tomato products (Xianquan *et al.*, 2005) (Rodriguez, 2003).

Selenium is a mineral, not an antioxidant nutrient. However, it is a component of antioxidant enzymes. Plant foods like rice and wheat are the major dietary sources of selenium in most countries. The amount of selenium in soil, which varies by region, determines the amount of selenium in the foods grown in that soil. Animals that eat grains or plants grown in selenium-rich soil have higher levels of selenium in their muscle. In the United States, meats and bread are common sources of dietary selenium. Brazil nuts also contain large quantities of selenium.

Vitamin A is found in three main forms: retinol (Vitamin A1), 3,4-didehydroretinol (Vitamin A2), and 3-hydroxyretinol (Vitamin A3). Foods rich in vitamin A include liver, sweet potatoes, carrots, milk, egg yolks and mozzarella cheese (Baublis et al., 2000). Vitamin C is also called ascorbic acid and can be found in high abundance in many fruits and vegetables and is also found in cereals, beef, poultry, and fish.

Vitamin E, also known as alpha-tocopherol, is found in almonds, in many oils including wheat germ, safflower, corn and soybean oils, and is also found in mangoes, nuts, broccoli, and other foods (Herrera & Barbas, 2001).

1.15. Plant as a source of antioxidants:

Plants are good sources of natural antioxidants. There is a list of plants which contain antioxidant property. Some of renowned plants which are rich with different natural antioxidants are *Cuscuta reflexa, PIcrorrhiza kurroa, Tinospora cordifolia, Curcuma domestica, Daucus carota, Emblica officinalis, Foeniculum vulgare, Glycyrrhiza glabra, Mangifera indica, Momordica charantia, Ocimum sanctum, Psoralea corylifolia* etc. Various types of natural antioxidants such as SOD, CAT, GTx, glutahthione reductse and some minerals such as Se, Mn, Cu, Zn are found in these plants. (Gupta & Sharma, 2006)

Attempts have been made to study the antioxidant potential of a wide variety of vegetables like potato, spinach, tomatoes, and legumes (Furuta S, 1997). There are several reports showing antioxidant potential of fruits (Wang H, 1996).

Strong antioxidants activities have been found in berries, cherries, citrus, prunes, and olives. Green and black teas have been extensively studied in the recent past for antioxidant properties since they contain up to 30% of the dry weight as phenolic compounds (Lin*et al*, 1998).

Screening of these plants are done by measuring the antioxidant activity by various *in vitro* methods such as DPPH radical scavenging method, DMPD method, ABTS scavenging assay, ORAC method, TBARS method etc and *in vivo* method such as rats or mice.

1.16Gastrointestinal transit activity test:

Gastrointestinal (GI) disorders are commonly associated with chronic conditions such as diabetes, obesity, and hypertension. Direct consequences are obstipation or diarrhea as opposite aspects of the irritable bowel syndrome, and more indirectly, alteration of appetite, feeling of fullness, flatulence, bloatedness, and eventually leading to altered absorption of nutrients. Moreover, GI retention and passage times have been recognized as important factors in determining the release site and hence the bioavailability of orally administered drugs. To facilitate the understanding of physiological and pathological processes involved, it is necessary to monitor the gut motility in animal models. Here, we describe a method for studying the GI transit time using technetium-labeled activated charcoal diethylenetriaminepentaacetic acid (^{99m}Tc-Ch-DTPA) detected by single-photon emission computed tomography (SPECT).

Measurement of gastrointestinal (GI) transit time is a very useful clinical and research technique for evaluating the motility disorders in the gastrointestinal tract. A number of testing methods are available and used frequently by the clinicians to evaluate the symptoms and causes of the GI transit, motility, and drug release in humans. These measurements will provide an assessment of the overall motility. The abnormality in gastrointestinal transit is considered as an unfathomable symptom. Normally, symptoms appearing in one part of the gut may overlap with symptoms from another; so, using direct *in vivo* measurements in transit studies is an important tool for diagnostic evaluations.

A variety of methods have been used for assessing GI transit times, motility, and drug release. Most well known is the use of X-ray and scintigraphic techniques which have been used to observe orally ingested capsules containing radiopaque substances or gamma emitters, respectively. On the other hand, non-invasive techniques such as ultrasound metaldetectors magnetic field detectors , and dyes have been used to avoid the adverse effects of ionizing radiation. However, all these methods have limitations due to fundamental constraints such as low temporal or spatial resolution, lack of balancing anatomical information, or incomplete spatial information. Furthermore, such capsules being foreign in nature and having considerably large size may not reflect an accurate physiology of the GI tract.

1.17 Thrombolytic activity:

Since ancient times,herbal preparations have been used for the treatment of several diseases.The

Leaves and twigs,stem,bark and underground parts of plants are most often used for traditional medicines.Herbal products are often perceived as safe because they are natural(Gesler,1992).Cerebral venous sunus thrombosis (CVST)is a common disorder which accompanied by significant morbidity and mortality(watson et al,.2002).Heparin,an anticoagulant agent,is the first line treatment for CVST,because of its efficacy,safety and feasibility(biousse and Newman,2004).Thrombolytic drugs like tissue plasminogen activator(t-pA),urokinase,Streptokinase etc.Play a crucial role in the management of patients withCVST(Baruah,2006).Thus, the aim of the present study was to investigate the thrombolytic ativity of methanolic extracts and its different fractions of *Stephania japonica.*

Thrombolysis also known as thrombolytic therapy, is a treatment to dissolve dangerous clots in blood vessels, improve blood flow, and prevent damage to tissues and organs. Thrombolysis may involve the injection of clot-busting drugs through an intravenous (IV) line or through a long catheter that delivers drugs directly to the site of the blockage. It also may involve the use of a long catheter with a mechanical device attached to the tip that either removes the clot or physically breaks it up.Thrombolysis is often used as an emergency treatment to dissolve blood clots that form in arteries feeding the heart and brain -- the main cause of heart attacks and ischemic strokes -- and in the arteries of the lungs (acute pulmonary embolism).

Thrombolysis is also used to treat blood clots in:

- Veins that cause deep vein thrombosis (DVT) or clots in the legs, pelvic area, and upper extremities; if left untreated, pieces of the clot can break off and travel to an artery in the lungs, resulting in an acute pulmonary embolism.
- Bypass grafts
- Dialysis catheters

CHAPTER 2

OBJECTIVE OF THE STUDY

2. Objective of Study:

In this project I worked on a medicinal plants named by *stepanania japonica* and *Trapabisphinosa*. This experiment is to search some activity of this plant by whole plant (Both) parts which can be established as a beneficial drug in medicine world**.**

The main aim of this project-

- **Search the antioxidant activity of *Stephania japonica* and *Trapa bisphinosa* plant parts.**
- **Search the Thrombolytic activity effect of the plant *Stephania japonica* on human blood sample.The aim of the present study was to investigate the thrombolytic activity of methanolic extracts of *Stephania japonica* plant.**
- **Pharmacological activity of the gastrointestinal transit test of the *Trapa bisphinosa* plant parts.**

Plants have formed the basis for traditional medicine systems which have been used for thousands of years in many countries of the world. These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization that approximately 80% of the world's inhabitants mainly on traditional medicines for their primary health care.

For doing a pharmacological study on a suitable plant is a very essential and decisive step. Mainly the plants are selected on basis of their traditional use, chemical content, toxicity, randomized selection or a combination of several criteria. The most common strategy is careful observation of the use of natural resources in folk medicine in different cultures; which is usually known as ethnobotany or ethnopharmacology. Keeping these selection criteria in mind, I selected two medicinally important plants for my research work.

My two plants are- *Staphania japonica,Trapa bispinos,*.These plants that are very commonly distributed in Bangladesh. Ethnopharmacological data reveals that these plants are very widely used by various ethnic groups to treat various health problems. So these plants could be very good source of discovering novel compounds with medicinal properties.

Encouraged by these ethnobotanical data on the traditional uses of these plants, I wanted to explore some pharmacological effects of these plant samples which were previously collected as a part of the on-going research project conducted and supervised by **Dr. Shamsun Nahar Khan**. In my study I mainly focused to determine the antioxidant activity of two plant samples and Thrombolytic activity test by the *staphania japonica* and gastrointestinal transit test by the *Trapha bisphinosa* plants.

CHAPTER 03

INTRODUCTION

TO THE PLANT

3. Introduction to the plant

3.1 *Trapa bispinosa*:

Trapa bispanosa also *Trapa bispinosa* Roxb (water chestnut), known as Water chestnut, Caltrop, Singhara nut (hindi), Karimphola (Malayam), Cimkhara (Tamil), Kubjakamu (Telegu), is an annual, floatingleaved aquatic plant found in freshwater wetlands, lakes, ponds, and sluggish reaches of rivers in India. It is one of the most popular vegetables used in Asia, due to its special feature and medicinal values. *Trapa bispinosa* is an aquatic floating herb which belongs to the family Trapaceae. It has flexuose stem, ascending in the water; the submerged parts are furnished with numerous opposite pairs of green root-like spreading pectinate organs. Leaves are alternate, crowded on the upper part of the stem; 3.8–5 cm long, rhomboid, somewhat truncate at the base, irregularly inciso-serrate, reddish-purple beneath; petiole dilated near the apex. Flowers are few, auxiliary, solitary, pure white. Fruits obovoid, angular, 2.2–5 cmlong, and broad,with a spreading flattened very sharp spinous horn at either side, indehiscent, one seeded, seeds while starchy. It contains carbohydrates, minerals, calcium, phosphate, iron, copper, manganese, magnesium, sodium and potassium. The kernels contain some vitamins like thiamine, riboflavin, nicotinic acid, vitamin C, vitamin A, Damylase and considerable amount of phosphorylases.

(Rodrigues *et al*, 1964)

Fig3.1.Trapa bisphinosa. (Feedtrade,2013)

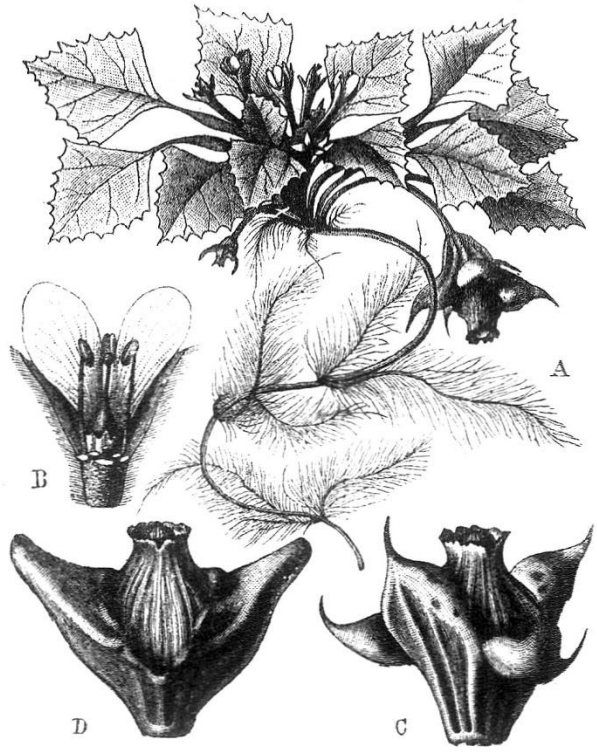


Fig 3.2: *Trapa bispanosa*(Feedtrade,2013)

3.1.1. Distribution:

Trapa bispinosa is an annual aquatic plant found in tropical and subtropical and temperate zones of the world. Their natural range of growth includes Southern Europe, Africa and Asia. It has been grown in Europe since Neolithic times. It is commonly used as food by ancient Europeans as an easy growing plant. *Trapa bispinosa* is commonly grown throughout India and locally known as water chestnut. (Singh *et al.*, 2011)

In addition to being important for aquatic ecosystems, *Trapa bispinosa* species are also food for humans and animals in India, China, and Southeast Asia. It is grown throughout Asia and tropical Africa in lakes and ponds and is often cultivated for its edible fruit. The medicinal values of the whole herb and fruit have long been recognized in folklore medicine as a cure for various diseases. (Rahman *et al.*, 2001)

3.1.2 Taxonomy

Kingdom : Plantae

Subkingdom : Tracheobointa

Division : Magnoliophyta

Class : Magnoliopsida

Subclass : Rosidae

Order : Myrtales

Family : Trapaceae

Genus :*Trapa*

Species :*bispinosa*

(Imtiaz, 2013)

3.1.3 Traditional uses:

It is used in many Ayurvedic preparations as nutrient, appetizer, astringent, diuretic, aphrodisiac, tonic, cooling and antidiarrheal agents. It is also useful in lumbago, sore throat, bilious affections, bronchitis, fatigue and inflammation. The medicinal values of the whole herb and fruit have long been recognized in folklore medicine as a cure for various diseases5. The whole herb has been reported for hepatoprotective activity, antimicrobial activity, antibacterial activity, antitumor activity, antioxidant activity and free radical scavenging activity. Further, the fruits have been used as intestinal astringent, aphrodisiac, antiinflammatory, antileprotic agent and in urinary discharges, fractures, sore throat, bronchitis and anemia. In addition to this, the juice of the fruit has been used for diarrhea and dysentery. Fruits are also being used in making liniments for the cure of rheumatism, sores and sunburn. It is also said to have cancer-preventing properties. Stem juice is used in ophthalmic preparations. Due to high activity of enzymes and phenolic contents, the color of water caltrop hulls easily changes from original pink to dark brown during transportation and processing. (Chandana,2013).

3.2.*Stephania japonica*:

Stephania japonica, known as snake vine, is a common scrambler often seen in sheltered areas near the sea.

3.2.1Description:

A dioecious vine without prickles. Greenish small flowers form on compound umbels, growing from the leaf axils in the warmer months. Inflorescences are 4 to 8 cm long. The fruit is an oval shaped, orange or red drupe, 2 to 5 mm long. A feature of this plant is the peltate leaves, (the stem is attached to the leaf, away from the leaf edge).

Fig.3.3. *Stephania japonica* leaf (Feedtrade,2013)

3.2.3 Distribution:

A widespread vine seen as far south as Eden, New South Wales, north through Queensland.Also seen in Japan, IndiaNepal and many other areas of south-east Asia and the Pacific region. The original specimen was collected in Japan, hence the specific epithet “japonica”. The variety in New South Wales is known as *bicolor*, as the under-side of the leaf is somewhat paler than above.

3.2.4 Chemistry

Protostephanine is an alkaloid from Stephania japonica (Menispermaceae). Antihypertensive agent.

3.2.5 Scientific classification:

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
Order:	Ranunculales
Family:	Menispermaceae
Genus:	*Stephania*
Species:	*S. japonica*

Binomial name

Stephania japonica
(Thunb.) Miers

3.2.6 Medicinal uses

Used in Ayurveda and Sidha. Whole plant hypotensive and spasmolytic. Roots and leaves in bowel disorders, stomachache, dyspepsia, dysentery, diarrhea, dropsy, cough, fever, birth control, piles. Root paste taken for vertigo, fever, diarrhea, dysentery, indigestion and urinary troubles; root for heart troubles; root tuber mixed with root juice of Flemingia stricta taken for asthma. Leaf paste applied on septic ulcer, headache; leaves extract for birth control, leucorrhea; leaf juice applied on forehead to cure headache and for cooling; leaves with honey for diarrhea, jaundice and urinary complaints; stem bark and leaves for diarrhea, dysentery(CRC, 2011).

Historically used to treat fever and diarrhea; continue use where antibiotics prescribed. Stephania japonica (stephania) showed MDR- reversing activity (CBM.2012)

Stem and Root Processing: Dry under the sun. Method of Administration: Oral (decoction); Topical(paste of fresh root or herb).

Folk Medicinal Uses:

l) Abdominal pain2) Common cold 3) Diarrhea 4) Feebleness caused by pulmonary tuberclosis 5) Sore-throat 6) Tooth ache 7) Stomach-ache 8) Edema 9) Beriberi 10) Urine difficulty 1 l) Eczema of vulva 12) Rheumatic arthritis 13) Snake bite 14) Carbuncle (Folk Medicine,2012)

Fig.3.4.*Stephania japonica*.(Feedtrade,2013)

In Japan and Taiwan decoction of the plant is used as a drink to treat malaria and to invigorate. In Indonesia, the roots are used to assuage stomachaches, and a paste of the fruit is applied to cancer of the breast. The antimalarial properties of the plant are very likely owed to the interesting

array of isoquinolines, which abound in the plant, including homostephanoline, hasubanon ine, prometaphanine, epistephanine, cyclanoline, hasubanol, isotrilobine, and trilobine. 5–

13 Hall and Chang

14 made the interesting observation that isotrilobine in reverse doxorubicin resistance in human breast cancer cells might hold some potential for chemotherapy. Note that weight loss

phytopharmaceuticals containing Stephania tetrandra S. Moore are banned from the Europ ean market because of their hazardous effect on the kidneys .[Medicinal Plants of Asia and the Pacific]

(Root): Stephanine, protostephanine, epistephanine, hypoepistephanine, homostephanoline,

metaphanine, prometaphanine, hasubanonine, insularine, cyclanoline, steponine, stephanoline, stepinonine. Treats nephritic edema, urinary tract infection, rheumatic arthritis, sciatic neuralgia.

Fig3.5:.*Stephania japonica* fruit (Feedtrade,2013)

CHAPTER 04

LITERATURE REVIEW

4. Literature review:

4.1. *Trapa bispinosa* Roxb.

Water chestnut (*Trapa bispinosa* Roxb.) is an aquatic plant of temperate and tropical freshwater wetlands, rivers, lakes, ponds and estuaries.It is now a species of conservation concern in Europe and Russia. It has been used in traditional system of medicine like Unani and Ayurveda since centuries for many medical conditions like strangury, dysuria, polyuria, sexual debility, general debility, sore throat, lumbago, bilious affections and dysentery etc. Modern researches have supported its traditional uses and also explored other important properties such as Analgesic, antibiotic, antidiabetic, immunomodulatory, neuroprotective etc. (Imtiaz *et al*, 2013)

4.1.1. Bioactive molecules

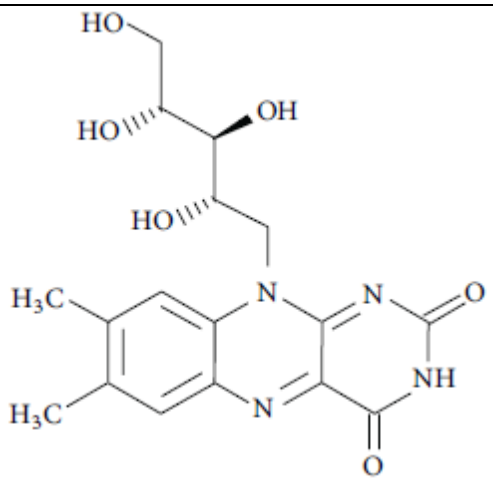
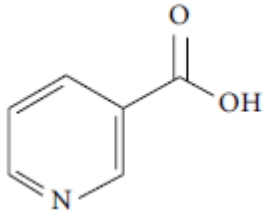
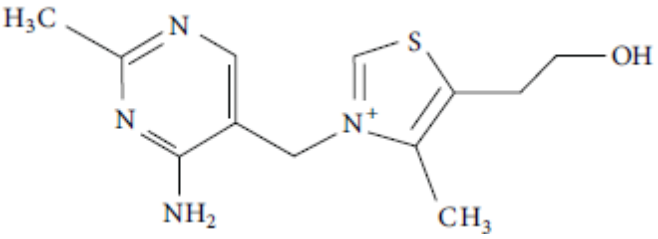
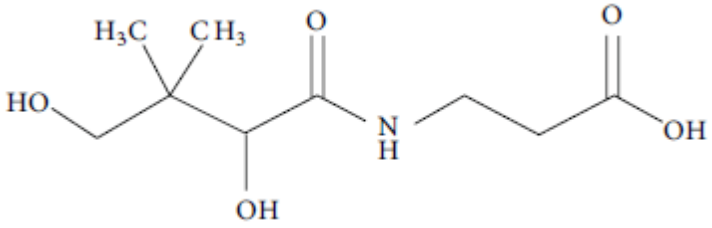
Trapa bispinosa (singhara) contains many organic and inorganic constituents which are mentioned below.

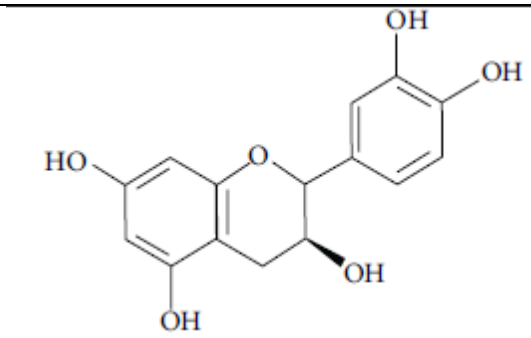
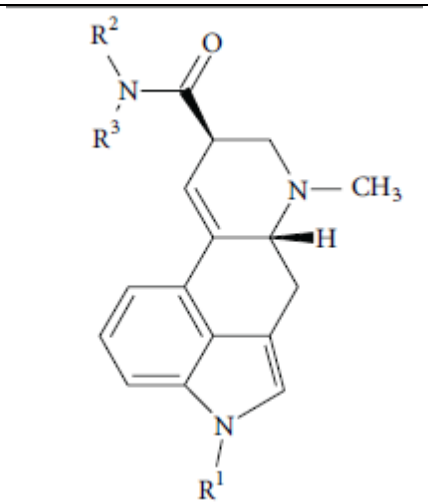
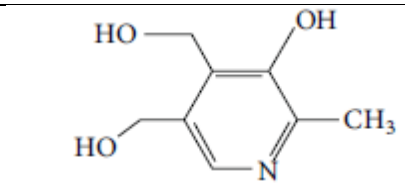
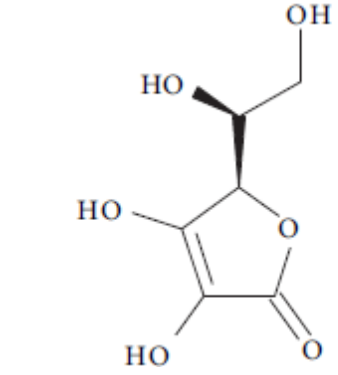
(a) Inorganic Constituents: Acids, minerals, calcium, phosphorus, iron, copper, manganese, magnesium, sodium and potassium.

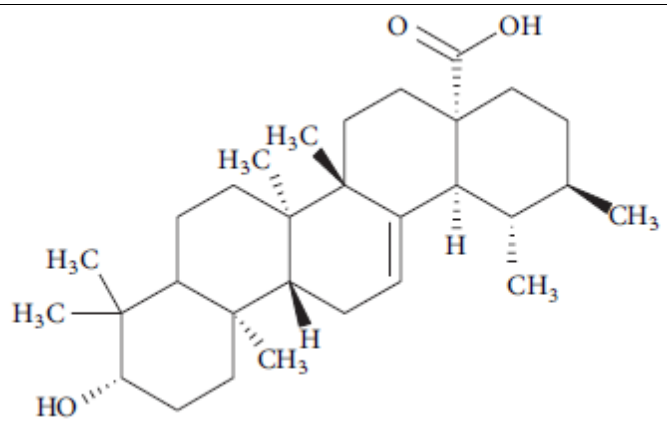
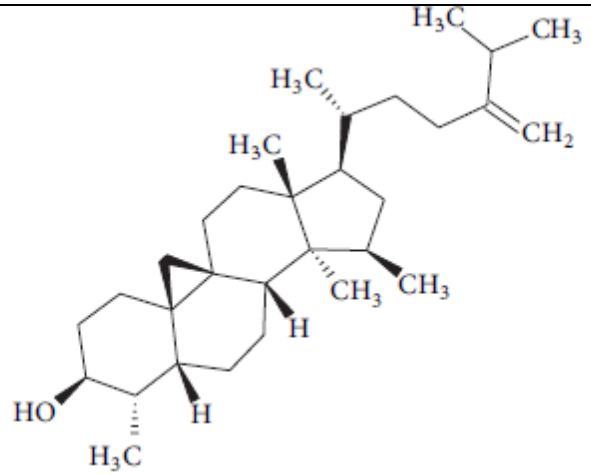
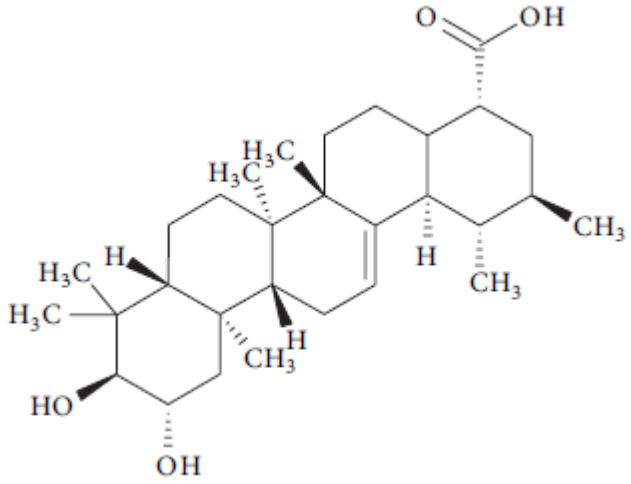
(b) Chemical Composition of Water Chestnut Kernel. Chemical analysis of the fruit showed that the moisture content of *Trapa bispinosa* kernel was 81.12% (wet basis). Fresh nuts having onsiderable water content are taken at breakfasts and are believed to suppress stomach and heart burning. The total soluble solids content of the fruit was 7.2%. The total acid in terms of citric acid present was 0.142%. Negligible amount of fat content was noticed in the fruit as 0.36% which substantiates its importance as dietary food. Also reported low crude lipid content in Chinese water chestnut was 0.06%. The potassium content of 0.41% has been reported as the major mineral present with iron and manganese contents which were 0.21 and 0.08%, respectively, being the minor minerals present. Crude fiber content of the water chestnut kernel was found to be 0.72% slightly higher than reported in Chinese *Trapa bispinosa* as 0.60%. The total protein content calculated in the fruit was 1.87%. It contains carbohydrates and vitamins, namely, Vitamin B-complex (thiamine, riboflavin, pantothenic acid, pyridoxine, nicotinic acid),

vitamin-C, vitamin-A, D-amylase, amylase, and considerable amount of phosphorylase. Cycloeucalenol, ursolic acid, and 2β,3α,23-trihydroxyurs-12-en-28-oic acid.

(Khare, 2007)

Riboflavin (vitamin B2)	
Nicotinic acid/Niacin (vitamin B3)	
Thiamine (vitamin B1)	
Pantothenic acid (vitamin B5)	

Flavonoids	
Alkaloids	
Pyridoxine (vitamin B6)	
Ascorbic acid (vitamin C)	

Triterpenoids (ursolic acid)	
Triterpenoids (cycloeucalenol)	
(2β, 3α)-2,3-Dihydroxy-urs-12-en-28-oic acid	

(Adhkar *et al*., 2014)

4.1.2. Pharmacological activity:

The following pharmacological activities have been reported on the genus:

4.1.2.1. Antimicrobial activity:

The methanolic extract of this plant at the concentration of 200 µg/disc showed a more potent antimicrobial activity against Gram positive (*Bacillus subtilis, B. cereus, B. megaterium, Staphylococcus aureus* and *Staphylococcus β-haemolyticus*) and Gram negative (*Escherichia coli, Klebsiella, Pseudomonas aeruginosa, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Shigella boydii, Salmonella typhi* A and *Salmonella typhi* B-56) bacteria than the activity shown by ethyl acetate, chloroform and petroleum ether extracts. The most significant cytotoxic activity in the brine shrimp lethality assay was observed for the chloroform extract.
(Agarwal *et al*, 2011)

4.1.2.2. Analgesic activity:

The methanolic extract of *Trapa natans* L. var. *bispinosa* Roxb. roots had shown potential analgesic ctivity on tested animals. Analgesic activity of the methanolic extract of the *T. bispinosa* root at a dose of 200 mg/kg and 400 mg/kg was evaluated by tail flick and tail immersion method against the standard drug Pentazocine at a dose of 30 mg/kg. The result suggest a significant analgesic activity which was observed by centrally acting drug.
(Arahari et.al, 2010) (Saxena & Brahman, 1995)

4.1.2.3. Anti-inflammatory activity:

Fruits of *Trapa natans* L. var. *bispinosa*, commonly known as Shingoda, were reported to be potential antiinflammatory agent in traditional literatures. Antiinflammatory activity was performed by using Carrageenan induced hind paw edema model. The aqueous extract of pericarp had shown significant anti-inflammatory activity by decreasing paw volume on the 3rd and the 5th hour, while the aqueous extract of seed showed significant antiinflammatory activity by decreasing the paw volume at the 5th hour only. (Patel *et al*, 2001)

4.1.2.4. Antioxidant activity:

The effect of hydroaccoholic extract of *T. bispinosa* (TB) was studied on fluorescence product and biochemical parameter like peroxidation catalase activity and glutathione peroxidase activity in brain of female Albino mice. Ageing was accelerated by the treatment of 0.5 ml of 5% D–glucose for 15 days. This resulted in increased fluorescence product showed an increase in lipid peroxidase and decrease the antioxidant enzyme like glutathione peroxides and catalase in cerebral cortex. After cotreatment with hydroalcoholic extract of TB (500 mg/kg) there was decrease in fluorescence product in cerebral cortex. Moreover, TB inhibited increase lipid peroxidation and restores glutathione peroxidase and catalase activity in cerebral cortex as compare to ageing accelerated control group. Thus the extract was found to be effective as an antioxidative agent which could reverse D-galactose induced ageing changes resulting due to oxidative damage. (Kar *et al.,* 2004)

4.1.2.5. Noortropic activity

TB extract showed significant facilitatory effect on aversively investigated for its noortropic activity using various experimental paradigms of learning and memory, *viz.* transfer latency (TL) on elevated plus-maze, passive avoidance response (PAS) and object recognition test. The investigation reported that TB 500 mg/kg significantly reduced the TL on 2nd and 9th day while TB 250 mg/kg was found effective on 9^{th} day. TB 250 and 500 mg/kg significantly increased the step down latency in the PAS at acquisition and retention test. Moreover the TB (250 & 500) motivated learning and memory in mice as well as improvement of memory in absence of cognitive deficit. From the above experiment it was proved that the hydroalcoholic extract of TB had significant noortropic activity. (Ambikar *et.al,* 2008)

4.1.2.6. Imunomodulatory activity:

Aqueous extract of fruits of *T. bispinosa* (TBAE) showed promising immunomodulatory function. The immunomodulatory effect was assessed in rats against sheep red blood cells (SRBC) as antigen by studying cell-mediated delayed type hypersensitivity reaction (DTH), humeral immunity response and percent change in neutrophil count. Macrophage phagocytosis assay was carried out by carbon clearance method in mice. Oral administration of TBAE dependently increased immunostimulatory responses. Delayed type hypersensitivity reaction was

found to be augmented significantly ($p<0.05$) by increasing the mean foot pad thickness at 48 hr and production of circulatory antibody titre (humoral antibody response) was significantly ($p<0.05$) increased in response to SRBC as an antigen. Immunostimulatory property of TBAE further **c**onfirmed by elevating neutrophil counts significantly ($p<0.01$), as compared to control. The results of the present study suggested that the aqueous extract of fruits of *T. bispinosa* could stimulate the cellular and humoral response in animals. (Markare *et.al*, 2001) (Momin, 1987)

4.1.2.7. Antidiabetic activity:

Antidiabetic activity of methanolic extract of *T. napans* fruit peels (METN) was studied in Wistar rats. The effect of METN on oral glucose tolerance and its effect on normoglycemic rats were studied. Diabetes mellitus was induced in rats by single intraperitonial injection of Streptozotocin (STZ, 65 mg/kg body weight). Three days after STZ induction, the hyperglycemic rats were treated with METN orally at the dose of 100 and 200 mg/kg body weight daily for 15 days. Glibenclamide (0.5 mg/kg body weight, orally) was used as reference drug. The fasting blood glucose levels were measured on every 5 days during the 15 days treatment. METN, at the doses of 100 and 200 mg/kg, was found to be orally significant ($p<0.001$) and dose dependently improved oral glucose tolerance, exhibited hypoglycemic effect in normal rats and ant diabetic activity in STZ induced diabetic rats by reducing and normalizing the elevated fasting blood glucose levels as compared to those of STZ control group. *T. natans* fruit peel demonstrated promising antidiabetic activity in STZ-induced diabetis in Wistar rats. (Das *et.al*, 2011) (Singh, 2010)

4.1.2.8. Antiulcer Activity:

The antiulcer activity of the fruits of *Trapa bispinosa* was studied on Wistar rats. The antiulcer activity of 50% ethanolic extract at two dose levels was evaluated by using pyloric ligation and aspirin plus pyloric ligation models. The tests extract revealed significant antiulcer activity, which might be due to increase in total carbohydrate content and alter state of mucosal barrier of the stomach.The results indicate that the ethanolic extract of fruits of *Trapa bispinosa* is endowed with potential antiulcer activity. (Kar *et.al*, 2010)

4.1.2.9. Neuropharmacological activity:

The different doses (100, 250, 500mg/kg, p.o) of hydroalcoholic extract of *Trapa bispinosa* were administered in laboratory animals. The effects of extract on various parameters, like motor coordination, spontaneous locomotor activity, object recognition, transfer latency, anxiolytic activity, and sodium nitrite induced respiratory arrest and hypoxic stress, and so forth, were studied. The *Trapa bispinosa* (250 & 500mg/kg) was found to decrease time required to occupy the central platform (transfer latency) in the elevated plus maze and to increase discrimination index in the object recognition test, indicating nootropic activity. *Trapa bispinosa* (250 & 500mg/kg) showed significant increase in reaction time in hot plate analgesic activity. Moreover, it also showed significant reduction in spontaneous locomotory activity and latency memory which may be due to enhanced cholinergic function. It also showed significant analgesic activity. (Vyawahare & Ambikar, 2010)

4.1.2.10. Neuroprotective Activity:

Effect of hydroalcoholic extract of *Trapa bispinosa* was studied on fluorescence product and biochemical parameters like lipid peroxidation, catalase activity, and glutathione peroxidase activity in brain of female albino mice. Ageing was accelerated by the treatment of 0.5mL 5%D-galactose for 15 days. This resulted in increased fluorescence product, increased lipid peroxidation and decreased antioxidant enzyme like glutathione peroxidase and catalase in cerebral cortex. After cotreatment with hydroalcoholic extract of *Trapa bispinosa* (500mg/kg, p.o) there was decrease in fluorescence product in cerebral cortex. Moreover, *T. bispinosa* inhibited increased lipid peroxidation and restored glutathione peroxidase and catalase activity in cerebral cortex as compared to ageing accelerated control group. (Agrahari *et al.*, 2010)

4.1.2.11. Enzymatic Activity:

The activities of some enzyme like amylase, cellulose, invertase, lipase, and protease were studied in locally available two varieties (green and red) of water chestnuts, Asian aquatic fruits popular for its nutritive value and medicinal properties. All the tested enzyme activities were found slightly higher in green variety than in red variety. The amylase, cellulase, invertase, lipase, and protease activities were 0.3532, 0.1922, 0.0587, 0.0234, and 0.0548mg/mL/min, respectively, in green variety and 0.2514, 0.1221, 0.0520, 0.0204, and 0.0515mg/mL/min,

respectively in red variety. From the enzyme activity assay it was found that water chestnuts might be used as a source of some enzymes such as amylase, cellulase, invertase, lipase, and protease. These enzyme activities could be major factor for determining the nutritive and medicinal value of the water chestnuts. (Adhkar *et.al*, 2014).

4.2 stephania japonica:

Review of literature:

4.2.1. Phytochemical studies:

4.2.1.1 Phytochemical screening:

The freshly prepared crude extract was qualitatively tested for thepresence of chemical constituents.Phytochemical screening of the extract was performed using thefollowing reagents and chemicals.Alkaloids with Dragendorff,s reagent.;flavonois with the use of Mgand HCL;tannins with ferric chloride and potassiumdichromate solutions and saponins with ability toproduce stable foam and steroids with Libermann-Burchard reagent.Reducing sugars with Benedict'sreagent.These were identified by charachteristc color changes using standard procedures.(Ghanni,2003).

4.2.1.2In vivo analgesicc screening:

Acetic acid induced writhing test:

The analgesic activity of teh samples was also studied using asetic acid-induced writhing test model in mice.Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered intraperitonially 15 min before injection of acetic acid.After an interval of 5 min, the mice were obsereved for specific contraction of body referred to as 'writhing' for the next 10 min. (Ahmed *et al*,2004)

4.2.1.3 In vitro tests for antioxidant screening:

Free radical scavenging activity measured by 1,1-diphenyl-2-picryl-hydrazyl(DPPH):The free Radical Scavenging activity of MeOH extract based on the Scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl(DPPH) free radical was determined by the method described by Braca

et al.(2001).Plant extract (0.1 ml) was added to 3 ml of a 0.004% MeOH solution of DPPH.Absorbance at %17 nm was determined after 30 min and the percentage inhibition activity was calculated from [(A0-A1)/A0]×100,where A0 is the absorbance of the control and A1 is the absorbance of the extract/standard.IC50 value was calculated from the equation of line obtained by plotting a graph of concentrations (μg mL¯1) Versus% inhibition (Rahman,2011).

4.2.1.4 Evaluation of Post-Coital Pregnancy Interceptive Activity:

Female rats exhibiting normal estrus cycle were selected for the study. These female rats were caged overnight with coeval males of proven fertility in the ratio of 2:1 and the vaginal smears of female rats were checked on the following morning. The day of presence of spermatozoa in thevaginal smear was considered as day 1 of pregnancy. Mated rats were isolated, randomized into various treatment groups and treated orally with the test agent or distilled water (vehi-cle) during first 7d of postcoitum. The animals were laparotomised under light ether anesthesia and sterile conditions on day 10 of pregnancy. Both horns of the uterus were observed for the number and status of implants and corpora lutea. The rats were allowed to recover and deliver after fullterm.

4.2.1.5DeterminingVariations in Estrus Cycle:

The estrus cycle of rats were monitored for 12d intwogroups, viz., con-trol and dose of test substance with best activity. Acyclic rats and rats with prolonged cycles were screened and eliminated. The process included examination of vaginal smear from each animal under microscope to observe different phases and duration of the estrus cycle 2.(Mukherjee *et al*, 2006)

CHAPTER 05

MATERIALS AND METHOD

5. Materials and Method

Two plant samples were selected to conduct this thesis work; these are *Trapa bispinosa,* and *Stephania japonica*. All two sample plants are subjected to antioxidant test, thrombolytic activity and gastrointestinal transit test. Among all samples only the fraction of methanolic extract of *Trapa bispinosa* (n-hexane and ethyl acetate fraction) is subjected to antioxidant assays and thrombolytic activity test by the *Stephania bisphinosa* and Gastrointestinal transit test by the *Trapa bisphinosa* medicinal plant parts.

5.1 Materials

5.1.1. Lists of Glass Wares

Glass rod, pipette, pasteur pipette, test tube, vial, conical flask, separating funnel, beaker (large, medium and small), round bottomed flask, flat bottomed flask, volumetric flask, funnels, reagent bottle, measuring cylinders, petri dish.

5.1.2. Lists of Other Material

Aluminium foil paper, spatula, pipette pumper, micropipette tip, cotton wool cloth, gas burner, forceps and tongs, labels and masking tape, filter paper, permanent marking pen, scissors, pH meter, pencil and pen, scale, tissue paper.

5.1.3. Lists of Equipments

UV-Visible Spectrophotometer, hot air oven, centrifuge machine, electric balance, rough balance, distilled water plant.

5.1.4 Lists of Solvents

Methanol, ethanol, n-hexane, ethyl acetate, distilled water.

5.1.5 Lists of Reagents

Folin-Ciocalteu reagent, salicylic acid, sodium carbonate, DPPH (2,2-diphenyl-2-picrylhydrazyl), ascorbic acid, monobasic sodium phosphate, dibasic sodium phosphate,

potassium ferricyanide, trichloroacetic acid, ferric chloride, potassium dichromate, Sulphanilamide, naphthylethylenediamine dihydrochloride, phosphoric acid, Sodium Nitroprusside.

5.1.6. Lists of Plant Sample

1. *Stephania japonica*
2. *Trapa bispinosa*

5.2. Total phenolic Content Assay

5.2.1. Introduction

The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, It has been reported that there is an inverse relationship between the antioxidative status occurrence of human diseases.

In addition, antioxidant compounds which are responsible For Such antioxidants activity, could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders.

5.2.2. Principle

In the alkaline condition phenols ionize completely. When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution become blue. The intensity of the color change is measured in a spectrophotometer at 700 nm. The absorbance value will reflect the total phenolic content of the compound.

5.2.3. Materials & Methods

Total phenolic content of crude extracts was measured employing the method as described by Arpona Hira *et al.* (2013) involving Folin-Ciocalteu reagent as oxidizing agent and salicylic acid as standard.

Table 5.2.1: Composition of Folin-Ciocalteu Reagent

Water	57.5ml
Lithium Sulfate	15.0mg
Sodium Tungstate Dihydrate	10.0mg
Hydrochloric Acid 25%	10.0mg
Phosphoric Acid 85 % solution in water	5.0mg
Molybdic Acid Sodium Dihydrate	2.5mg

5.2.3.1. Standard Curve Preparation

Salicylic acid was used here as standard. Different concentration of Salicylic acid solution were prepared having a concentration ranging from 10 mg/ml to 0.625 mg/ml. 5.0 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 4.0 ml of Na_2CO_3 (7.5 % w/v) solution was added to 100µl of Salicylic acid solution. The mixture was incubated for 1 hour at room temperature. After 1 hour the absorbance was measured at 700 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

5.2.3.2. Sample Preparation

10 mg of crude extract was taken and dissolved in 1ml of methanol to get a sample concentration of 10mg/ml in every case.

5.2.3.3. Determination of Phenolic Content of Samples

100 µl solution of crude extract mixed with 5.0 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 4.0 ml of Na_2CO_3 (7.5 % w/v) solution. The mixture was incubated for 1 hour at room temperature. After 1 hour the absorbance was measured at 765 nm.

Using the absorbance of the sample,

total phenolic content is measured by using following equation ---

$$T = \frac{C \times V}{M} \text{ mg/gm}$$

Where,

T = Total phenolic content

C = Concentration from linear regression equation

V = Volume of sample

M = Mass of sample

5.3. DPPH Radical-Scavenging Activities

5.3.1. Introduction

Lipid peroxidation is one of the main reasons for deterioration of food products during processing and storage. Synthetic antioxidant such as *tert*-butyl-1-hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are widely used as food additives to increase self life, especially lipid and lipid containing products by retarding the process of lipid peroxidation. However, BHT and BHA are known to have not only toxic and carcinogenic effects and humans, but abnormal effects on enzyme systems. Therefore, the interest in natural antioxidant, especially of plant origin, has greatly increased in recent years.

5.3.2. Principle

The free radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl) is determined spectrophotometrically. When DPPH reacts with an antioxidant

compound, which can donate hydrogen, it was reduced. The changes in color (from deep violet to light yellow) were measured at 517 nm on a UV-visible light spectrophotometer.

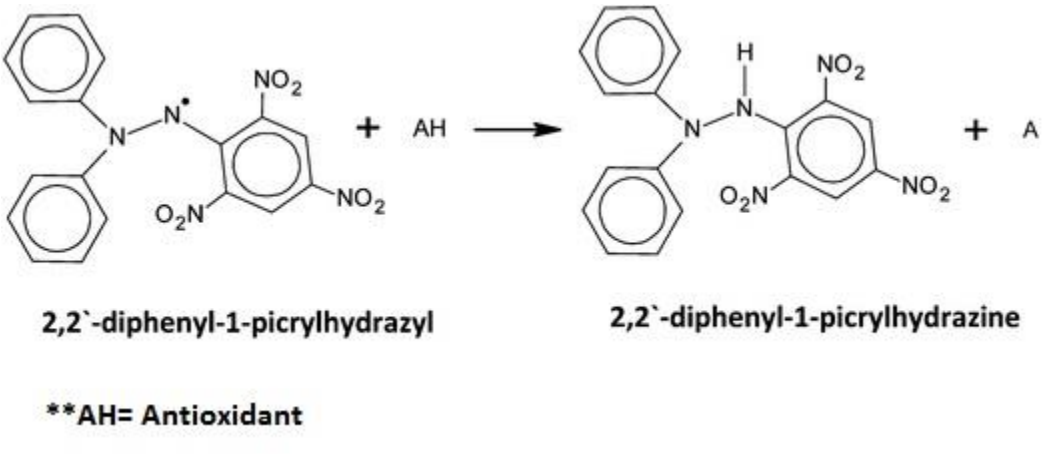


Figure 5.1.: Reaction of DPPH with antioxidant.

5.3.3. Material and Method

DPPH was used to evaluate the free radical scavenging activity of crude extracts was measured employing the slightly method described by Arpona Hira et al. (2013) involving DPPH as oxidizing agent and Ascorbic acid as standard.

5.3.3.1 Preparation of DPPH Solution

A dry 250ml conical flask was cleaned and covered with an aluminium foil protect its contents from light. Accurately weighted 2mg of DPPH placed in conical flask and 50ml methanol was added to prepare 0.1mmol/L or 40µg/ml DPPH solution.

5.3.3.2 Preparation of Sample Solution

Accurately weighted 20 mg of plant extract was taken into a vial and 2ml of methanol was added and the concentration of final solution is 10µg/µl ten test tube were taken and covered with an aluminium foil protect its contents from light and these test tubes were marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from 0.98µg/ml to 500 µg/ml. The test was done three times.

5.3.3.3 Preparation of Standard Solution

Accurately weighted 20 mg of ascorbic acid as standard was taken into a vial and 2 ml of distilled was added and the concentration of final solution is 10µg/µl ten test tube were taken and covered with an aluminum foil to protect its contents from light and these test tubes were marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from 0.98µg/ml to 500 µg/ml. The test was done three times.

5.3.3.4. Measurement of DPPH Radical Scavenging Activity

2ml of a methanol solution of the extract at different concentration were mixed with 2ml of a DPPH methanol solution and this mixture was vigorously shacked and left at 25^0C for 60 minutes in the dark. After 60 minutes reaction period at room temperature in dark place the absorption was measured at 517nm of methanol as blank by UV spectrophotometer.

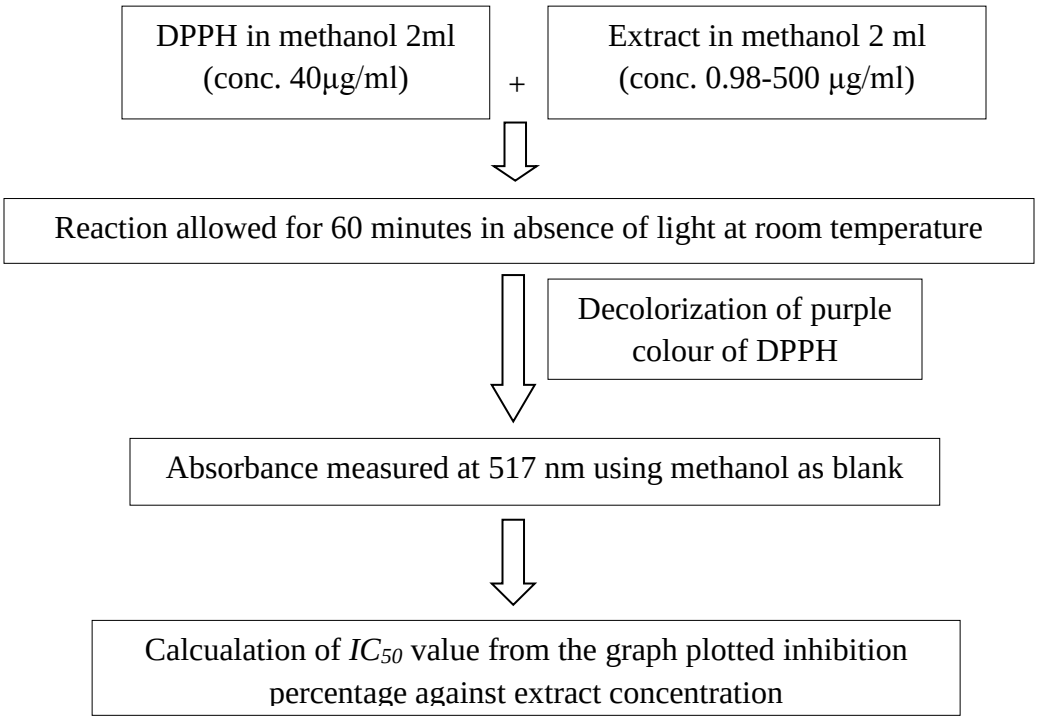


Figure 5.2.: Schematic representation of the method of assaying free radical scavenging activity.

5.4. Reducing Power Assay

5.4.1. Introduction

Antioxidants prevent the human system by neutralizing the free radicals interactively and synergi stically. Plants arerich source of free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavanoids, quinones, coumarins, alkaloids, amines, bet alains and other metabolites which are rich in antioxidant activity.

5.4.2. Principle

Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

$$\text{Potassium ferricyanide} + \text{Ferric chloride} \xrightarrow{\text{Antioxidant}} \text{Potassium ferrocyanide} + \text{ferrous chloride}$$

5.4.3. Material and Method

Reducing power assay of crude extracts was measured employing the method as described by Arpona Hira *et al.* (2013) involving Phosphate Buffer (2.5ml, 0.2M, pH 6.6), 1% Potassium Ferricyanide [$K_3Fe(CN)_6$], 10% Trichloroacetic acid 0.1% $FeCl_3$ and Ascorbic acid as standard.

5.4.3.1. Preparation of Sample Solution

Accurately weighted 20 mg of plant extract was taken into a vial and 2ml of methanol was added and the concentration of final solution is 10µg/µl ten test tubes were taken and marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from 0.98 µg/ml to 500 µg/ml. The test was done triplicate.

5.4.3.2. Preparation of Standard Solution

Accurately weighted 20 mg of ascorbic acid as standard was taken into a vial and 2 ml of distilled was added and the concentration of final solution is 10µg/µl ten test tube were taken and marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from0.98 µg/ml to 500 µg/ml. The test was done triplicate.

5.4.3.3. Procedure

1ml of stock mixture (concentration 0.98 µg/ml to 500 µg/ml) is mixed with 1ml of distilled water added with 2.5ml of Phosphate Buffer and 2.5ml of 1% Potassium Ferricyanide. The reaction mixture is incubated at 50^{o}C for 20minute. After incubation 10% Trichloroacetic acid is added. The mixture is centrifuged for 10min at 3000rpm. After centrifugation Upper layer was taken (2.5ml) dissolved with 2.5ml distilled water and 0.5ml of $FeCl_3$. Absorbance was measured at 700nm.

5.5. Nitric Oxide (NO) Scavenging Activity

5.5.1 Introduction:

Antioxidants can produce their protective role against free radicals by as variety of different mechanism including (a) the catalytic systems to neutralise or divert ROS, (b) binding or inactivation of metal ions prevents generation of ROS by Haber-Weiss reaction, (c) suicidal and chain breaking antioxidants scavenge and destroy ROS, (d) absorb energy, electron & quenching of ROS. In 21st century, demand for intake of antioxidant food or dietary antioxidant increasing with the hope of keep body healthy and free from diseases.

5.5.2 Principle

This assay relies on diazotization reaction that was originally described by Griess in 1879. Modifications have been made to the original reaction through the years. This procedure is based on the chemical reaction through the years. This procedure iittein thrrs based on the chemical reaction shown below, which uses sulfanilamide and napthylethylenediamine dihydrochloride (NEDD) under acidic condition.

Sulfanilamide and NED compete for nitrite in the Griess reaction. The reagent detects NO_2 in a variety of biological and experimental liquid samples such as plasma, serumand tissue culture medium. The limit of detection is 2.5 uM (125 pmolt) nitrite (in distilled deionized water); however the sensitivity will vary depending upon the sample used. Individual researcher must determine the limits for their individual experiments and referred to the absorbance at 546 nm at standard solutions of potassium nitrite, treater in the same way with Griess reagent.

The percentage scavenging of nitric oxide of Plant extract and standard compounds was calculated using the following formula:

NO Scavenged (%) = $(A_{cont} - A_{test}) / A_{cont} \times 100$
Where, A_{cont} is the absorbance of control reaction and A_{test} is the absorbance in the presence of the sample.

5.5.3. Materials and method:

Nitric oxide scavenging assay of crude extracts was measured employing the method as described by Arpona Hira et al. (2013) involving Sodium Nitroprusside, Phosphate Buffered Saline (NaCl, KCl, Na_2HPO_4, KH_2PO_4), Griess Solution (1% Sulphanilamide, 2% Phosphoric acid, and 0.1% naphthylethylenediamine dihydrochloride) and ascorbic acid as standard.

5.5.3.1 Preparation of Phosphate Buffered Saline (PBS Buffer)

8g of NaCl, 0.2g of KCl, 1.44g of Na_2HPO_4 and 0.24g of KH_2PO_4 was dissolved in 1 liter distilled H_2O. The pH was adjusted to 7.4 with HCl.

5.5.3.2.Preparation of 5mmol Sodium Nitroprusside in Phosphate Buffer Saline:

0.130 gm of Sodium Nitroprusside was accurately weighed and dissolved in 100 ml Phosphate Buffered Saline (PBS Buffer).

5.5.3.3 Preparation of Griess reagent

Firstly, 1gm of Sulphanilamide was dissolved into 100ml distilled water. (Solution a). Then 0.1gm of naphthylethylenediamine dihydrochloride was dissolved into 100ml of 2% phosphoric acid. (solution b). 1 part of solution 'a' was mixed with 1 part of solution 'b'. This reagent was cautiously put into amber glassed bottle to protect it from light as this reagent is very light sensitive.

5.5.3.4 Preparation of Sample Solution

Accurately weighted 20 mg of plant extract was taken into a vial and 2ml of methanol was added and the concentration of final solution is 10µg/µl ten test tubes were taken and marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from 0.98 µg/ml to 500 µg/ml. The test was done triplicate.

5.5.3.5. Preparation of Standard Solution

Accurately weighted 20 mg of ascorbic acid as standard was taken into a vial and 2 ml of distilled was added and the concentration of final solution is 10µg/µl ten test tube were taken and marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from0.98µg/ml to 500 µg/ml. The test was done triplicate.

5.5.3.6 Procedure:

2ml 5mmol Sodium Nitroprusside in Phosphate Buffer Saline (PBS Buffer) was mixed with different concentration (0.98µg/ml - 500µg/ml) of sample dissolved in 2ml of methanol. A control without the test compound but with an equivalent amount of methanol was taken and was incubated at 25ºC for 30minute. After 30min, 1.5mL of the incubation solution was removed and diluted with 1.5mL of Griess solution. The absorbance was measured at 546nm. The NO radical scavenging activity was expressed at the inhibition percentage (I%) and calculated as per,

I (%)=$[(A_{blank} - A_{sample})/A_{blank}]\times100$, Where A_{blank} is the absorbance of the control reaction (containing all reagents except test compound). Finally IC_{50} value is determined.

5.6. THROMBOLYTIC ACTIVITY

5.6.1: Introduction

Since ancient times,herbal preparations have been used for the treatment of several diseases.The Leaves and twigs,stem,bark and underground parts of plants are most often used for traditional medicines.Herbal products are often perceived as safe because they are natural(Gesler,1992).Cerebral venous sunus thrombosis (CVST)is a common disorder which accompanied by significant morbidity and mortality(watson et al,.2002).Heparin,an anticoagulant agent,is the first line treatment for CVST,because of its efficacy,safety and feasibility(biousse and Newman,2004).Thrombolytic drugs like tissue plasminogen activator(t-pA),urokinase,Streptokinase etc.Play a crucial role in the management of patients withCVST(Baruah,2006).Thus, the aim of the present study was to investigate the thrombolytic ativity of methanolic extracts and its different fractions of *stephania japonica.*

5.6.2 Materials and methods

5.6.2.1: Preparation of sample:

The thrombolytic activity of all extractives was evaluated by a method using streptokinase(SK) as standard substance.10 mg of methanolic extracts and its different fractions of *Stephania japonica were* taken in different vials to which 1 ml distilled water was added.

5.6.2.2: Streptokinase (SK):

Commercially available lyophilized altepase(streptokinase) vial(Beacon Pharmaceuticals Ltd)of 15,00,000 i.u., was collected and 5 ml sterile distilled water was added and mixed properly.This suspension was used as a stock from which 100 micro litre(30000 i.u) was used for *in vitro* thrombolysis.

5.6.2.3: Blood sample:

Whole blood(n=10)was drawn from healthy human(myself blood)volunteers without a history of oral contraceptive or anticoagulant therapy and 1ml of blood was transferred to the previously weighted sterile vials and was allowed to form clots.

5.7. Thrombolytic activity:

Aliquots (5 ml)of venous blood were drawn from myself and distributed in ten different pre weighted sterile vials(1ml/tube) and incubated 37 degree c for 45 minutes. After clotformation,the serum was completely removed without disturbing the clot and each vial having clot was again weighted to determine the clot weight(clot weight=weight of clot containing tube-weight of tube alone).

To each vial containing reweighted clot,100 micro litre aqueous solutions of different partitioates along with the crude extracrs was added separately. A a positive control,100 micro litre of streptokinase(SK),and as a negative non thrombolytic control,100 microlitre of distilled water were separeately added to the control vials.All the vials were then nicuvated at 37 degree c for 90 minutes and ovserved for clot lysis.After incuvation,the released of fluid was removed and vials were again weighed to ovserve the difference in weight after clot disruption.Didderence obtained in weiht taken before and after clot lysis was expressed as percentages of clot lysis as shown below:

% of clot lysis=(wt of released clot/clot wt)*100

5.8. Evaluation of Laxative activity

5.8.1 Experimental Animals

Swiss albino mice of either sex (20-25gm) were obtained from the Animal house of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55-65%, r.t. 23.0±2.0°C and 12 h light: dark cycle). The animals were fed with standard diet and water ad libitum.

Figure 5.3.*Swiss albino* Mice.

4.8.2 Equipments

large beaker (1000 ml), small beaker (50ml), pipette, filter paper (Whatman 40), vial (5ml), mice oral needle, petri dishes, distilled water, Scissors, masking tape, permanent marking pen, aluminium foil paper, test tube, analytical balance (ELH 3000, Shimadzu, Japan), refrigerator, pencil, scale, container.

5.8.3 Drugs and Chemicals

5.8.3.1Chemical Agents

1. 5% CMC (Vehicle) 10ml/kg as negative control,
2. 0.3 mL of charcoal meal of distilled water suspension containing 10% gum acacia, 10% activated charcoal and 20% starch.

5.8.3.2 Standard Drug

1. Bisacodyl (5mg/kg, p.o.) used as positive control
2. Atropine (10 mg/kg, i.p.)

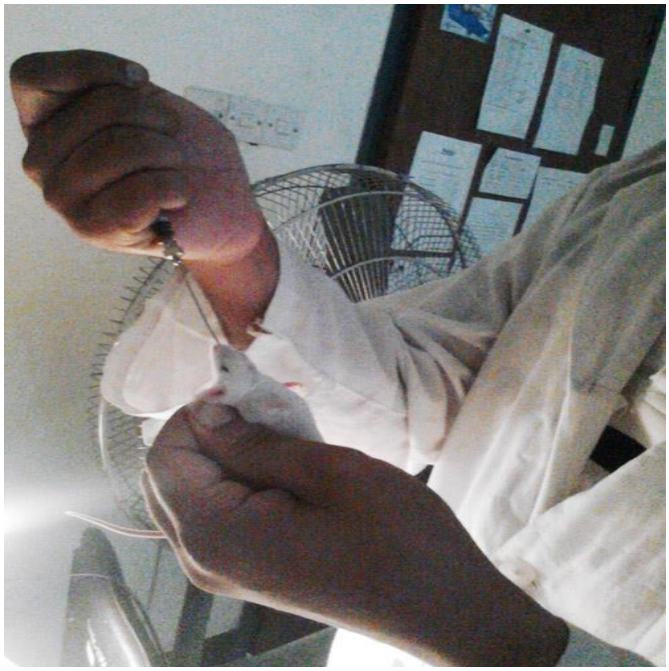
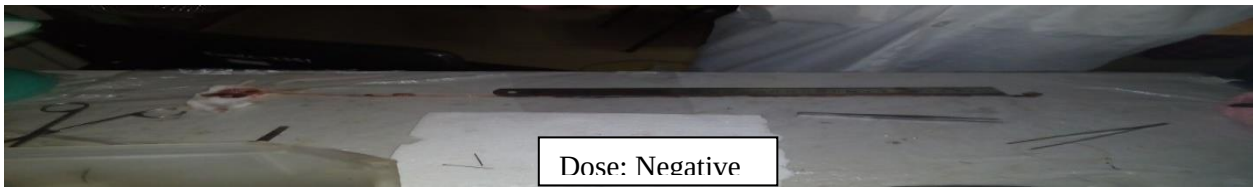


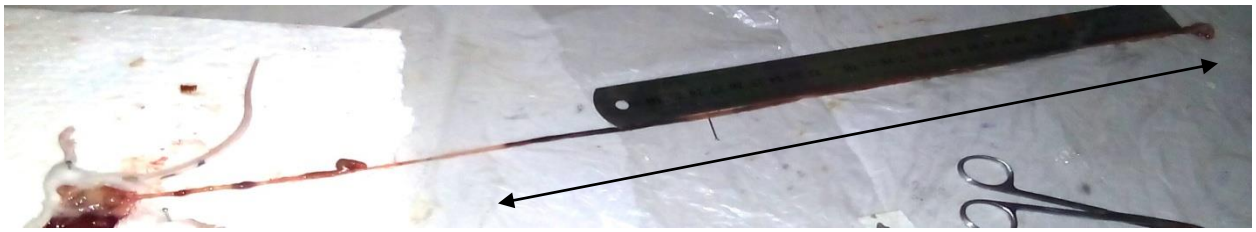
Figure 5.4. Oral administration into mouse.

5.9 Charcoal meal GI transit test

The method described by (Al-Qarawi et al., 2003) was followed with slight modifications. Mice fasted for 12 h were divided into 8 different groups (5 animals in each). Three of the groups were treated per oral (p.o.) with increasing doses of 200mg/kg, 400 mg/kg & 800mg/kg of the plant *Trapa bisphinosa*acting as the test groups. One group was taken as negative control, treated with 5%CMC (10 mL/kg). The next group was administered Bisacodyl (5 mg/kg) as the positive control. After 15 min, the animals were given 0.3 mL of charcoal meal of distilled water suspension containing 10% gum acacia, and 20% starch. The animals were sacrificed after 30 min and the abdomen was opened to excise the whole small intestine. The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport ratio or percentage. In order to assess the involvement of acetylcholine (ACh)-like prokinetic effect of the extract, further groups of mice were pretreated with intraperitonial (i.p.) injection of atropine (10 mg/kg) 15 min prior the administration of the extract.

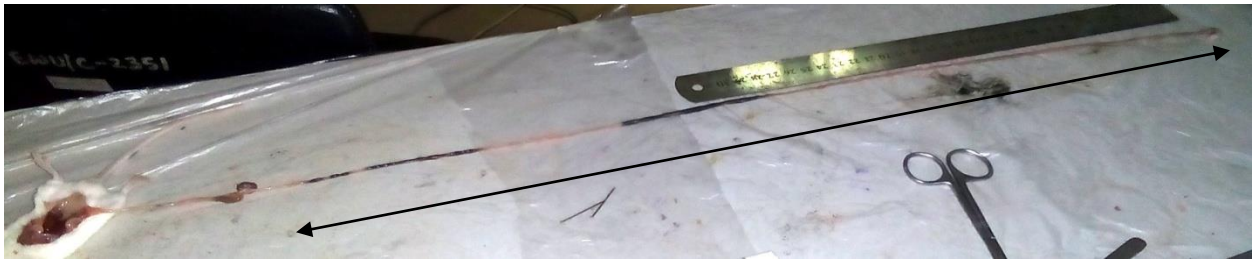


Dose: 200mg/kg

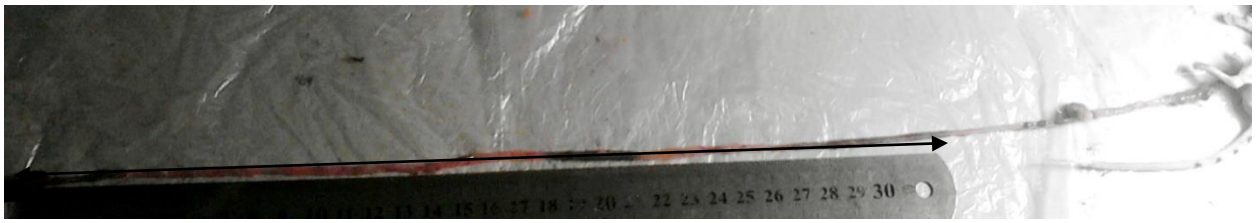


Dose: 400mg/kg

Dose: 800mg/kg



Dose: Positive



Dose: 400mg/kg + Atropine

Dose: 800mg/kg + Atropine

Dose: Positive + Atropine

Figure5.5: GI transit into mouse

CHAPTER 06

RESULTS AND DISCUSSION

6. Results and Discussion

6.1 Total Phenolic Content Assay

The Ethyl acetate fraction of methanolic extract of *Trapa bispanosa*, N-hexane fraction of methanolic extract of *Trapa bispanosa*, Methanolic extract of *Stephania japonica*were subjected to Total Phenolic Content Assay by the method described by (Arpona et al., 2013). Here, Total Phenolic Content was measured as Salicylic acid equivalence.

Table 6.1.1:Standard Curve Preparation by Using Salicylic Acid

Standard Curve Preparation by Using Salicylic Acid

SL	Concentration mg/ml	Absorbance	Regression Equation	R^2
1	0.625	0.433	Y = 0.109x + 0.418	0.983
2	1.25	0.528		
3	2.5	0.768		
4	5	0.998		
5	10	1.480		

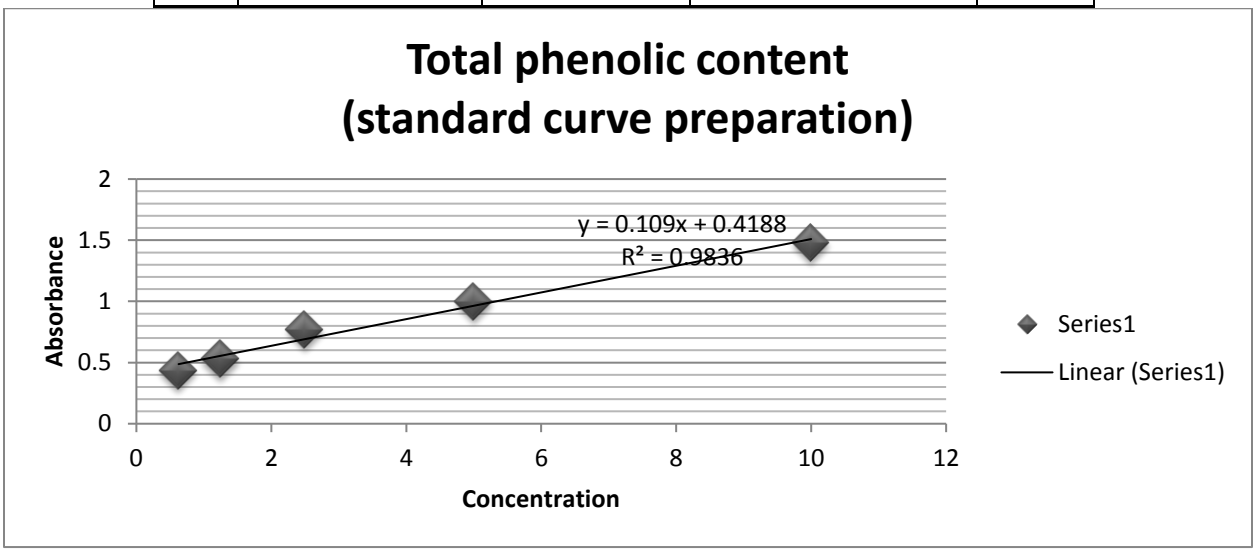


Fig 6.1.1.: Total Phenolic Content (Standard Curve)

Table 6.1.2: Total Phenolic Content of *Trapa Bispanosa*:

Absorbance	mgSAE/g	Mean
0.636	328.62	325.56
3.9	319.44	
0.986	328.62	

Table 6.1.4: Total Phenolic Content of *Stephania japonica:*

Absorbance	mgSAE/g	Mean
0.959	49.63	53.81
1.058	58.71	
0.997	53.11	

Comparison of Total Phenolic Content of *Trapa bisphinosa and Stephania japonica* Extract:

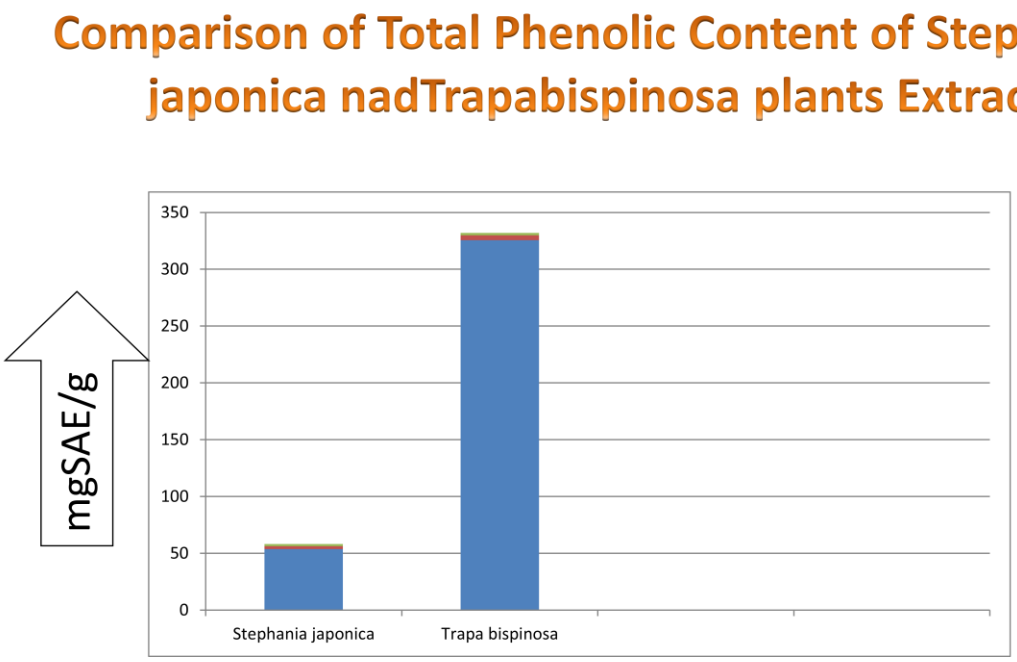


Fig 6.1.2.Comparison of Total Phenolic Content of *Trapa bisphinosa and Stephania japonica* Extract.

Discussion: From the above result we can see that among Two experiment ethyl acetate fraction of methanolic extract of *Trapa bispanosa* have Higestamount of phenolic content (325.56 mgSAE/g) than N-haxen extract of *Trapa bispanosa have phenolic* content (119.93mgSAE/g). and methanolic extract of *Stephania japonica*(53.81 mgSAE/g) have the least amount of phenolic content among all Two extracts.

So from the above data it can be said that, ethanolic extract of ethyl acetate fraction of methanolic extract of *Trapa bispanosa* have highest amount of phenolic content (152.13 mgSAE/g) thus it can be a good source of phenol.

Evaluation of Antioxidant Activity

6.2. DPPH Radical Scavenging Assay

The methanolic extract of *Stephania japonica* and Ethyl acetate and n-hexane fraction of methanolic extract of *Trapa bispanosa* were subjected to DPPH Radical Scavenging Assay according to method described by (Arpona *et al*., 2013) and ascorbic acid was used as reference standard in this experiment.

Table 6.2.1: DPPH Radical Scavenging Assay of Ascorbic Acid (Standard)

Absorbance of Blank	Concentration µg/ml	Log Concentration	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.625	0.98	- 0.009	0.536	14.24	4.35
	1.95	0.290	0.508	18.72	
	3.91	0.592	0.365	41.60	
	7.81	0.893	0.198	68.32	
	15.63	1.194	0.098	84.32	
	31.25	1.495	0.058	90.72	
	62.5	1.796	0.042	93.25	
	125	2.097	0.027	95.68	
	250	2.398	0.021	96.32	
	500	2.699	0.015	97.60	

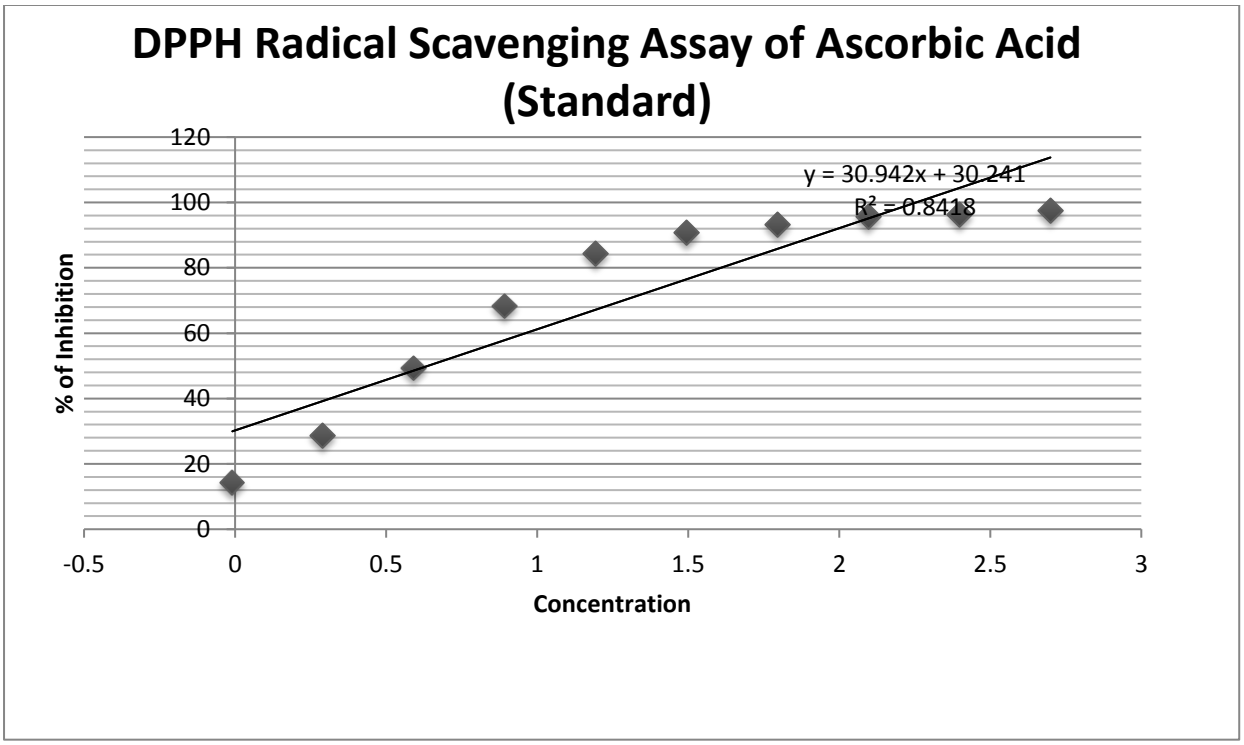


Fig 6.2.1: DPPH Radical Scavenging Assay of Ascorbic Acid (Standard)

Table 6.2.2: DPPH Radical Scavenging Assay of *Trapa Bispanosa*plant extract:

Absorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.612	0.98	0.608	0.653	55.66
	1.95	0.590	3.59	
	3.91	0.500	18.30	
	7.81	0.381	47.41	
	15.63	0.142	76.79	
	31.25	0.038	93.79	
	62.5	0.024	96.07	
	125	0.022	96.40	
	250	0.019	96.89	
	500	0.012	98.03	

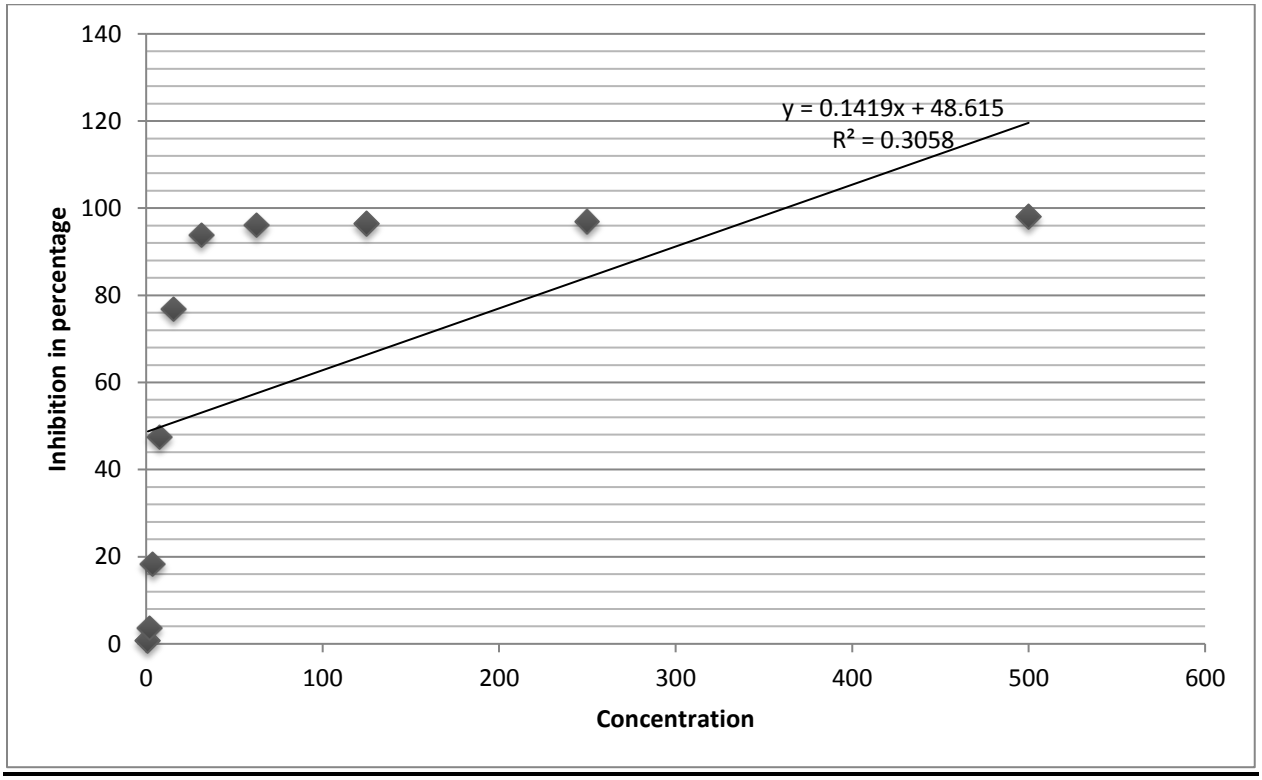


Fig 6.2.2: DPPH Radical Scavenging Assay of *Trapa Bispanosa*plant extracts.

Table 6.2.4: DPPH Radical Scavenging Assay of *Stephania japonica:*

Absorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.470	0.98	0.457	2.76	25.14
	1.95	0.450	4.25	
	3.91	0.438	6.80	
	7.81	0.425	9.57	
	15.63	0.407	13.40	
	31.25	0.351	25.32	
	62.5	0.274	41.70	
	125	0.145	69.15	
	250	0.092	80.42	
	500	0.032	93.19	

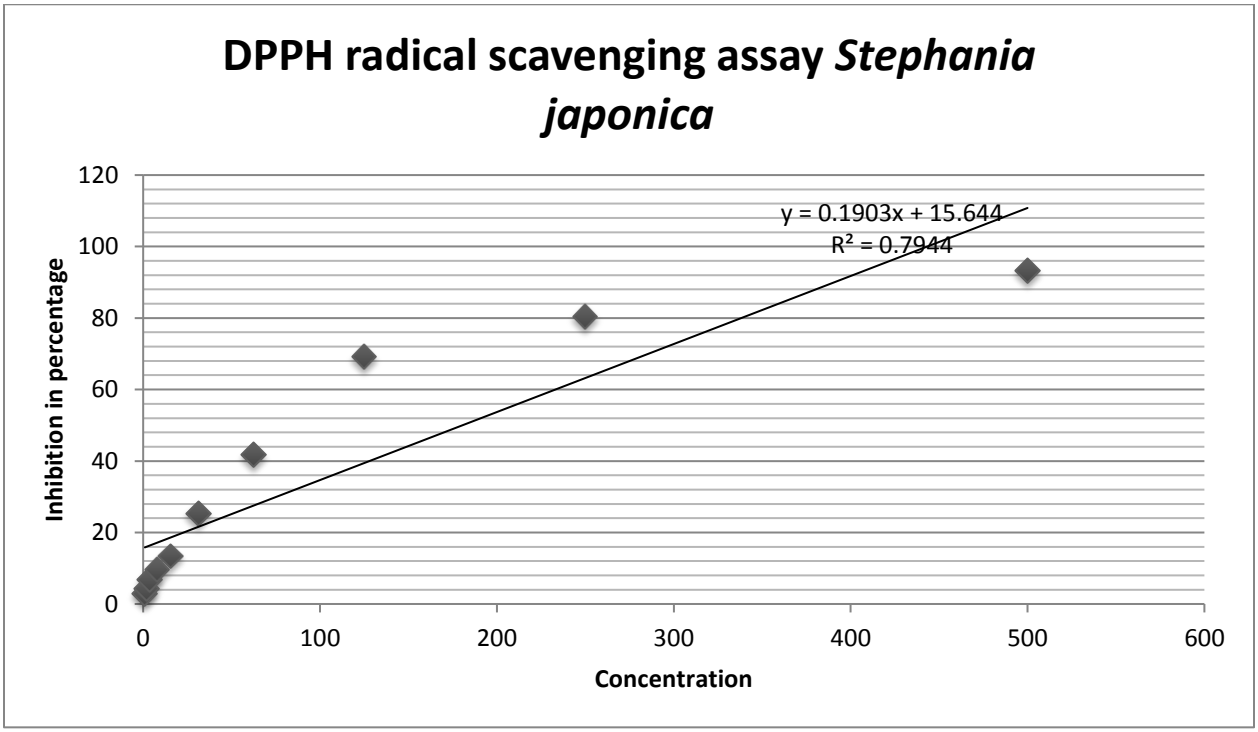


Fig 6.2.4: DPPH Radical Scavenging Assay of *Stephania japonica*

Comparison of DPPH of the IC50 between Standard and Extracts

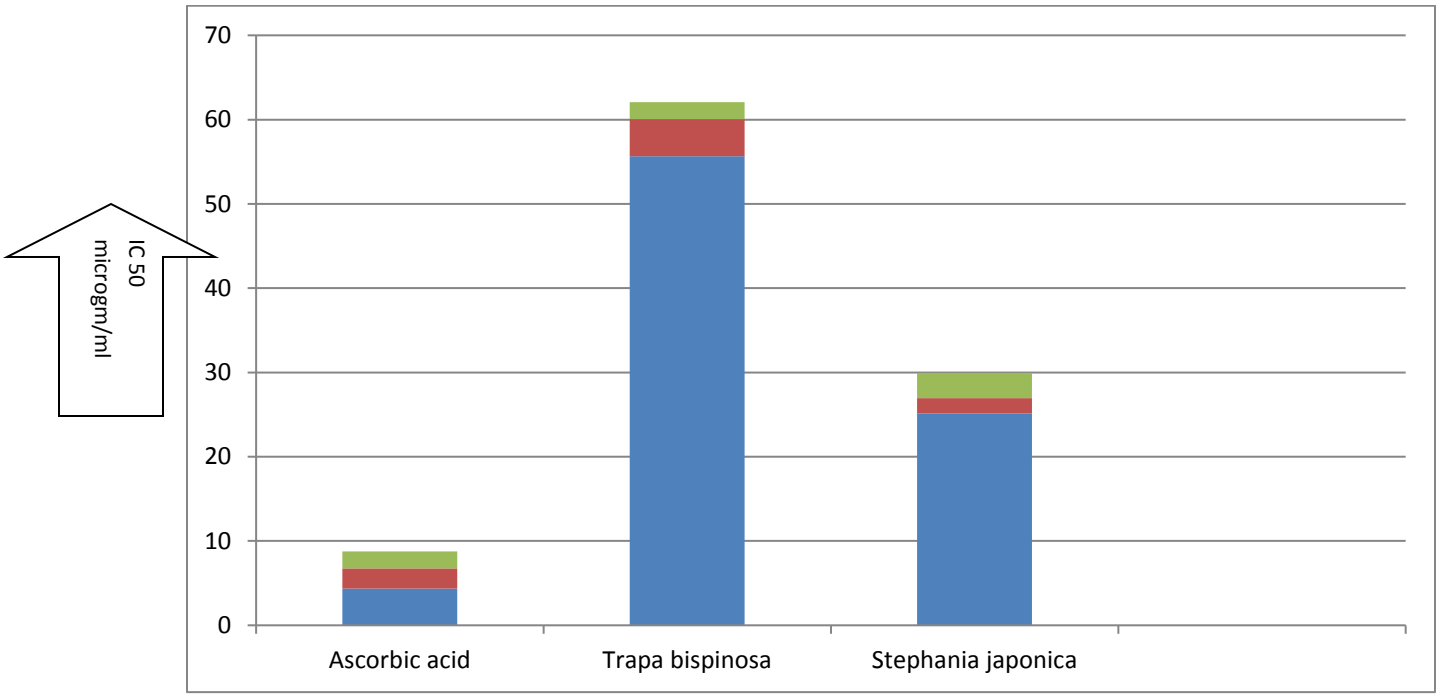


Fig 6.2.5.Comparison of IC_{50} between Standard and Extract.

Discussion: The result of the tests is present in the following figure. The extract demonstrated an antioxidant activity by using DPPH Where DPPH radical activity of methanolic extract of *Stephania japonica*is good (IC_{50}25.14 µg/ml) after which is by n-hexane and ethyl acetate extract of *Trapa bispanosa*(IC_{50} 53.32 µg/ml and 55.66 µg/ml).

6.3. Nitric oxide Scavenging Assay

The methanolic extract of *Stephania japonica* and Ethyl acetate and n-hexane fraction of methanolic extract of *Trapa bispanosa* were subjected to Nitric oxide Scavenging Assay according to method described by (Arpona *et al*., 2013) and ascorbic acid was used as reference standard in this experiment.

Table 6.3.1: Nitric oxide scavenging assay of ascorbic acid (standard)

Absorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.030	0.98	0.032	6.66	18.29
	1.95	0.038	26.6	
	3.91	0.041	36.6	
	7.81	0.048	60	
	15.63	0.050	66.6	
	31.25	0.052	73.3	
	62.5	0.060	100	
	125	0.067	123.33	
	250	0.098	226.67	
	500	0.168	460	

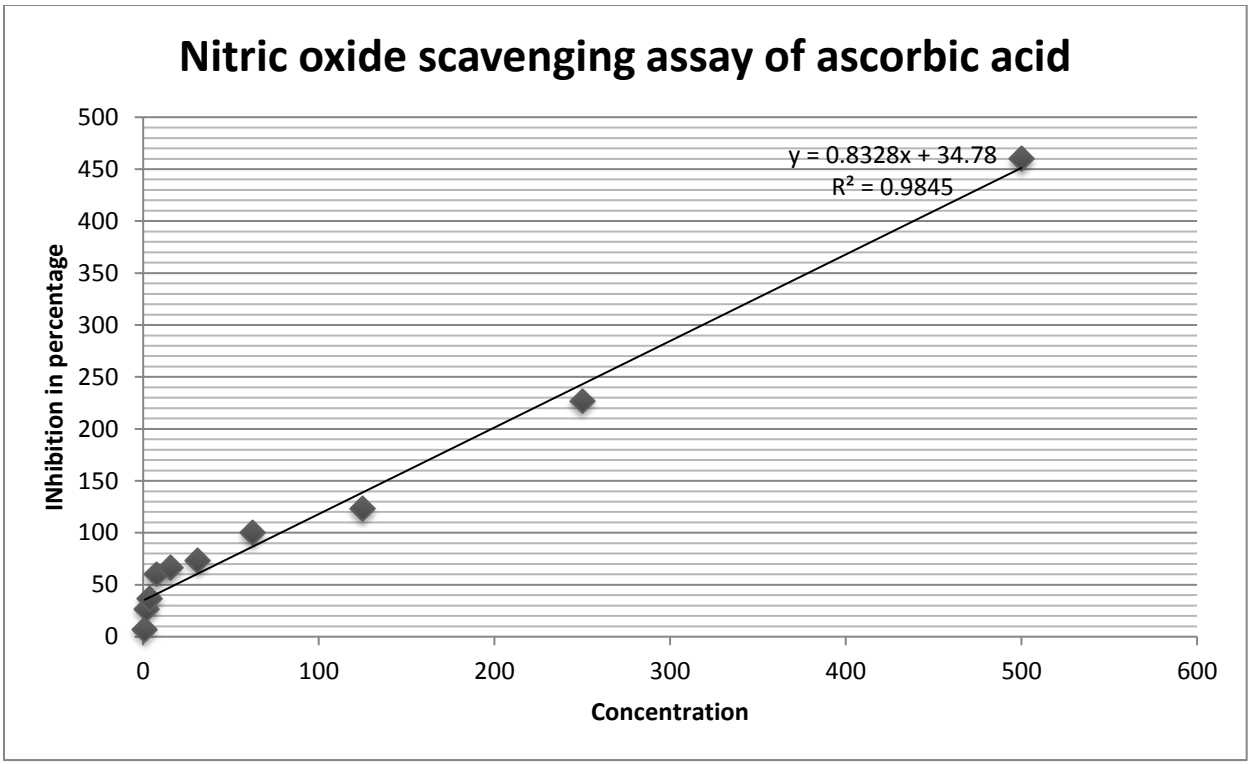


Fig 6.3.1: Nitric oxide scavenging assay of ascorbic acid (standard)

Table 6.3.2: Nitric oxide scavenging assay of *Trapa Bispanosa* :

Absorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.062	0.98	0.063	1.61	15.96
	1.95	0.070	12.9	
	3.91	0.114	83.87	
	7.81	0.061	1.61	
	15.63	0.079	29.03	
	31.25	0.084	37.09	
	62.5	0.128	108.06	
	125	0.270	337.09	
	250	0.528	753.22	
	500	0.761	1129.03	

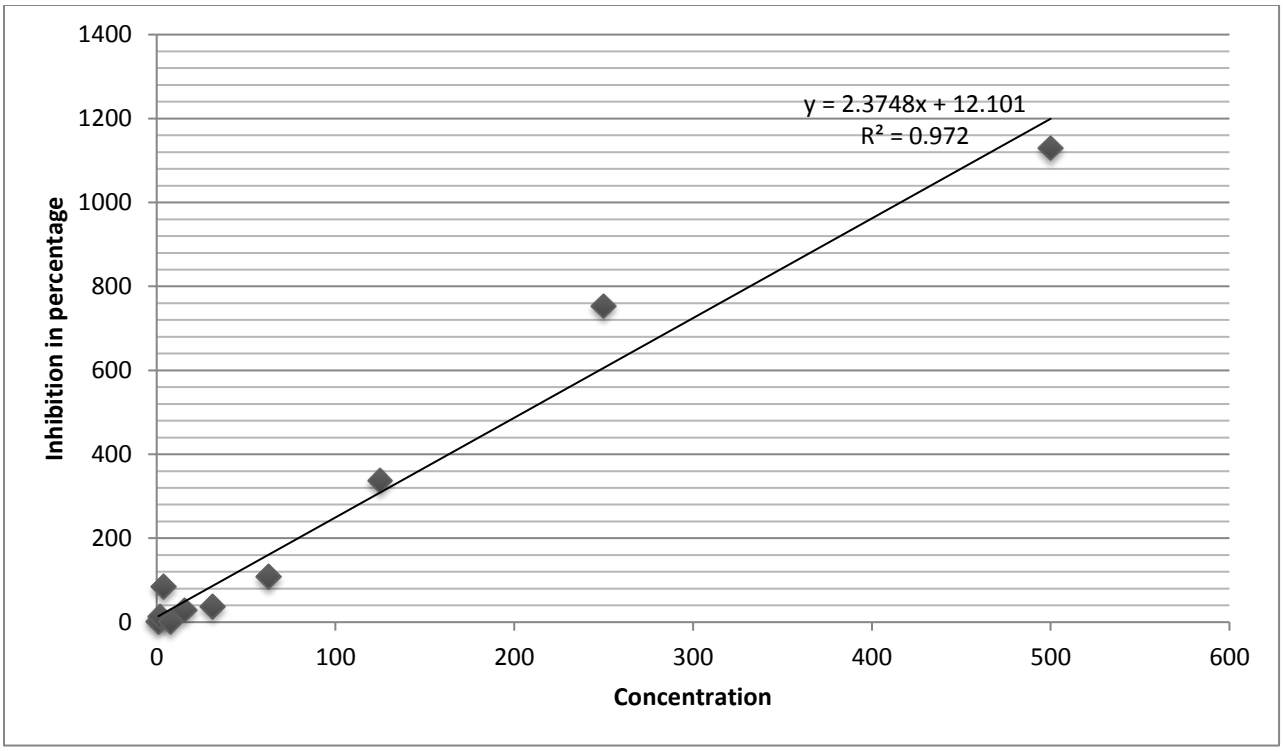


Fig 6.3.2: Nitric oxide scavenging assay of *Trapa bisanosa*.

Table 6.3.4: Nitric oxide scavenging assay of *Stephania japonica:*

Absorbance Abosorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.035	0.98	0.039	11.43	85.03
	1.95	0.040	14.28	
	3.91	0.041	17.14	
	7.81	0.042	20.00	
	15.63	0.044	25.71	
	31.25	0.044	25.71	
	62.5	0.046	31..43	
	125	0.051	45.71	
	250	0.074	111.43	
	500	0.130	271.43	

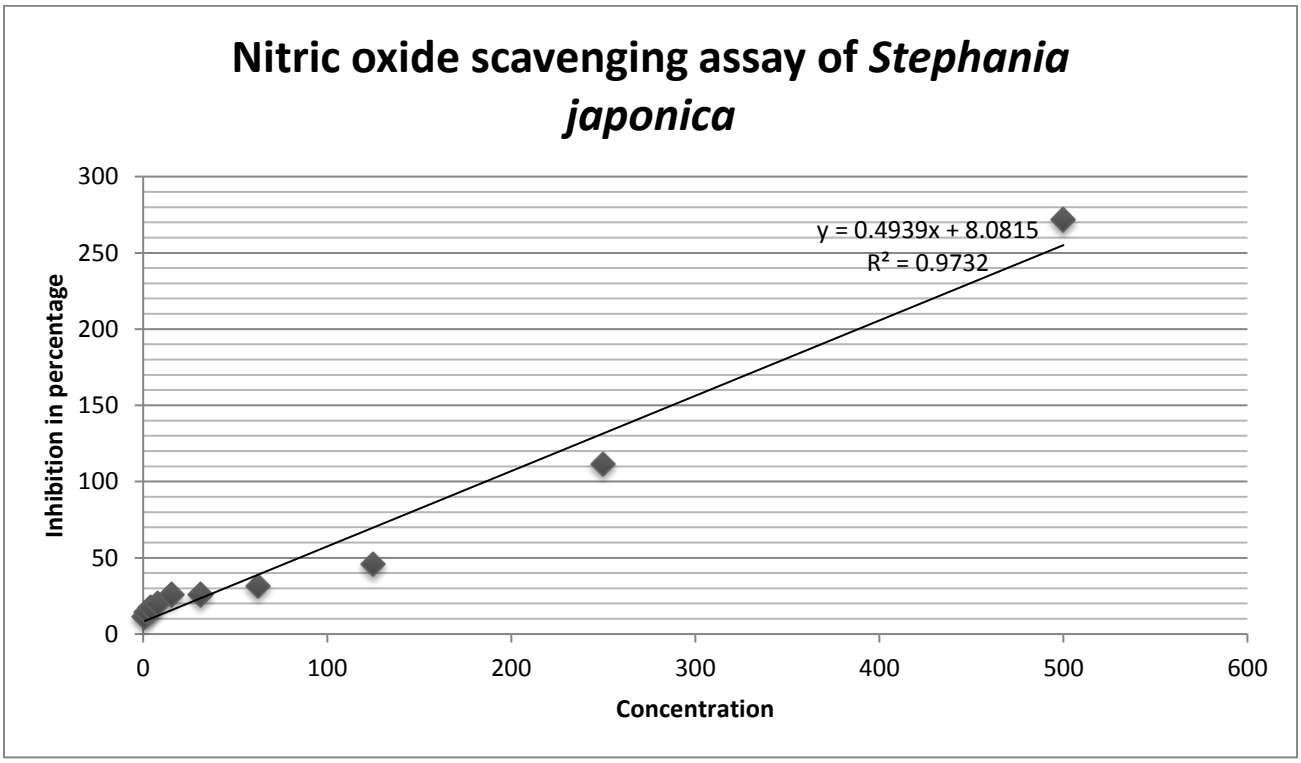


Fig 6.3.4: Nitric oxide scavenging assay of *Stephania japonica.*

Comparison of IC_{50} values between standard and *Trapha bisphinosa and Stephaniajaponica* in Nitric oxide scavenging assay

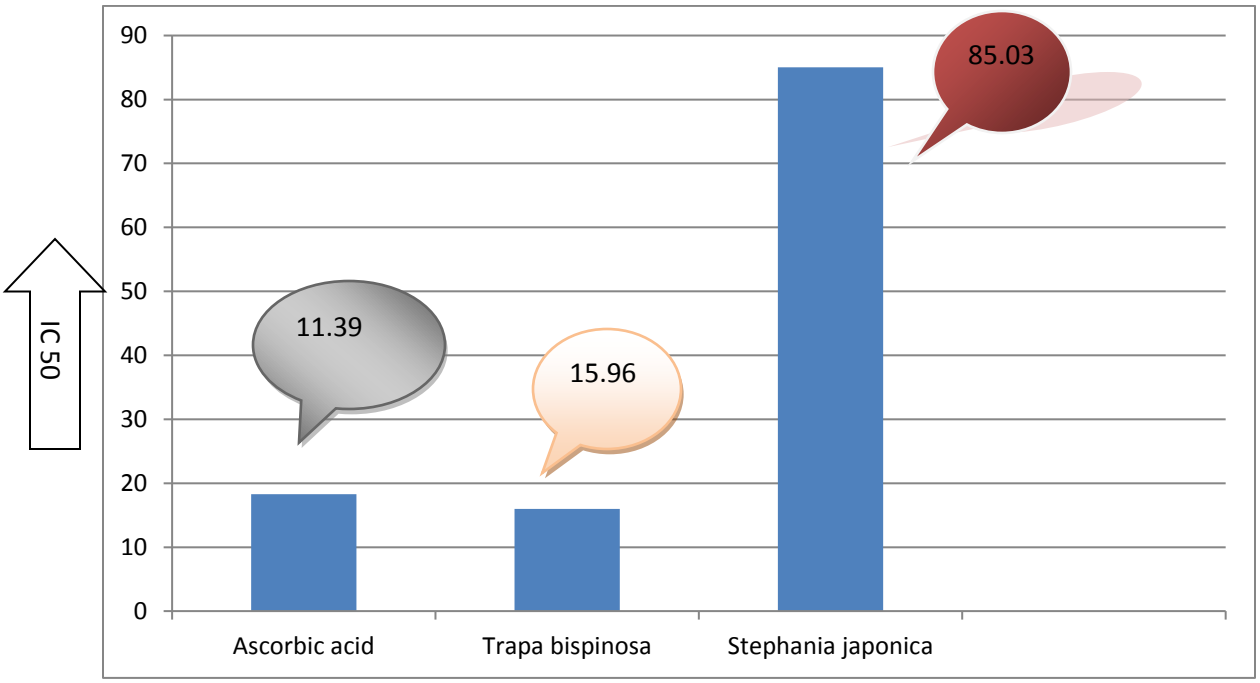


Fig 6.3.5: Comparison of IC_{50} between Standard and Extract.

Discussion: The result of the tests is present in the following figure. The extract demonstrated an antioxidant activity in Nitric oxide scavenging assay where NO scavenging activity of methanolic extract of *Trapa bispanosa* (IC_{50} 15.96 µg/ml). *Stephania japonica*(IC_{50} 85.03 µg/ml) showed least activity.

6.4. Reducing Power Assay

The methanolic extract of *Stephania japonica* and Ethyl acetate and n-hexane fraction of methanolic extract of *Trapa bispanosa* were subjected to Reducing Power Assay according to method described by (Arpona *et al*., 2013) here, ascorbic acid was used as reference standard.

Table 6.4.1Reducing Power Assay of Ascorbic acid (Standard)

SL.	Concentration µg/ml	Absorbance			Mean
		1	**2**	**3**	
1	0.98	0.099	0.098	0.097	0.098
2	1.95	0.101	0.105	0.103	0.103
3	3.91	0.143	0.145	0.146	0.144
4	7.81	0.221	0.222	0.221	0.221
5	15.63	0.283	0.284	0.285	0.284
6	31.25	0.486	0.488	0.489	0.487
7	62.5	0.952	0.955	0.953	0.953
8	125	1.434	1.435	1.434	1.434
9	250	1.978	1.978	1.976	1.977
10	500	2.567	2.567	2.566	2.566

Table 6.4.3.: Reducing Power Assay of *Trapa bispanosa*

SL.	Concentration µg/ml	Absorbance			Mean
		1	**2**	**3**	
1	0.98	0.043	0.042	0.043	0.043
2	1.95	0.062	0.065	0.064	0.063
3	3.91	0.086	0.087	0.088	0.087
4	7.81	0.098	0.097	0.095	0.096
5	15.63	0.116	0.112	0.114	0.114
6	31.25	0.121	0.123	0.127	0.123
7	62.5	0.167	0.169	0.168	0.168
8	125	0.192	0.194	0.192	0.192
9	250	0.328	0.327	0.329	0.328
10	500	0.522	0.521	0.522	0.521

Table 6.4.4: Reducing Power Assay of *Stephania japonica.*

SL.	Concentration µg/ml	Absorbance			Mean
		1	**2**	**3**	
1	0.98	0..044	0.043	0.046	0.044
2	1.95	0.063	0.062	0.066	0.063
3	3.91	0.076	0.074	0.076	0.075
4	7.81	0.087	0.088	0.087	0.087
5	15.63	0.098	0.098	0.099	0.098
6	31.25	0.113	0.110	0.114	0.112
7	62.5	0.179	0.176	0.179	0.177
8	125	0.234	0.232	0.235	0.233
9	250	0.398	0.399	0.400	0.399
10	500	0.578	0.576	0.578	0.577

Comparison of Reducing Powerassay Between Standard and Trapa bispinosa and Stephania japonica Crude Extact:

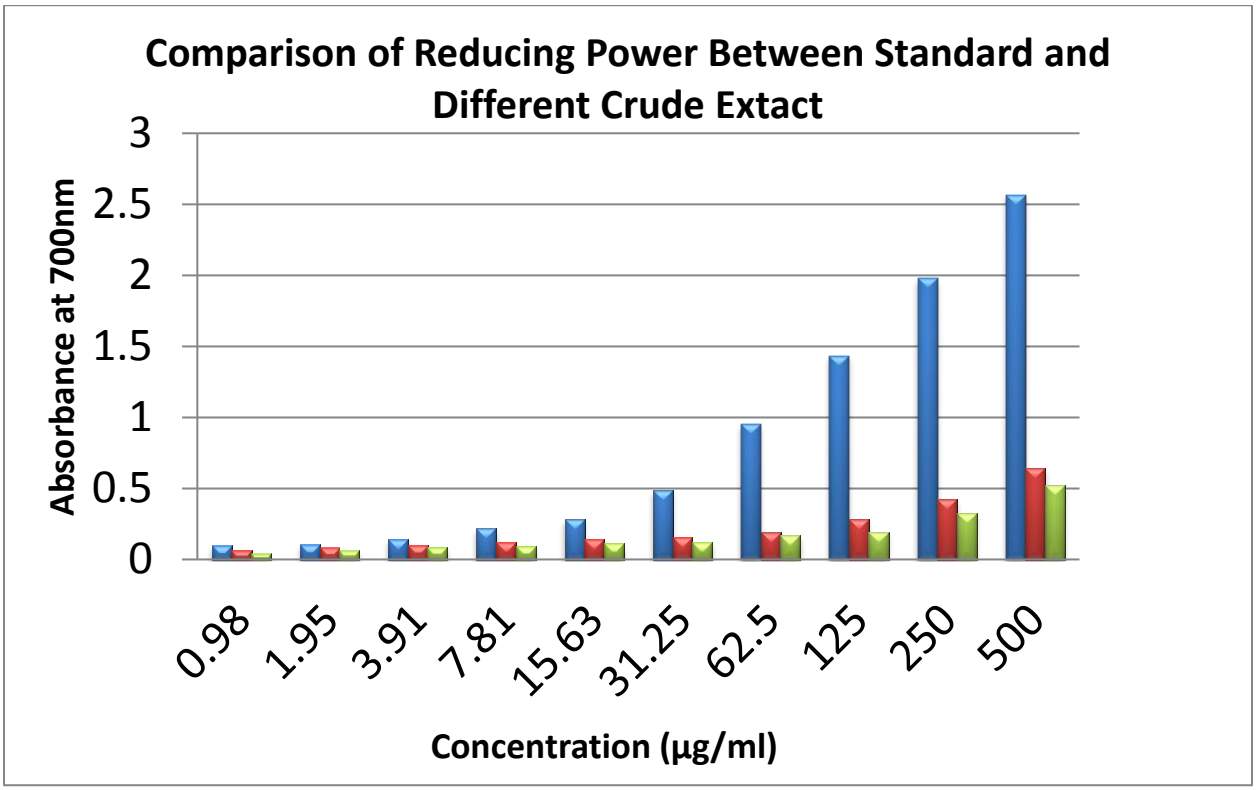


Fig 6.4.1: Comparison of IC50 values between standard and different crude extract.

Discussion:

In reducing power assay higher absorbance of the reaction mixture indicates higher reductive potential. In the graph above we can see that with the increase of concentration absorbance is also increase. From the above graph we can concluded that among Two different extract *Stephania japonica* showed highest level of reducing power where as *Trapa bispinosa.*The reducing capability of a compound may serve as a significant indicator of its potential antioxidant activity. Further studies will help in identifying the individual compounds that aids in the reducing power and to identify the synergistic effect.

Also a correlation between the reducing power and antioxidant activity can be derived. In the present investigation, we have warranted the concentration dependent reducing abilityof the extracts of methanolic extract of *Stephania japonica* and *Trapa bispinosa.*

6.5. Results and discussion of the thrombolytic activity of *Stephania japonica:*

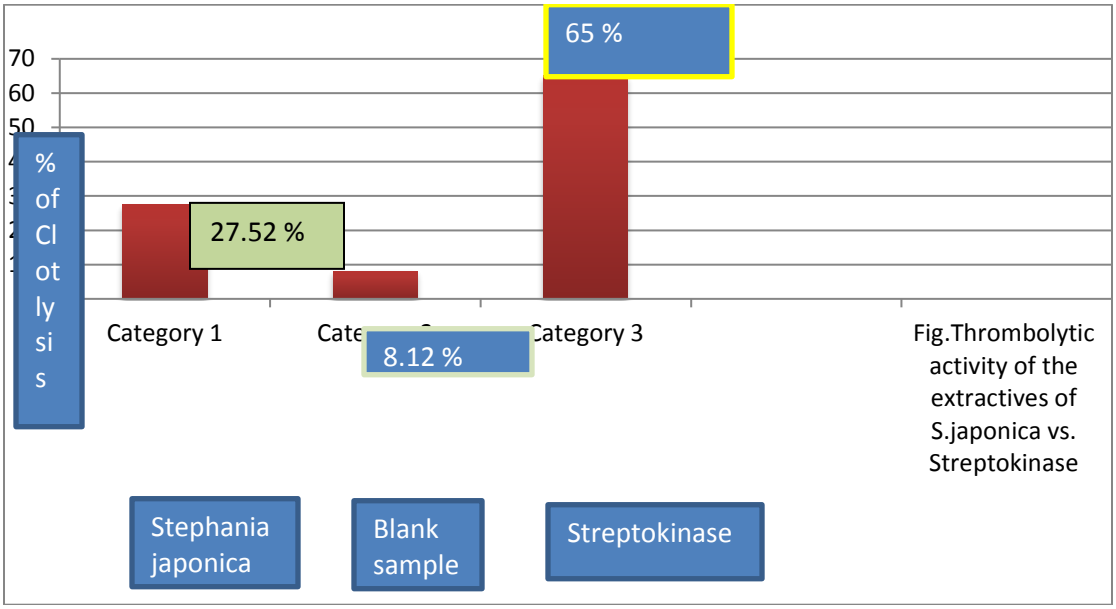
As a part of discovery of cardio protective drugs from natural sources,the extract of stephania japonica assessed for thrombolytic activity and the results are presented in table of 100 micro litre SK,a positive control(30000I,U)to the clots and subsequent incubation for 90 minutes at 37 degree showed 65 percent lysis of clot on the other hand,distilled water was treated as negativecontrol whichexhibited a negligible percentage of lysis of clot 8.12%.The mean difference inclot lysis percentages between positive and negative control was found very significant.

In this study, the methanolic extract of *S.japonica* exhibited 27.52 % activity.

6.5.1. Table: Thrombolytic activity of the extractives of *S.japonica*:

Sample	Weight of empty vial W1 g	Weight of clot containing vial before clot disruption W2 g	W3 weight of clot containing vial after clot disruption w3 g	Weight of clot before clot disruption clot W4=W2-W1 g	Weight of clot after clot disrtuption clot W5=W3-W1 g	% of lysis(W4-W5)/W4* 100%
Extract	**15.170**	**16.390**	**16.385**	**1.22**	**1.215**	**27.52**
Blank	**4.72**	**5.09**	**5.06**	**0.37**	**0.34**	**8.12**
SK	**4.65**	**5.05**	**4.79**	**0.4**	**0.14**	**65**

Comparison thrombolytic activity with standard:



6.5.1. Fig. Comparison of Thrombolytic activity of the methanolic extract of *Stephania japonica* and *Streptokinase.*

Here is,Category 1: is the % of clot lysis of extract Stephania japonica.
Category 2: is the % of clot lysis of blank sample.
Category 3: is the % of clot lysis of streptokinase.

6.6.Results and discussion Laxative effect test:

6.6.1. Charcoal meal GI transit test:

For the determination of laxative effect, charcoal meal GI transit test was done upon the administration of the crude extract of *Trapa bisphinosa.* Table-6.6.1 and figure-6.1 show the laxative effect by charcoal meal GI transit test of the crude extract of *Trapa bisphinosa*on normal and atropine induced test mice. For the determination of GI transit rate, the length of small intestine and the distance between the pylorus region and front of the charcoal meal was measured to obtain the charcoal transport ratio or percentage.The test was carried out to find out the effects of extract on the transit of the gastrointestinal tract. Comparative evaluation of the extract with the reference motility drug, bisacodyl, and Negative control group showed that the extract significantly increase gastrointestinal motility in mice table-1. A total 8 doses, e.g. 200 mg/kg, 400 mg/kg & 800mg/kg , 400mg/kg + atropine, and 800mg/kg + atropine of crude extract of *Trapa bisphinosa* were used for the gastrointestinal transit test.

Table 6.6.1: Effects of crude extract of *Tapa bisphinosa* on the Gastrointestinal Transit Test:

Group No.	Treatment	Dose (ml/kg or mg/kg)	Mean of % length of small intestine
1	**5% CMC (p.o)**	**10**	**32.70±1.39*** **
2	**Bisacodyl (p.o)**	**5**	**83.57±1.68*** **
3	**Dose-1, H.Kurzii(p.o)**	**200**	**55.42±1.68*** **
4	**Dose-2, H.Kurzii(p.o)**	**400**	**70.50±1.22*** **
5	**Dose-3, H.Kurzii(p.o)**	**800**	**80.0±1.61*** **
6	**Bisacodyl (p.o)+Atropine (i.p)**	**5 + 10**	**71.67±1.90*** **
7	**Dose-1(p.o)+ +Atropine (i.p)**	**400+10**	**64.23±1.76** **
8	**Dose-2(p.o)+ +Atropine (i.p)**	**800+10**	**75.0±1.33*** **

Dose-1=200mg/kg (*Trapa bisphinosa*)

Dose-2= 400 mg/kg (*Trapa bisphinosa*)

Dose-3= 800 mg/kg (*Trapa bisphinosa*)

p.o=Per oral; i.p= Intraperiotinal

All the test group of crude extract except group-3 showed significant increased transit ($p<0.001$, $p<0.01$ and $p<0.05$) of GI motility test at a dose of 200 mg/kg, 400mg/kg and 800 mg/kg. For each doses (Dose-1, 2, 3) two groups of animals were tested. One group was given only dose and another group was pretreated with atropine (i.p) before administration of doses. Among all the test groups of crude extract, dose-3 (group-5) showed highest percentage of the intestinal length traversed by the charcoal (80.0 ± 1.61**,** $p<0.001$). The reference drug bisacodyl showed the percentage of length 81.40 ± 0.75 ($p<0.001$) at a dose of 5mg/kg.

In case of the test group of the animals pre-treated with atropine also showed significant increase of percentage of length travelled by charcoal. Crude extract of group 8 showed most prominent percentage of the intestinal length traversed by the charcoal, that is (75.0 ± 1.33**,** $p<0.001$) and the reference drug showed the percentage of length (74.67 ± 1.20**,** $p<0.001$).

GI transit test of *Trapa Bisphinosa*

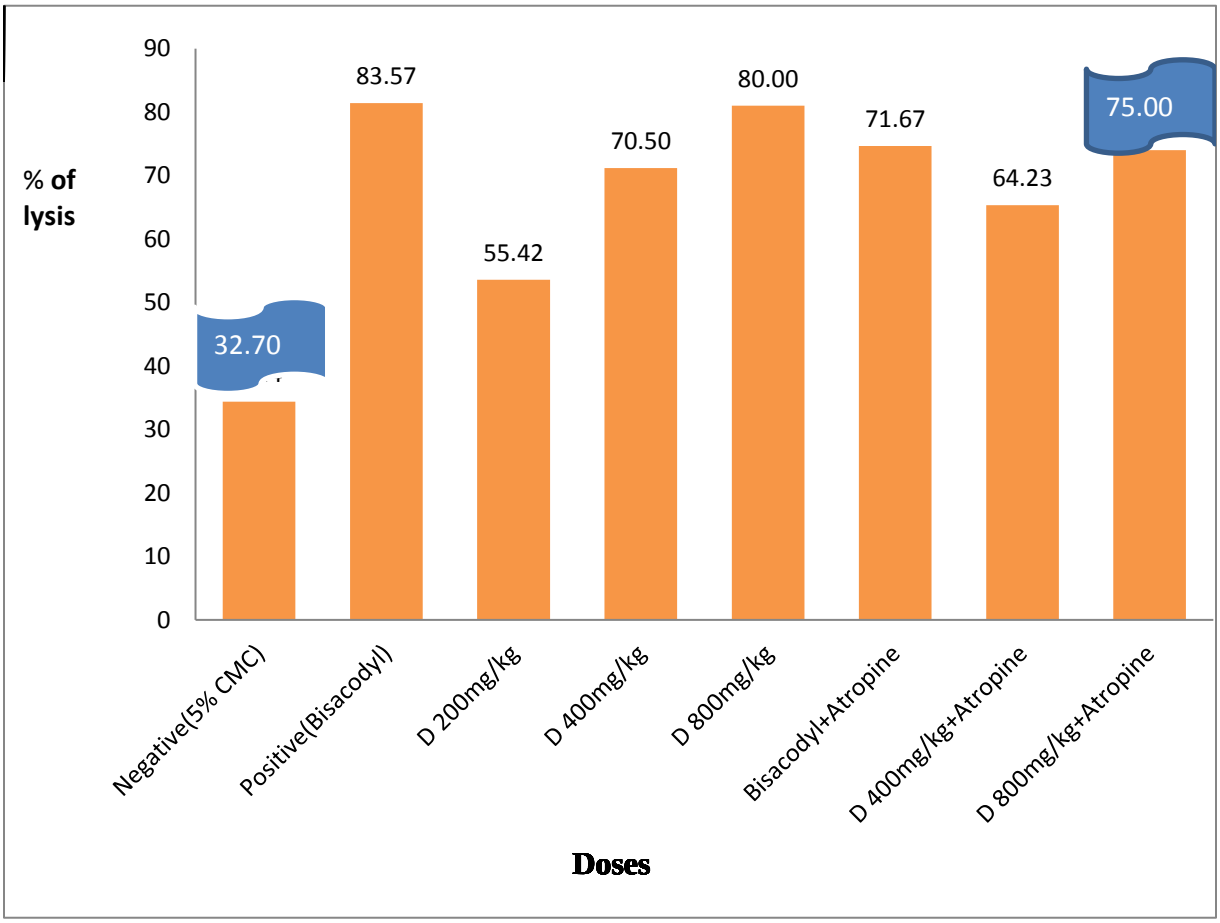


Figure 6.1: Bar diagram showing the dose-dependent effect of crude extract on the travel of charcoal meal through small intestine of mice, in the absence and presence of atropine. Each bar shown represents mean of 5 animals per group.

CONCLUSION

Conclusion

Many substances consumed by man either through foods, drinks and inhalation, even effect of exogenous material (ultraviolet radiation) on the skin may be destructive to the health and thus, shortening the life span of man. Free radicals when generated in the body system of man causes damage to which eventually leads to death at shorter time. Continuous usage of the same vegetable oil, smoking, chronic alcoholism and exposure to pollution are the reason for which oxidative stress occurs in body. Report has shown that proper intake of antioxidant will help quench all these inevitably free radicals in the body thus, improving the health by lowering the risk of various diseases such as cancer. Synthetic antioxidants are recently reported to be dangerous to human health. Thus the search for effective, nontoxic natural compounds with antioxidative activity has been intensified in recent years. In addition to endogenous antioxidant defense systems, consumption of dietary and plant-derived antioxidants appears to be a suitable alternative. Dietary and other components of plants form a major source of antioxidants. Higher intake of foods with functional attributes including high level of antioxidants in functional foods is one strategy that is gaining importance. The RDA has been previewed therefore, the consumption of antioxidant rich diet will lead to lower health risks and people live longer and have fewer disabilities.The study indicated that medicinal plants like *Trapa bispinosa and Stephania japonica* are good sources of **antioxidant** and also *S.japonica* showed very poor **thrombolytic activity** against **streptokinase** and also Laxative effect test of Gastrointestinal transit test**(GIT)**activity showed very well by the above experiment with *Trapa bisphinosa*. Newer approaches utilizing collaborative research and modern technology in combination with established traditional health principles will yield dividends in near future in improving health, especially among people who do not have access to the use of costlier western systems of medicine.

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