ANTIOXIDANT ACTIVITY OF MEDICINAL PLANTS COMMONLY USED TO TREAT DIABETES

A thesis

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Submitted by

MD. HABIBUL BASAR

Examination Roll No: MS-080702

Session # 2007-08

BIOTECHNOLOGY AND GENETIC ENGINEERING DISCIPLINE
LIFE SCIENCE SCHOOL, KHULNA UNIVERSITY
KHULNA-9208, BANGLADESH

APRIL, 2011

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DEDICATED TO MY

MOST RESPECTABLE PARENTS

TITLE

ANTIOXIDANT ACTIVITY OF MEDICINAL PLANTS COMMONLY USED TO TREAT DIABETES

DECLARATION

This thesis report is the original research work of the authors, except as otherwise stated. The material presented has not submitted either in whole or in part for a degree for this or any other university. The findings of the research has not been/will not be published anywhere without the written concurrence of the concerned supervisor. Legal action would be taken in any case of infringement regarding the declaration.

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14.04.2011

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Date:

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The

author

April - 2011

ABSTRACT

Plants are the most important source of bioactive compounds providing health benefits. To seek out the potential source of bioactive compounds such as polyphenols, flavonoids, anthocyanins and antioxidant of different medicinal plants that are commonly used to treat diabetes, methanolic extract of fourteen anti-diabetic plants or plant's part were studied. Among the extracts, *Sonneratia apetala* (seed) had the highest polyphenols (300 mg GAE/g) contents followed by *Syzygium cumini* (294 mg GAE/g) and *Swertia chirata* (183 mg GAE/g) whether *Asparagus racemosus* had the lowest polyphenols (17.94 mg GAE/g) content followed by *Coccinia indica* (25.30 mg GAE/g) and *Trigonella foenumgraecum* (25.94 mg GAE/g). From the experiment it was seen that *Swertia chirata* contained the highest total flavonoids (77.92 mg CE/g extract) followed by *Bacopa nonnieri* (59.95 mg CE/g extract), *Syzygium cumini* (31.70 mg CE/g extract) and *Sonneratia apetala* (seed) (30.63 mg CE/g extract). Total flavonoid was lowest in the extract of *Swietenia mahagonee*, and *Trigonella foenumgraecum* (0.974 mg CE/g extract). The highest amount of anthocyanin was found in *Achyranthes aspera* (4.65 μmol/g extract) followed by *Sonneratia apetala* (seed) (2.3 μmol/g extract), *Ficus racemosa* (bark) (1.75 μmol/g extract) and *Coccinia indica* (1.24 μmol/g extract) Whereas it was lowest in case of *Asparagus racemosus* (0.09 μmol/g extract) followed by *Swietenia mahagonee* (0.15 μmol/g extract), *Sonneratia apetala* (pericarp) (0.25 μmol/g extract) and *Gymnema sylvestre* (0.26 μmol/g extract). *Sonneratia apetala* (seed) showed the most potent DPPH radical scavenging activity with an IC_{50} 4.32 μg/ml followed by *Syzygium cumini* (6.25 μg/ml), *Ficus racemosa* (bark) (10.28 μg/ml) and *Swertia chirata* (12.61 μg/ml). *Syzygium cumini* and *Sonneratia apetala* (seed) both showed the powerful NO radical scavenging activity (at 400 μg/ml concentration) above 90% with an IC_{50} 48.61 μg/ml and 49.41 μg/ml followed by *Swertia chirata* (110.55 μg/ml) and *Ficus racemosa* (bark) (161.76 μg/ml). At 200 μg/ ml concentration, *Sonneratia apetala* (seed) was the highest absorbance (O.D-2.452) followed by *Syzygium cumini* (O.D-1.744), *Ficus racemosa* (O.D-1.513) and *Swertia chirata* (O.D-1.505). These extracts also showed promising total antioxidant capacity with *Sonneratia apetala* (seed) showed the highest antioxidant capacity (310.24 mg AAE/g; 280.77 mg GAE/g). This was followed by *Syzygium cumini* (207.82 mg AAE/g; 171.31 mg GAE/g) and *Swertia chirata* (155.03 mg AAE/g; 140.30 mg GAE/g). Total antioxidant capacity was lowest in the extract of *Coccinia indica* (14.15 mg AAE/g; 11.67.96 mg GAE/g). The results concluded that among the extracts *Sonneratia apetala* (seed), *Syzygium cumini, Swertia chirata* and *Ficus racemosa* (bark) are potential sources of bioactive compounds- polyphenols, flavonoids, anthocyanins, antioxidants and these medicinal plants therefore have activities beneficial to health.

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INTRODUCTION

INTRODUCTION

Human beings have been utilizing plants for basic preventive and curative health care since time immemorial. Recent estimates suggest that over 9,000 plants have known medicinal applications in various cultures and countries, and this is without having conducted comprehensive research amongst several indigenous and other communities (Farnsworth and Soejarto, 1991).

However, it is only during the last decade that the real significance of the medicinal plants sector has begun to be realized. Interest in natural materials by the dominant economic powers had waned from the late 1960s to the early 1980s as new possibilities in biotechnology and the synthesization of drugs beckoned. But by the mid-1980s, there was a renewed interest in natural materials and approaches to health care, coupled with a recognition that technology alone could not solve the pressing health care needs of the world's population (Tempesta and King, 1994)

Medicinal plants used for personal health-care and environmental esthetics are ecologically sound. Medicinal plants come with the territory. They are integral planet–provided food and medicine for all inhabitants. They are in every forest, jungle, rock, seashore, mountain and garden. They have a great bio-diversity and they are representatives of plant evolution level. Medicinal plants include annual and perennial species. They include wild and cultivated plants. Medicinal herbs are very much like foods, and in many cases they are indistinguishable from them. They contain a variety of different nutritious and therapeutic constituents: Vitamins, minerals, trace elements as well as active ingredients with a variety of medicinal actions. These include volatile oils, tannins, mucilage, alkaloids, bitters and flavonoids.

Medicinal plants are used at the household level by women taking care of their families, at the village level by medicine men or tribal shamans, and by the practitioners of classical traditional systems of medicine such as Ayurveda, Chinese medicine, or the Japanese Kampo system. According to the World Health Organization, over 80% of the world's population, or 4.3 billion people, rely upon such traditional plant-based systems of medicine to provide them with primary health care (Bannerman et. al., 1983).

Plant or plant's part as a dietary provides not only essential nutrients needed for life but also other bioactive compounds for health promotion and disease prevention. Previous epidemiologic studies have consistently shown that diet (Plant or plant's part) plays a crucial role in the prevention of chronic diseases.

Consumption of fruits and vegetables, grains as well as medicinal plant (for treatment against diseases) has been strongly associated with reduced risk of oxidative stress, cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts, and age-related functional decline (Temple, 2000; Willett, 1995). Whereas, Allopathic medicine is known to have serious limitations in terms of untoward effects, drug resistance, high costs, technical expertise, etc.

This convincing evidence suggests that a change in dietary behavior such as increasing consumption of fruits, vegetables and grains is a practical strategy for significantly reducing the incidence of chronic diseases. The protection that fruits and vegetables or medicinal plants provide against diseases has been attributed to the various antioxidant compounds (phytochemicals) such as phenolic compounds, flavonoids, anthocyanins, lignins, catechins, isocatechins, tannins, proanthocyanidins, vitamins C, and E, beta-carotene and various plant or herbal extracts contained in them (Ames, 1983; Gey, 1990; Steinberg et al., 1989, 1991).

Vegetative parts of plants are the major sources of health promoting agents in the body. Among these agents, antioxidants are most important because they inhibit lipid peroxidation, scavenge free radicals, and chelate divalent captions. The antioxidant properties of these compounds are responsible for their anticancer, antiviral and anti-inflammatory properties (Cao et al., 1997). They can also prevent capillary fragility and platelet aggregation (Benavente-Garcia et al., 1997; Aviram, 1999). Medicinal plant as af fruits or vegetables contain a wide variety of phytochemicals such as phenolics and carotenoids that may help protection of cellular systems from oxidative damage and lower the risk of chronic diseases. It is estimated that more than 5000 phytochemicals have been identified, but a large percentage still remain unknown and need to be identified before their health benefits are fully understood. However, more and more convincing evidence suggests that the benefits of phytochemicals in fruits, vegetables and medicinal plants may be even greater than is currently understood because oxidative stress induced by free radicals is involved in the etiology of a wide range of chronic diseases (Ames, 1991). At present, there is overwhelming evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids. Free radicals may lie at the heart of the etiology or natural history of a number of diseases including cancer and atherosclerosis. Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of these disease states. (Sta¨helin et al., 1991; Willett, 1995). Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals".

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. Generally, free radicals attack the nearest stable molecule, "stealing" its electron. When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction and can be "thousand of events long" (Przedborski et al., 1998). Once the process is started, it can cascade, finally resulting in the disruption of a living cell (Jesberge et al., 1991).

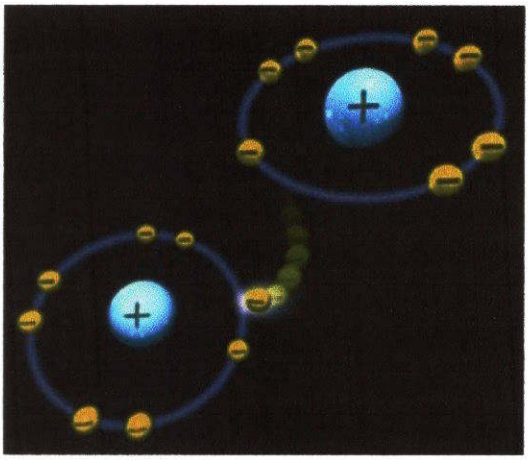


Fig.1: Free radical

In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell (Sies, 1997; Baillie et al,. 2009) However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level (Rhee, 2006).

The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and free radicals such as the hydroxyl radical (·OH) and the superoxide anion (O_2^-) (Valko et al., 2007). The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. This species is produced from hydrogen peroxide in metal-catalyzed redox reactions such as the Fenton reaction (Stohs and Bagchi, 1995). These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins (Sies, 1997). Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms (Nakabeppu et al., 2006; Valko et al., 2004), while damage to proteins causes enzyme inhibition, denaturation and protein degradation (Stadtman, 1992).

An **antioxidant** is a molecule capable of inhibiting 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. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997).

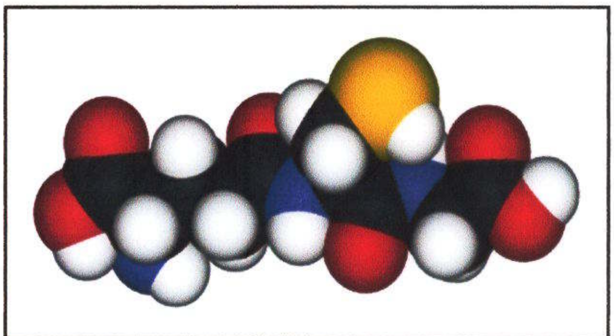


Fig.2: Space-filling model of the antioxidant metabolite glutathione. The yellow sphere is the redox-active sulfur atom that provides antioxidant activity, while the red, blue, white, and dark grey spheres represent oxygen, nitrogen, hydrogen, and carbon atoms, respectively.

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, cause 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. However, it is unknown whether oxidative stress is the cause or the consequence of disease.

Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer, coronary heart disease and even altitude sickness. 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 (Baillie et al., 2009; Bjelakovic et al., 2007). 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.

In recent years, one of the areas which attracted a great deal of attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage (Vani et al., 1997). An enormous variety of plants have been studied as new sources of phytochemicals but there are only a few reports about phenolic content and antioxidant activities from fruits and vegetables. Local medicinal plants especially that is commonly used to treat diabetes could be used as a potential source of new natural antioxidants and can serve as leads for the development of new drugs with the prospect of .improving the treatment of several diseases with improving diabetes. The formulation of preventive and healthy nutrition requires the information about total antioxidant activities based on numerous evidence of the strong biological activity of phytochemicals.

Medicinal plants used to treat hypoglycemic or hyperglycemic conditions are of considerable interest for ethno-botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and a number of plants have shown varying degree of hypoglycemic and anti-hyperglycemic activity (Grvoer et al., 2002) The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents (Grvoer et al., 2002).

Several species of medicinal plants are used in the treatment of diabetes mellitus, a disease affecting large number of people world-wide. Traditional plant medicines or herbal formulations might offer a natural key to unlock diabetic complications (Fabricant and Farnsworth, 2001). Diabetes mellitus is the major endocrine disorder responsible for renal failure, blindness or diabetic cataract, poor metabolic control, increased risk of cardiovascular disease including atherosclerosis and AGE (advanced glycation end) products. Antioxidants play an important role to protect against damage by reactive oxygen species and their role in diabetes has been evaluated. Many plant extracts and products were shown to possess significant antioxidant activity.

Therefore, in the present study, we have conducted the antioxidant activity of the crude extracts of selected fourteen medicinal (14) plant commonly used to treat the diabetes.

Objectives:

The objectives of the research work are mentioned below-

- **To determine the amount of polyphenols, flavonoids and anthocyanins contents in medicinal plants which are commonly used to treat the diabetes;**
- **To clarify the potentiality of the anti-diabetic plant to scavenge DPPH free radicals and NO;**
- **To know the reducing power of the medicinal plants commonly used to treat diabetes;**
- **To determine total antioxidant capacity of selected medicinal plants commonly used to treat diabetes.**
- **To know the medicinal plants which have high content of antioxidant and might be used to treat diabetes.**

CHAPTER: TWO

PLANT PREVIEW

PLANT PREVIEW

Achyranthes aspera (Leaves)

A coarse, rambling or erect, distantly branched annual herb, 0.5 to 2 m high. Leaves are oblong-ovate to elliptic or obovate, 6 to 15 cm long, pointed at both ends, more or less hairy, though often nearly smooth. Flowers are green and about 5 cm in length. Sepals 4 or 5, filaments connate at the base, the stamens and staminodes square toothed or fimbricate, pale purplish. Ovary oblong, 1-ovuled; flowers unisexual. The spikes are rigid, elongated and 10 to 15 cm long. Buds point upwards but when the flowers open, they spread out from the sides. Seeds oblong, brown and 2 to 3 mm long. Fruits utricles, oblong or ovoid, indehiscent.

Common name : Prickly Chaff Flower, Chaff-flower, Crocus stuff, Crokars staff, Devil's horsewhip Hindi-Chirchita.

Scientific classification :

Kingdom : Plantae

Order : Caryophyllales

Family : Amaranthaceae

Genus : *Achyranthes*

Species : ***A. aspera***

Binomial name : ***Achyranthes aspera*** L.

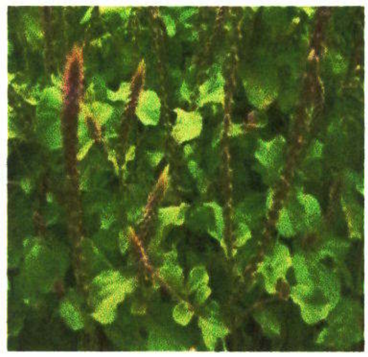


Fig.3: ***Achyranthes aspera***

Medicinal Uses :

Culinary

Leaf used as potherb,

Folkloric

- Cold with fever, heat stoke with headache, malaria, dysentery.
- Urinary tract lithiasis, chronic nephritis, edema.
- Rheumatic arthralgia (joint pain)
- It is also used for the treatment of diabetes.
- In Ayurveda, used for treatming vomiting, bronchitis, heart problems, hemorrhoids, itching, abdominal pains, ascites, etc.
- In **India**, used as abortifacient
- Decoction of leaves used in treatment of diarrhea and dysentery.
- Poultice of leaves used for rabies, hysteria, insect and snake bites.

Others

- Ash from burnt plant, mixed with mustard oil and pinch of salt, and used as powder for cleaning teeth.
- Dried twigs or fresh piece of root used as toothbrush.
- Ash is a rich source of potash. Used for washing clothes.

Alpinia nigria (Rhizome)

Alpinia nigra is a perennial rhizomatous herb, leaves sessile or very shortly petiolate, up to 50 x 15 cm, linear-lanceolate, acuminate, some pubescence on either side of the midrib below, otherwise glabrous; ligule c. 0.5 cm long, rounded, entire, pubescent; sheaths glabrous. Inflorescence paniculate, pubescent, usually rather lax with remote cincinni. Bracts membranous, up to 0.5 cm long (those at the base often much larger), each subtending a cincinnus of up to 4 shortly pedicellate flowers. Bracteoles tubular, membranous, splitting to the base readily.

Common name: Jangli ada (Beng.)

Scientific classification :

Kingdom **:** Plantae
Class : Liliopsida
Order : Zingiberales
Family : Zingiberaceae
Genus **:** *Alpinia*
Species **: *A. nigria***
Binomial name **:** ***Alpinia nigria* B.L. Burtt.**

Fig.4: ***Alpinia nigria***

Medicinal uses: Anti-diabetic, expectorant, carminative, rheumatism, sores, dyspepsia, bronchitis, diabetes, kidney disease.

Asparagus racemosus (Rhizome)

Asparagus racemosus is a creeper, 1 to 2 meters tall, that is common throughout India and the Himalayas. It prefers to take root in gravelly, rocky soils, high up in piedmont plains (1,300 - 1,400 meters elev.). It was botanically described in 1799. Satavar has odd little pine-needle-like leaves that are uniform, and shiny green. In July it has minute, white flowers on short, spiky stems, and in September it fruits blackish-purple, globular berries.

Common name : Asparagus, Wild Asparagus, Asparagus Root, Satavari.

Scientific classification :
Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Monocots
Order : Asparagales
Family : Asparagaceae
Genus : *Asparagus*
Species : ***A. racemosus***
Binomial name : ***Asparagus racemosus***

Fig.5: ***Asparagus racemosus***

Medicinal uses :. The roots are used in Ayurvedic medicine, following a regimen of processing and drying, with the name of Shatavari. It is used to treat the diabtes. It is also used as an anodyne, aphrodisiac and galactogogue. Shatavari is considered to be the main Ayurvedic rejuvenating female tonic for overall health and vitality. The reputed adaptogenic effects of Shatavari may be attributed to its concentrations of saponins, known as Shatavarins. In Sanskrit, Shatavari means "she who possesses a hundred husbands."

Adhatoda zeylanica Medic (Leaves)

An evergreen, gregarious, stiff, perennial, shrub, 1.2-6.0 m in height. Leaves elliptic-lanceolate or ovate lanceolate, entire, 5-30 cm long, hairy, light green above, dark below, leathery; flowers large, white with red- or yellow-barred throats, in spikes with large bracts; capsules clavate, longitudinally channelled, 1.9-2.2 cm x 0.8 cm; seeds globular.

Common name : Basok, vasaka, malabarnut etc.

Scientific classification :

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Lamiales
Family : Acanthaceae
Genus : *Adhatoda*
Species : ***A. zeylanica***
Binomial name: ***Adhatoda zeylanica*** **Medic**

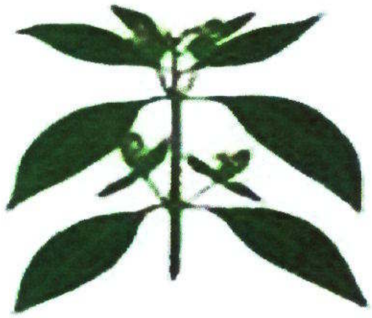


Fig.6: *Adhatoda zeylanica*

Medicinal uses : Antidiabetic, antioxidant and antibacterial activity to its wide range of pharmacological activities which includes antiulcer, hepatoprotective, anti-inflammatory, antitussive, bronchodialator, antitubercular and radioprotective activities.

Bacopa nonnieri (Floating leaves)

Bacopa monnieri is a perennial, creeping herb whose habitat includes wetlands and muddy shores. The leaves of this plant are succulent and relatively thick. Leaves are oblanceolate and are arranged oppositely on the stem. The flowers are small and white, with four or five petals. Its ability to grow in water makes it a popular aquarium plant. It can even grow in slightly brackish conditions. Propagation is often achieved through cuttings.

Common name : Coastal Waterhyssop, Brahmi, Thyme-leafed gratiola, Water hyssop

Scientific classification :

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Eudicots
(unranked) : Asterids
Order : Lamiales
Family : Scrophulariaceae
Genus : *Bacopa*
Species : ***B. monnieri***
Binomial name: ***Bacopa monnieri*** L.

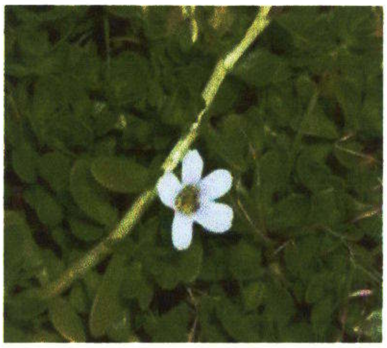


Fig.7: ***Bacopa nonnieri***

Medicinal uses :

This plant has a number of uses in Ayurveda. It is a traditional treatment for epilepsy and asthma. It has antioxidant properties, reducing oxidation of fats in the bloodstream. However, anti-epilepsy properties seem to be in very high toxic and near lethal doses, so it's only used—at much lower non-toxic dosage— as an additive to regular epilepsy medication. Studies in humans show that an extract of the plant has antianxiety effects. It's floating leafs is used to treat the diabetes for the diabetic patient.

It is listed as a notropic, a drug that enhances cognitive ability. Laboratory studies on rats indicate that extracts of the plant improve memory capacity and motor learning ability. Recent studies suggest bacopa may improve intellectual activity. The sulfhydryl and polyphenol components of *Bacopa monniera* extract have also been shown to impact the oxidative stress cascade by scavenging reactive oxygen species, inhibiting lipoxygenase activity and reducing divalent metals. It is used in Rebirthing therapy to accelerate trauma release and make continuous breathing easier. Bacopa monnieri is a well known nootropic plant reported for its tranquilizing, sedative, cognitive enhancing, hepatoprotective and antioxidant action.

Coccinia indica (Leaves)

It is an annual creeper which is found spreading on ground and twilling around the trees and supports around it. The stem is pentagonal in shape. Leaves are triangular or pentagonal in shape; it is dentate and has a length of 2 to 5 inch and breath of 2 inch. Flowers are monocieus and are bid in size. They are placed in bunches having about 2 to 4 flowers. These are of white color. Fruit id slimy in touch, pulpy and is barrel shaped. It is 1 to 2 inch in length. it is green in color when it is raw form which turns to scarlet red color when it ripes. It also possesses about ten white stripes on its posterior portion. This fruit posses numerous seeds inside. Its roots are long and resemble tuberous roots. According to ayurveda there are two types of varieties.

Common Name : Hindi name- kundru, **Sanskrit name-** imbi / tundika, **Gujarati name** – tindora, Telakucha in Bangla.

Scientific classification :

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Cucurbitales

Family : Cucurbitaceae

Genus : *Coccinia*

Species : ***C. indica***

Binomial name: ***Coccinia indica***

Fig.8: ***Coccinia indica***

Medicinal uses :

It is kapha pitta suppressant. It is a good wound healer and reduces any kind of inflammation occurring in body. It is good appetizer and helps in improving digestion. It is a good laxative and stimulates liver for proper secretion of bile juices. It is also used in wormal infestation especially in amoebiasis caused by entamoeba hystolitica. It also purifies blood. It is also helpful in expelling the extra amount of mucus accumulated in the respiratory tract. It also controls the frequency of micturation and widely used in diabetes as it controls the glucose level in the blood. It also helps in opening the pores in the skin so as to facilitate the easy secretion of sweat thus expelling out the toxin in the body.

Ficus racemosa (Bark and Fruits)

Ficus racemosa, a member of the fig family, is an evergreen tropical fig tree native to Asia and Australia. It is sometimes used as a bonsai. This tree has edible fruit and works well as a shade tree. There are some characteristics that all Ficus racemosa trees have in common. Ficus racemosa can grow over 40 feet tall and 20 to 40 feet wide. Plant Ficus racemosa in a location that receives full sun to part shade. The oval leaves measure 3 to 4 inches long and are dark green. Because it is an evergreen tree, it does not lose its leaves. The fruit of Ficus racemosa is 3/4 inch to 2 inches long, circular and grows directly on the trunk.

Common name : Ficus racemosa is the botanical name of this tree; it is also sometimes known as Ficus glomerata. The common names are cluster fig, clustertree, country fig, redwood fig, Indian fig, crattock and ju guo rong.

Scientific classification :

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Rosales
Family : Moraceae
Genus : *Ficus*
Species : ***F. racemosa***
Binomial name : ***Ficus racemosa*** Roxb.

Fig.9: ***Ficus racemosa***

Medicinal usea :

The roots, bark-skin, fruits, lates and leaves of udumbara have great medicinal value. Udumbara is used both, internally as well externally, externally; the latex is applied on chronic infected wounds to alleviate edema, pain and to promote the healing. The tender leaf buds are applied on the skin, in the form of paste, to improve the complexion; the decoction of leaves is salutary in washing the wounds for better cleansing and healing. The decoction of its bark-skin is an effective gargle in stomatitis and sore throat. Application of latex alleviates the edema in adenitis, parotitis, orchitis, traumatic swelling and toothache.

Internally, udumbara is used in vast range of maladies. The decoction of bark skin is extremely useful in diarrhea, dysentery and ulcerative colitis. In children, the latex is given along with sugar to combat diarrhea and dysentery. The cold infusion of ripened fruits mixed with sugar, is salutary in Rakta pitta is effectively controlled with the decoction of bark-skin. In diabetes, the ripe fruits or bark-skin decoction is beneficial, as it works well as anti-diuretic. The decoction of leaves is an effective remedy in glandular swelling, abscess, chronic wounds, cervical adenitis etc. In uterine bleeding, abortion, leucorrhea and vaginal bleeding the decoction of its bark-skin is given orally, as well as in a form of basti (enema). The latex mixed with sugar is benevolent in sexual debility in males. The juice of its fruit is a panacea for hiccup. The powder of the bark-skin works well as an anorexient, hence, beneficial in gyperphagia-bhasmaka

Gymnema sylvestre (Leaves)

Gymnema sylvestre is a herb native to the tropical forests of southern and central India where it has been used as a naturopathic treatment for diabetes for nearly two millennia. Large climbers, rooting at nodes, leaves elliptic, acuminate, base acute to acuminate, glabrous above sparsely or densely tomentose beneath; Flowers small, in axillary and lateral umbel like cymes, pedicels long; Calyx-lobes long, ovate, obtuse, pubescent; Corolla pale yellow campanulate, valvate, corona single, with 5 fleshy scales. Scales adnate to throat of corolla tube between lobes; Anther connective produced into a membranous tip, pollinia 2, erect, carpels 2,unilocular; locules many ovuled; Follicle long, fusiform1

Common name : Sanskrit Name : Meshasringi, Madhinasini or madhoolika, Hindi: Gurmar, Tamil and Malayalam are Sirukurinchaan, Amudhapushpam, Chakkarakkolli.

Fig.10: ***Gymnema sylvestre***

Scientific classification :

Kingdom : Plantae

Order : Gentianales

Family : Asclepiadaceae

Genus : *Gymnema*

Species : ***G. sylvestre***

Binomial name : ***Gymnema sylvestre*** R. Br.

Medicinal uses :

While it is still being studied, and the effects of the herb are not entirely known, the herb has been shown to reduce blood sugar levels when used for an extended period of time. Additionally, *Gymnema* reduces the taste of sugar when it is placed in the mouth, thus some use it to fight sugar cravings. From extract of the leaves were isolated glycosides known as Gymnemic acids, which exhibit anti-sweet activity (kinghorn and CM Compadre, 1991). Extracts of Gymnema is not only claimed to curb sweet tooths but also for treatment of as varied problems as hyperglycemia, obesity, high cholesterol levels, anemia and digestion. According to the Sushruta of the Ayurveda it helps to treat Madhumeha ie glycosuria.

Memordica chinensis (Fruits)

Momordica is a genus of about 80 species of annual or perennial climbers herbaceous or rarely small shrubs belonging to the family Cucurbitaceae, natives of tropical and subtropical Africa and Asia and Australia. Most species produce floral oils and are visited by specialist pollinators in the apid tribe Ctenoplectrini. Some *Momordica* species are grown in cultivation for their fleshy fruit, which are oblong to cylindrical in shape, orange to red in colour, prickly or warted externally, and in *Momordica charantia (also called as* ***Memordica chinensis)*** burst when ripe, generally with elastic force, into irregular valves.

Common name : called *balsam apple, balsam pear, bitter cucum-ber, bitter pear, carilla cundeamor, fu gwa*, or *karolla*.

Scientific classification :

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Cucurbitales
Family : Cucurbitaceae
Subfamily: Cucurbitoideae
Tribe : Joliffieae
Subtribe : Thladianthinae
Genus : ***Momordica***
Species : ***M. chinensis***
Binomial name : ***Momordica chinensis*** L.

Fig.11: ***Memordica chinensis***

Medicinal uses : Fruit, seeds, seed oil, leaves; uses: antidiabetic, antiinfective, antipyretic, anthelmintic, laxative, possible antifungal, androgenic, antiviral, antimalarial actions; possibly useful for infertility; precautions: pregnancy, lactation, children, patients taking hypoglycemic medications; may cause uterine bleeding or contractions, hepatotoxicity; seeds are toxic to children.

Sonneratia apetala (Seed and Pericarp)

Sonneratia mangrove is a small to medium size tree, which can attain a height of about 20m and a girth of about 2.5m. The tree occurs on newly accreted soil in moderately to strongly saline areas and is considered as a pioneer species in ecological succession. The species is distributed in India, Bangladesh, and Myanmar. The leaves are simple opposite, entire and leathery. Bark thin, light brown, irregularly fissured. Flowers apetalous, cream coloured, arranged in axillary 3-flowered of 7 flowered dichasial cyme. The flowers have no petals, but 4 prominent green sepals. Cream colored mass of stamens give the flowers a cream colored look. The most interesting part of the flower is the style , which consists of a white, 2-3 cm long, curved, stigma, falttened like umbrella or mushroom. The upper portion is reddish in color.

Common name: Sonneratia mangrove, Maram (Tamil), Keora (Bengali), Kyalanki (Telugu), Khirwa (Oriya), {Kandal, Chipi} (Marathi)

Scientific classification :

Kingdom : Plantae
Phylum : Magnoliophyta
Class : Magnoliopsida
Order : Myrtales
Family : Lythraceae
Genus **:** *Sonneratia*
Species : ***S. apetela***
Binomial name : ***Sonneratia apetala*** Buch-Ham**.**

Fig.12: ***Sonneratia apetala***

Swertia chirata (Whole part)

The plant can be grown in a variety of soils with sandy loam rich in carbon and humus. It is also found in open ground and recently slash-and-burnt forests10. *S. chirayita* has an erect, about 2–3 ft long stem, the middle portion is round, while the upper is four-angled, with a prominent decurrent line at each angle. The stems are orange brown5 or purplish in colour9, and contain large continuous yellowish pith. The root is simple, tapering and stout, short, almost 7 cm long and usually half an inch thick8, Flowering in *S. chirayita* is in the form of numerous small, axillary, opposite, lax cymes arranged as short branches and the whole inflorescence is 2 ft long. Flowers are small, stalked, green-yellow, tinged with purple colour, rotate and tetramerous 6, 9. The corolla is twice as long as the calyx and divided near the base into four ovate–lanceolate segments. The upper surface of the petal has a pair of nectaries covered with oblong scales and ending as fringes9. Fruit is a small, one-celled capsule with a transparent yellowish pericarp. It dehisces from above, septicidally into two valves. Seeds are numerous, minute, many-sided and angular. Floral characteristics such as colourful corolla and presence of nectaries support crosspollination in the species. Generally, bees (Apoidae, Hymenoptera) are the pollinators of *S. chirayita*

.

Common name: Chirayata, Kirata-tikta, Kiryat-charayatah , Bhunimba, Bhuchiretta, Charayatah, Chiretta, Chiraita, Indian Gentian, Jwaran- thakah, Kirata, kiraita, Kiriath, Kiriyattu, Kiryat-charayatah, Mahatita, Nila-vemu, Nila-vembu, Qasabuz-Zarirah.

Scientific classification :

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Eudicots
(unranked) : Asterids
Order : Gentianales
Family : Gentianaceae
Genus : *Swertia*
Specises : ***S. chirata***
Binomial name : ***Swertia chirata***

Fig.13: ***Swertia chirata***

Medicinal uses: It is an excellent medicine for strengthening the stomach and promoting its action thus used in treating stomach disorders like dyspepsia and diarrhoea. its anthelmintic properties help in destroying intestinal worms. An infusion of the herb is taken for this purpose. The root of the plant is useful in curing hiccups and vomiting. The herb can also be used for range of other diseases and conditions including leprosy, leucoderma, scabies, neuro-muscular disorders, menorrhagia, menstrual irregularity, urinary disease, heart disease, asthma, cough , dyscrasia, Ulcer, jaundice and anaemia. It also reduces the blood glucose level.

Syzygium cumini (Seed)

The evergreen **Jamun** plant is originally from Indonesia and India; it now grows abundantly in Suriname. It has dispersed spontaneously in the wild as the seeds are sought by birds.

An evergreen tropical tree 50 to 100 ft. tall, with oblong opposite leaves that are smooth, glossy; they have a turpentine smell. The bark is scaly gray and the trunk forks into multiple trunks about 3 - 5' from the ground.

Jamun has fragrant white flowers in branched clusters at stem tips and purplish-black oval edible berries. The juicy fruit-pulp contains resin, gallic acid and tannin; it tastes usually from acid to fairly sweet. The somewhat astringent, jamun fruit can be utilized for juice.

Common name :

Java plum, jambul, jamun, jaman, black plum, faux pistachier, Indian blackberry, jambol, doowet, jambolan.

Scientific classification :

Kingdom : Plantae
Order : Myrtales
Family : Myrtaceae
Genus : *Syzygium*
Species : ***S. cumini***
Binomial name : ***Syzygium cumini*** L.

Fig.14: ***Syzygium cumini***

Medicinal uses :

All parts of the java plum can be used medicinally and it has a long tradition in alternative medicine. The bark has anti-inflammatory activity and is used In India for anemia, the bark and seed for diabetes which reduce the blood sugar level quickly, the fruit for dysentery and leave's juice for gingivitis (bleeding gums). In the Philippines and Suriname wine is made from the fermented fruit. In Surinam also, the whole leaves are used by women as a feminine sitz-bath to shrink the vagina after giving birth and getting rid of mucus and odors. This bath from the Jamun leaves is also used at any other time to shrink the vagina. This gives the ultimate in sexual pleasure for both partners!

Swietenia mahagoni (Seed)

Swietenia is a genus of trees in the mahogany family Meliaceae. As a timber, both *Swietenia macrophylla* and *Swietenia mahogoni* are both grown in plantations in several Asian countries such as Fiji, Indonesia, India, and Bangladesh

It is usually taken to consist of three species, geographically separated. They are medium-sized to large trees growing to 20–45 m tall, and up to 2 m trunk diameter. The leaves are 10–30 cm long, pinnate, with 3-6 pairs of leaflets, the terminal leaflet absent; each leaflet is 5–15 cm long. The leaves are deciduous to semi-evergreen, falling shortly before the new foliage grows. The flowers are produced in loose inflorescences, each flower small, with five white to greenish-yellowish petals. The fruit is a pear-shaped five-valved capsule 8–20 cm long, containing numerous winged seeds about 5–9 cm long.

Common name : Big Leaf Mahogany, Big-leaf Mahogany, Bigleaf Mahogany, broad-leaved Mahogany, Brazilian Mahogany, Honduras Mahogany, Large-leaved Mahogany, genuine mahogany, Sky Fruit, Tropical American Mahogany.

Scientific classification :

Kingdom : Plantae
Order : Sapindales
Family : Meliaceae
Genus : *Swietenia*
Species : ***S. mahagonee***
Binomial name : ***Swietenia mahagonee*** L.

Fig.15: ***Swietenia mahagonee***

Medicinal uses : The fruits of *Swietenia macrophylla* are called "sky fruit", because they seem to hang upwards from the tree. The "sky fruit" concentrate is sold as a natural remedy that is said to improve blood circulation, skin and reduce blood glucose level. It is also said to have Viagra-like qualities regarding erectile dysfunction. Consumption of the sky fruit concentrate is approved by Malaysia Ministry of Health.

Trigonella foenumgraecum (Seed)

An erect annual to 50 cm. which may be branched, the leaves are trifoliate and the leaflets oblong-lanceolate, to 5 cm. Its yellowish flowers 12 – 18 mm long (1 – 2) are in the leaf axils. The fruits are almost straight and flattened with a pronounced beak ;they are 50 – 110 mm long excluding the beak of 10 – 35 mm. Chromosome number 2n=16. The seeds are brownish, about 1/8 inch long, oblong, rhomboidal, with a deep furrow dividing them into two unequal lobes; they are contained, ten to twenty together, in long, narrow, sickle-like pods. There are about 50 000 seeds per kilogramme.

Common names :

Fenugreek; fenugrec (France), fieno greco (Italy) alholva, feno-greco (Spain), helba (Arabic), methi (India, Pakistan).

Scientific classification :

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Fabales
Family	: Fabaceae
Genus	: *Trigonella*
Species	: ***T. foenum-graecum***

Binomial name :***Trigonella foenum-graecum*** L.

Fig.16: *Trigonella foenumgraecum*

Medicinal uses: abdominal colic, bronchitis, cough, sprains, treatment after childbirth, digestive, abortive, purgative, galactagogue, diabetes, emmenagogue, stomachic, anthelmintic, asthma, emphysema, aphrodisiac, reconstituent, sedative for palpitations, icterus, gastro-intestinal troubles, fevers, constipation, sterility.

CHAPTER: THREE
LITERATURE REVIEW

LITERATURE REVIEW

It is emerging evidence that the generation of reactive species (ROS) is one major factor in the onset and development of diabetes and its complications (Tesfamariam, 1994; Jones et al., 1988; Tilton et al., 1997; Cohen, 1993). The term oxidative stress, widely used in the literature refers to a serious imbalance between the production of free radicals and antioxidant defense, leading to potential tissue damage (Halliwell, 1995).

Researchers have demonstrated a wide range of biochemical and pharmacological effects, including anti-inflammatory and antioxidant actions, for a class of phytochemicals called polyphenols, flavonoids and anthocyanins. These effects are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidantion and chelating transition metals (Lee et al., 2003; Miliauskas et al., 2004; Atoui et al., 2005). Flavonoids are diphenylpropanes (C6-C3-C6) ubiquitous in plants and are an integral part of the human diet. They occur in foods generally as O-glycosides and are classified in flavonols, flavones, flavanones, catechins (flavanols), anthocyanidins, isoflavones, dihydroflavonols, and chalcones, according to their chemical structure (Paola et al., 2004). Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipids and protein (Scheen, 1997). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs (Lyra et al,. 2006).

In diabetic rats, the utilization of impaired carbohydrate leads to accelerate lipolysis, resulted in hyperlipidemia (Morel and Chisolm, 1989). Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies (Mitra et al., 1996; Shukla et al., 2000; Bhattaram et al., 2002; Mahomed and Ojewole, 2003; Hou et al., 2005; Huang et al., 2005). Antihyperglycemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycemic activity have been available in literature (Oliver-Bever, 1986; Rai, 1995). However, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, cartenoids, etc., that are frequently implicated as having antidiabetic effect (Loew and Kaszkin, 2002). Plants have played an important role in the treatment and control or many infectious and chronic diseases for centuries mainly in rural population. According to Gullo and Hughes (2005) in general 75% of new drugs originate from natural sources and are very useful in combating diseases.

Glucose oxidation during intermediate metabolism is believed to be the main source of free radicals (Maritim et al., 2003). When glucose is oxidized in a transition metal dependent reaction it produces enediol radical anions that are converted into reactive ketoaldehydes and superoxide anoin radicals. The superoxide anion radicals undergo dismutation to hydrogen peroxide, which when not degraded by catalase (CAT) or glutathione peroxidase (GSH-Px) can lead to the production of highly reactive hydroxyl radicals in the presence of transition metals (Maritim et al., 2003). High levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acid which eventually lead to cell death. There are several lines of evidence to suggest that antioxidant defence levels may be low in diabetes. The activities of the antioxidant enzymes CAT, superoxide dismutase (SOD) and GSH-Px are all reduced in diabetic patiens (Laight et al., 2000).

Antioxidants may counter the action of free radicals in three ways:

- Increase in levels of enzymes that degrade free radicals (Rahimi et al., 2005)
- Increase in levels of proteins such as transferring that can bind the metals which stimulate the production of free redicals (Rahimi et al., 2005)
- Chemical antioxidants such as vitamin C and E which act as direct free radical scavengers (Rahimi et al., 2005)

Antioxidant therapy can be achieved by supplementation with pharmaceutical preparations of antioxidant nutrients which may confer both cardiovascular and metabolic benefits in diabetes (Laight et al., 2000). Plants have numerous/substantial amounts of antioxidants and their action is an important property of plant medicines associated with diabetes (McCune and Johns, 2002)

Polyphenols have the ability to interfere with cell/tissue based assays in diabetes research by causing damage to cellular proteins, membrane lipids and nucleic acid. During the past decade the interest in polyphenols, including flavonoids has increased considerably with nutritionist, epidemiologist and food manufacturers taking a special interest in polyphenols this is mainly due to their various biological properties like their anti-oxidant effects. Polyphenols include other suclassed such as phenolic acids, stibenges, lignans, tannins and oxidized polyphenols (George et al., 2005).

The importance of antioxidants in preventative medicine has been recognized for several years and polyphenols have become the focus of research interest due to the perceived health beneficial effects (Wollgast and Anklam, 2000). Diseases that are believed to be caused or partially enhanced by oxidative stress include cardiovascular and cerebrovascular disease, some forms of cancer and other disorders such as diabetes and rheumatoid disease (Wollgast and Anklam, 2000)

Polyphenols constitute one of the most numerous and sidely distributed groups of substances in the plant kingdom, with more than 8000 phenolic structures currently being identified (Wollgast and Anklam, 2000). Polyphenols are products of the secondary metabolism of plants and originate from two main synthetic pathwasys; the shikimate pathway and the acetate pathway, both of which are derived or occur from glucose metabolism within plants (Wollgast and Anklam, 2000). A group of polyphenolic compounds are also called tannins at times as they are powerful reducing agents, and have been known to inhibit the activity of digestive enzymes including that of trysin and amylase (Thompson et al., 1984). Polyphenols may also exert their effect as chelators of divalent cations.

CHAPTER: FOUR
METERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS

Collection of Sample

The anti-diabetic plants were collected from Khulna, Satkhira, Narail and Jessore.

Preparation of samples

Used part of the collected anti-diabetic plants was washed by water to remove undesirable materials. Then it is turned into convenient form (shape, size).

Preparation of Extract

The convenient form of plants or plant's part of anti-diabetic plants was given shed dried until all the water evaporated. The dried samples were grinded by a grinding machine into powder form. Twenty five grams of powder of each sample was taken in 100ml of 100% methanol and kept in air tight bottle. After 3 days the methanol was filtered by Whitman No. 1 filter paper. After filtration the filtrate was air dried and the solid extracts were kept in the refrigerator. Finally 10 mg of the prepared extract was dissolved in 1ml 100% methanol (10 mg/ml) and used to conduct experiments.

Sample collection
↓
Sample processed in suitable form
↓
Drying (shed)
↓
Grinding into powder form
↓
Homogenization (sample powder into methanol)
↓
Filtration
↓
Evaporation
↓
Crude extract of sample
↓
Preparetion convenient sample concentration

Flow diagram- sample preparation (convenient concentration)

Chemicals, Glasswares and Instruments

- 200 mM phosphate buffer; P^H 6.6 (0.2 mM Na_2HPO_4, 0.2 mM NaH_2P0_4)
- Potassium ferricyanide
- Trichloroacetic acid
- Ferric chloride
- Sulfuric acid
- Sodium carbonate
- 1M NaOH
- L-Ascorbic acid (AA)
- Gallic acid (GA)
- DPPH (2, 2-diphenyl-1-picrylhydrazyl)
- Catechin
- Ammonium molybdate
- 50% ethanol
- Folin reagent (Ciocalteu's phenol reagent).
- Sulfanilamide
- Naphthalin
- Phosphoric acid
- Sodium nitroprusside

Instruments

Test tubes, Beakers, Micropipettes, Magnetic stirrer, P^H meter, Electronic balance, Vortex, Conical flask, Water bath, Centrifuge, UV Spectrophotometer.

METHODS

Determination of total phenolic content (TPH)

The total concentration of phenolics (TPC) in the extracts was determined according to the Folin-Ciocalteu method (Ough and Amerine, 1988) with gallic acid as the standard and expressed as (mg) GA equivalents (GAE/g) (Aoshima and Ayabe, 2007).

Total flavonoids content (TF)

The determination of total flavonoids content of anti-diabetic plants was measured according to the method of Zhishen et al., 1999.

Determination of anthocyanin content

The anthocyanin content of anti-diabetic plants was determined according to the method of Padmavati et al., 1997.

Determination of DPPH radical scavenging activity

The determination of DPPH radical scavenging activity was determined according to the mcthod of Blios, 1958; Aoshima ct al., 2004.

Determination of NO Scavenging Activity

Nitric oxide radical scavenging activity was determined according to the method reported by Marcocci et al., 1994.

Reducing power determination (RP)

The reducing power of the extract was determined according to the method of Oyaizu, 1986.

Total antioxidant capacity (TAC) measurement

The total antioxidant capacity of the extract was determined according to the method of Prieto et al., 1999.

CHAPTER: FIVE
RESULTS

RESULTS

Total Polyphenols (TPH)

Total Phenolic Contents (TPH) of methanolic extract of fourteen anti-diabetic plants is shown in **Fig. 17**. The TPH content was expressed as gallic acid (GA) equivalent. The TPH content ranged from 300.10 to 17.94 mg GAE/g of extract. The amount was highest in the extract of *S. apetala* (seed- 300 mg GAE/g) followed by *S. Cumini* (294.46 mg GAE/g) and *S. chirata* (183.fourteen mg GAE/g) whereas it was lowest in the extract of *A. racemosus* (17.94 mg GAE/g) followed by *C. indica* (25.30 mg GAE/g) and *T. foenumgraecum* (25.94 mg GAE/g).

Total Flavonoids (TF)

Total Flavonoids (TF) content of the methanolic extracts of fourteen anti-diabetic plants **(Fig. 18)** was expressed as catechin equivalent (CE). From the present experiment it was seen that *S. chirata* contained the highest total flavonoids (77.92 mg CE/g extract) followed by *B. nonniera* (59.95 mg CE/g extract), *S. cumini* (31.70 mg CE/g extract) and *S. apetala* (30.63 mg CE/g extract). TF was lowest in the extract of *S. mahagonee,* and *T. foenumgraecum* (0.974 mg CE/g extract).

Anthocyanin Determination Test

Anthocyanin determination test was measured at 530 and 657 nm O.D. and shown in **Fig. 19.** The present study showed that anthocyanin content was highest in *A. aspera* (4.65 µmol/g extract) followed by *S. apetala* (seed-2.3 µmol/g extract), *F. racemosa* (bark-1.75 µmol/g extract) and *C. indica* (1.24 µmol/g extract).This was lowest in case of *A. racemosus* (0.09 µmol/g extract).

DPPH Radical Scavenging Activity

The DPPH radical scavenging activities of fourteen anti-diabetic plants are also shown in **Fig. 20**. The level of free radical (DPPH) scavenging ranged from 93.58% to 1.64% at 100 µg/ ml of extract. At this concentration, *B. nonniera, F. racemosus* (bark), *F. racemosus* (fruits), *S. apetala* (seed), *S. chirata* and *S. cumini* showed the highest DPPH radical scavenging activity (all are above 92%) followed by *S. apetala* (pericarp-84.39%) and *G. sylvestre* (51.54%). *T. foenumgraecum* exerted the lowest activity (01.64%). Dose-dependent DPPH radical scavenging activities of *B. nonniera, F. racemosus* (bark), *F. racemosus* (Fruits), *S. apetala* (seed), *S. chirata* and *S. cumini* are shown in **Fig. 21**. The comparison of IC_{50} values for DPPH scavenging test of the most potent 6 anti-diabetic plants are shown in **Fig. 22** and correlation between total polyphenols and DPPH radical scavenging activity is shown in **Fig. 23**.

Nitric Oxide Scavenging Activity

Nitric oxide (NO) scavenging assay was measured at 570 nm O.D. and shown in **Fig. 24.** *S. apetal* (seed) showed the highest activity (93.00%) followed by *S. cumini* (92.66%) and *S. chirata* (82.39%) at a concentration of 400 µg/ml. *C. indica* showed the lowest NO scavenging activity (1.37%) whether *A. aspera, A. nigra, G. sylvestre, T. foenumgraecum* and *A. racemosus showed no activity.* Dose dependence activity of ***B. nonniera, F. racemosa (bark), F. racemosa (fruits), S. apetela (seed), S. apetela (pericarp), S. cumini*** and ***S. chirata,*** are given in **Fig. 25.** *Syzygium cumini* and *Sonneratia apetala* (seed) both showed the powerful NO radical scavenging activity (at 400 µg/ml concentration) above 90% with an IC_{50} 48.61 µg/ml and 49.41 µg/ml followed by *Swertia chirata* (110.55 µg/ml) and *Ficus racemosa* (bark) (161.76 µg/ml), are given in **Fig.26**. At 200 µg/ ml concentration **Fig.27** represents the co-relation between total polyphenols and NO scavenging activity at 400 µg/ml.

Reducing Power

The more antioxidant compounds reduce more oxidized form of ferric iron (Fe^{+3}) to ferrous iron (Fe^{+2}). In the present study, reducing powers of methanolic extracts of fourteen anti-diabetic plants were measured at 700 nm OD. Reducing powers of the extracts determined using the potassium ferricyanide method is shown in **Fig.28**. At 200 µg/ ml concentration, it was seen *S. apetala* (seed- 2.452) has the highest absorbance followed by *S. cumini* (1.744), *F. racemosus* (1.513) and *S. chirata* (1.505). Dose-dependent reducing power of *S. apetala* (seed, pericarp), *B. nonniera.* and *F. racemosa* (bark, fruits) are shown in **Fig.29.** Correlation between total polyphenols and reducing power is also shown in **Fig.30.**

Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) which was expressed as ascorbic acid equivalents (AAE) and gallic acid equivelents (GAE) of methanolic extracts of fourteen anti-diabetic plants is shown in **Fig. 31**. *S. apetala* (seed) showed the highest antioxidant capacity (310.24 mg AAE/g; 280.77 mg GAE/g).This was followed by *S. cumini* (207.82 mg AAE/g; 171.31 mg GAE/g) and *S. chirata* (155.03 mg AAE/g; fourteen0.30 mg GAE/g). *C. indica* showed the lowest antioxidant capacity (fourteen.15 mg AAE/g; 11.67.96 mg GAE/g). Correlation between total polyphenols and total anti-oxidant capacity is also shown in **Fig. 32.**

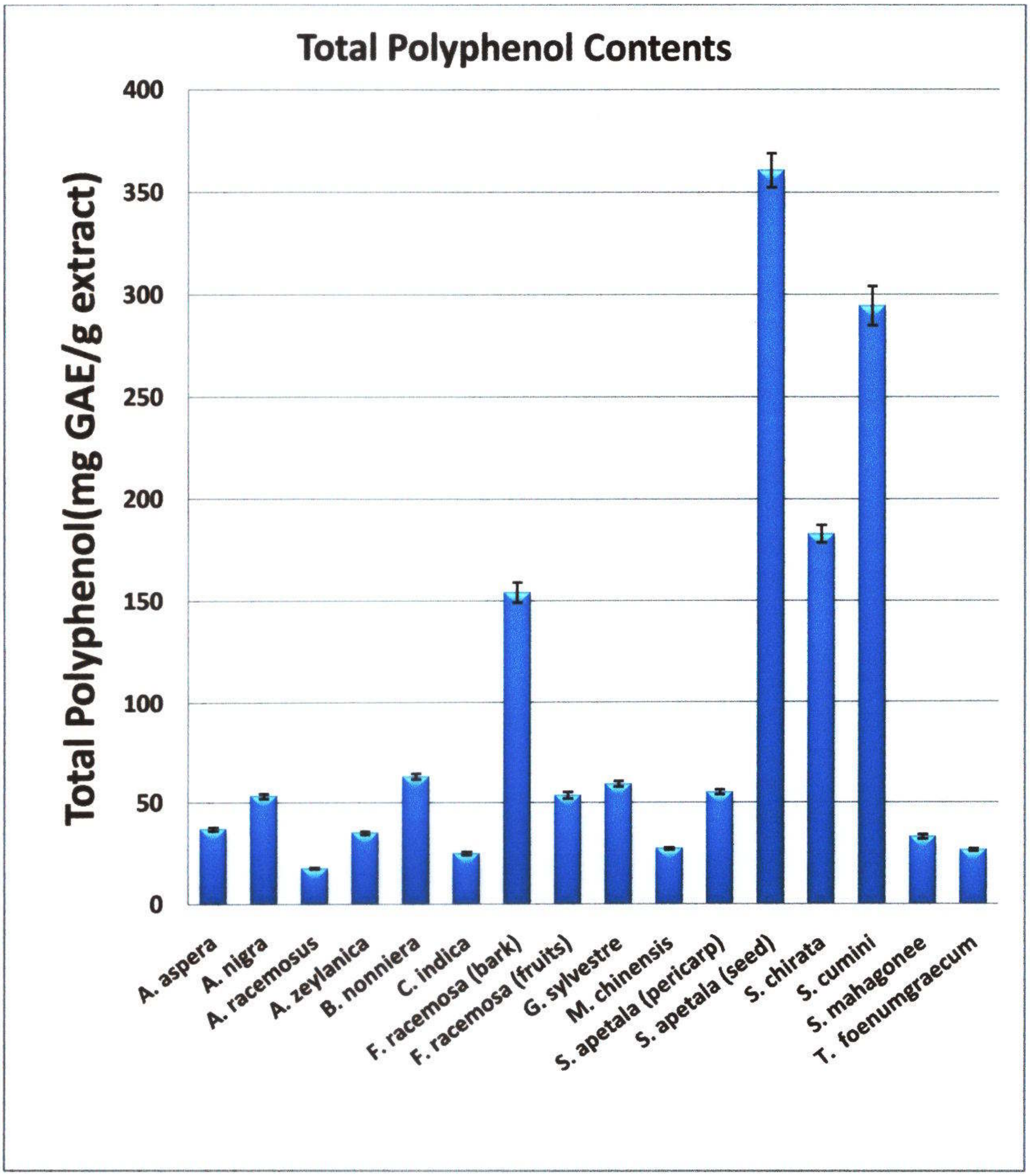


Fig.17: Total polyphenol contents (mg GAE/g extracts) of fourteen medicinal plants commonly used to treat diabetes. GAE= Gallic Acid Equivalent. Values of total polyphenols content are means ± SDs (bars) from three experiments. n=3 or 5.

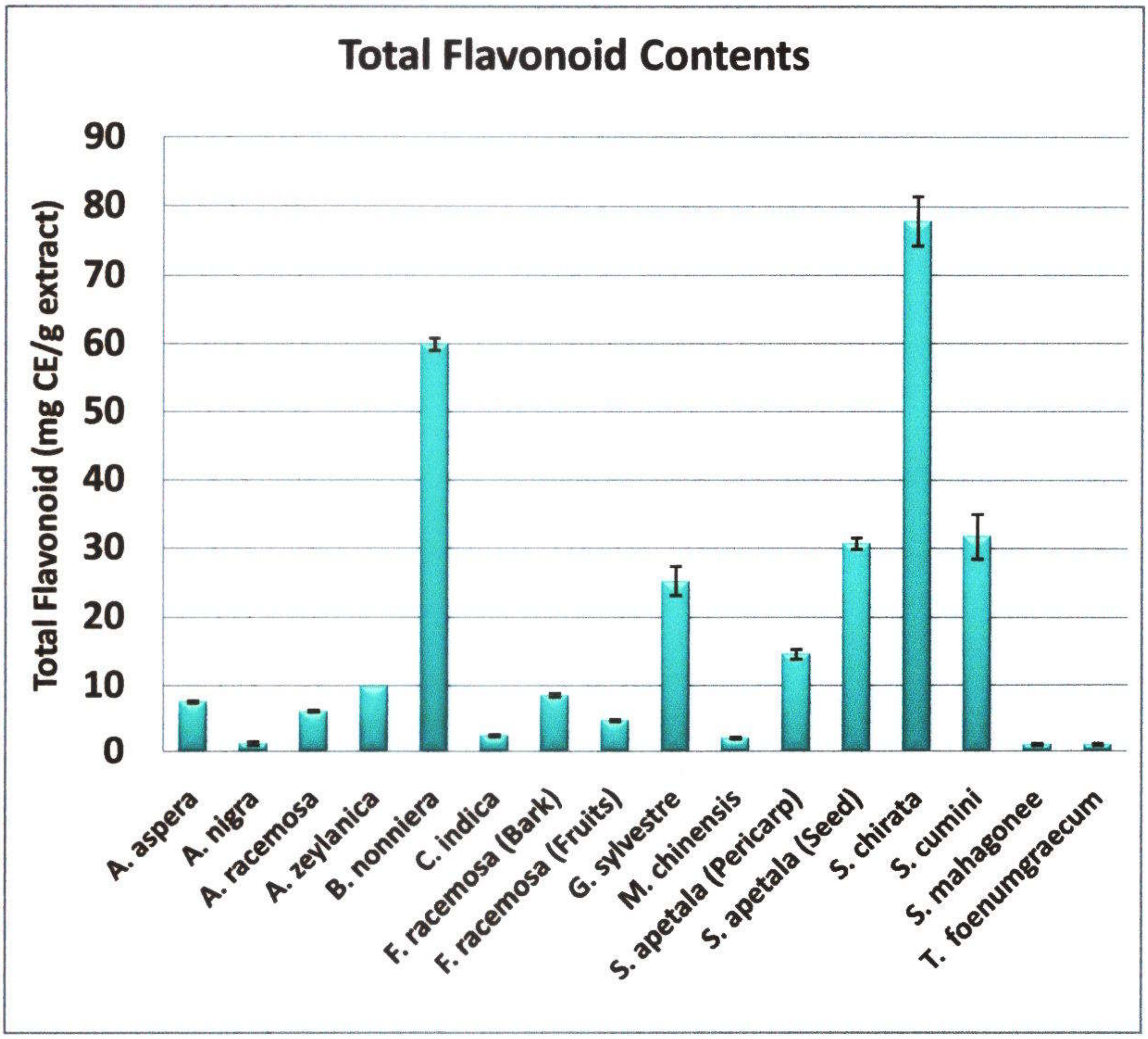


Fig.18: Total flavonoids contents (mg CE/g extract) of fourteen medicinal plants . CE= Catechin Equivalent. Values of total flavonoids contents are means ± SDs (bars) from three experiments.

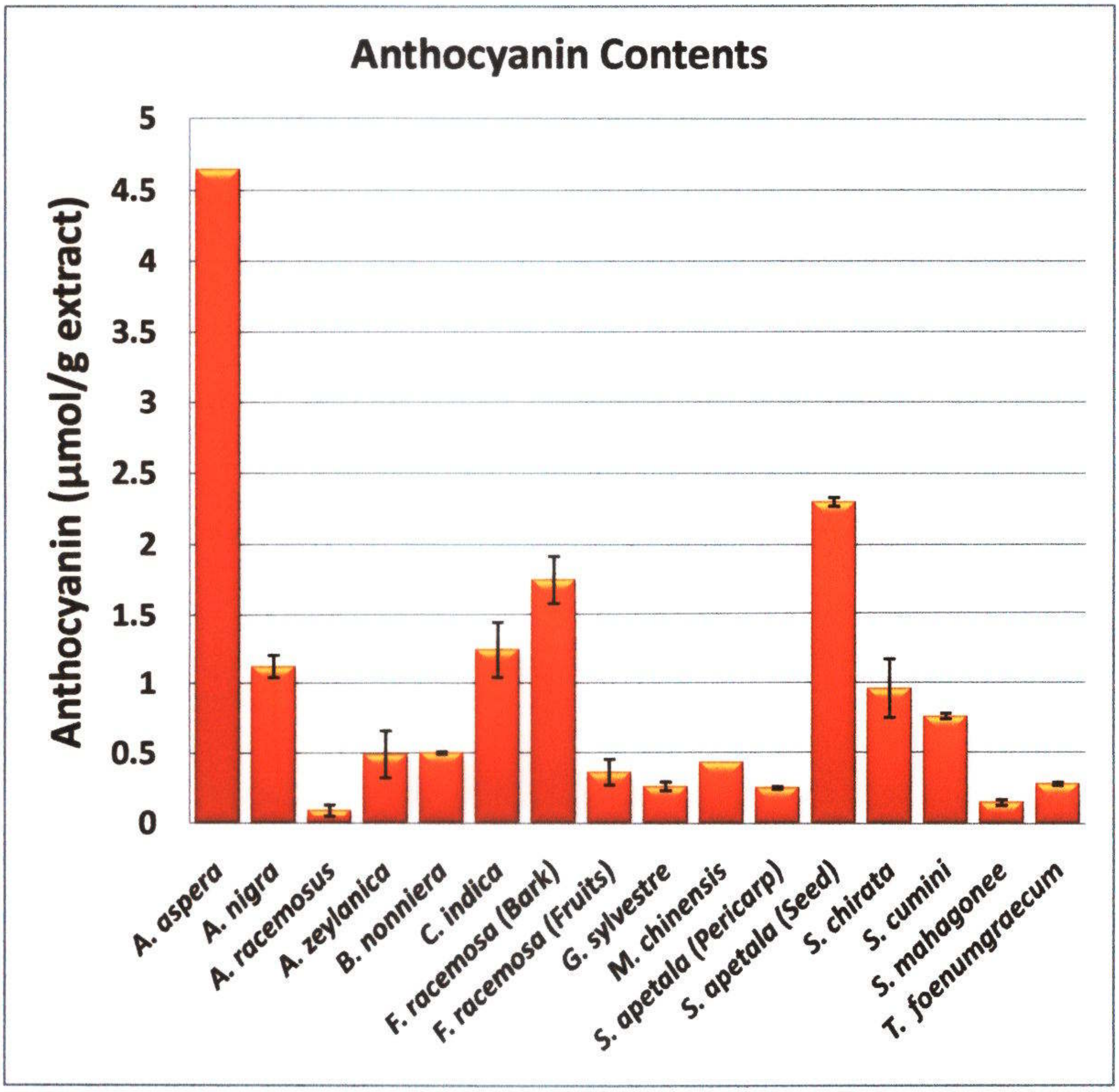


Fig.19: Measurement of anthocyanin contents (µmol/g extract) of fourteen medicinal plants. Results are mean ± SDs (bars).

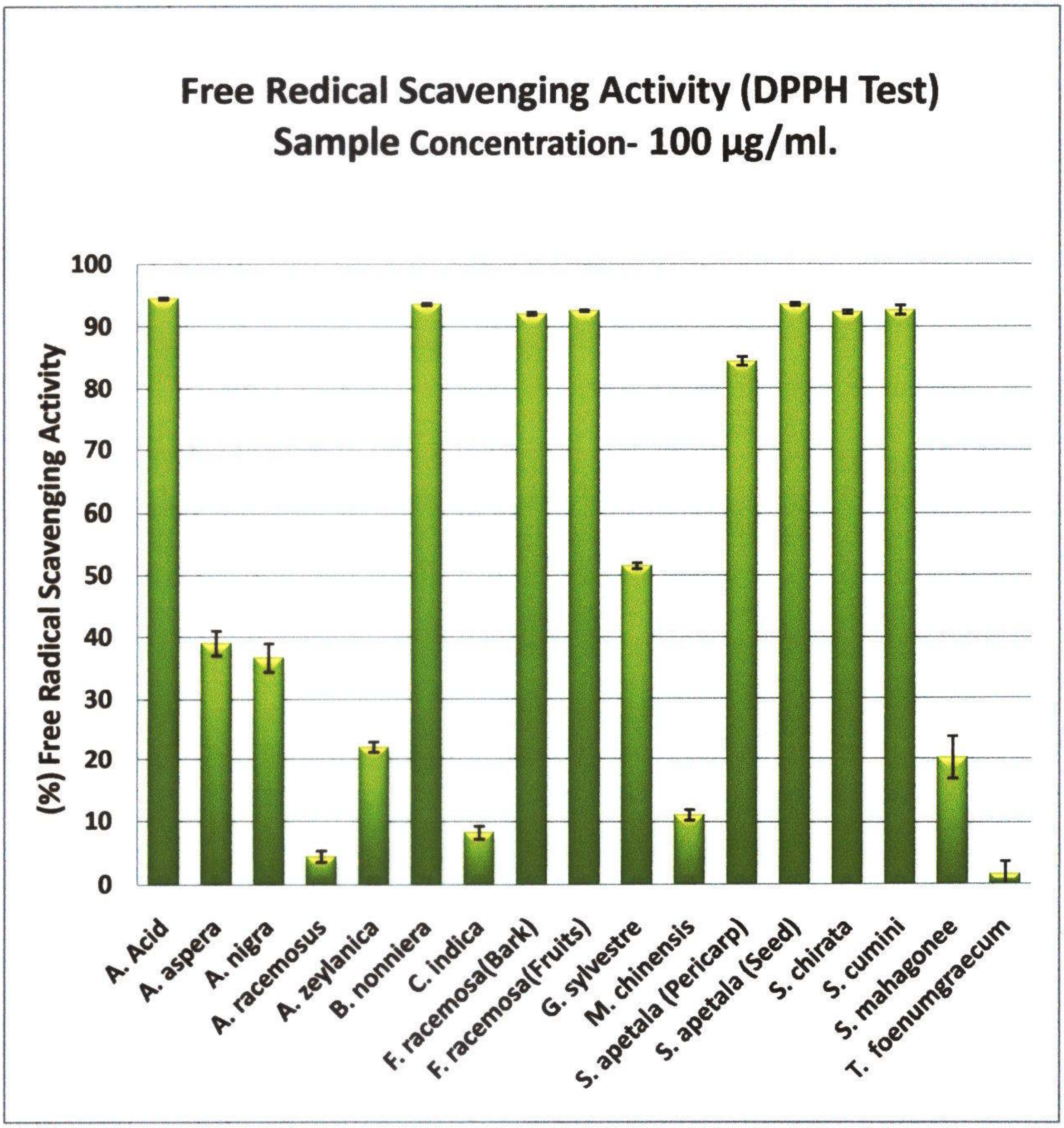


Fig.20: DPPH Free Radical Scavenging activity of the methanolic extracts of fourteen medicinal plants at 100 µg/ml concentration. The data are presented as mean ± SDs (bars) of at least three experiments.

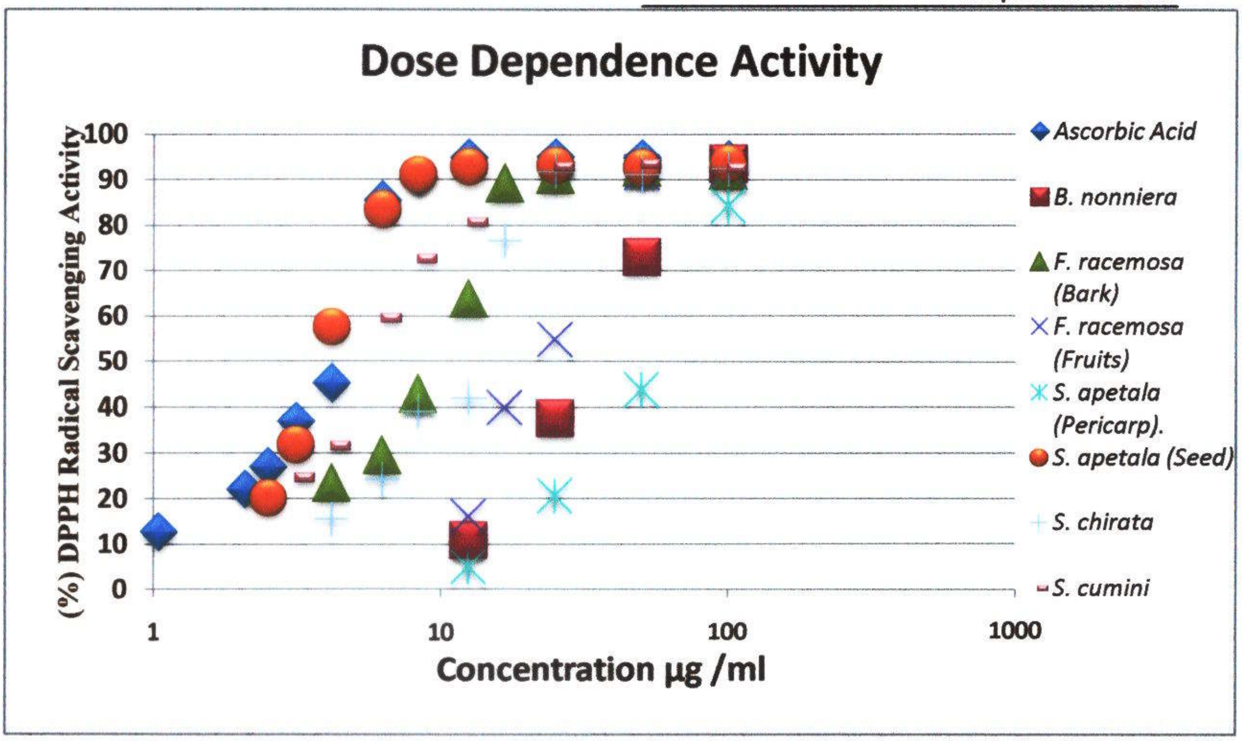


Fig.21: Dose-dependence activity of DPPH free radical scavenging. Data are mean ± SDs (bars), n=3 or 5.

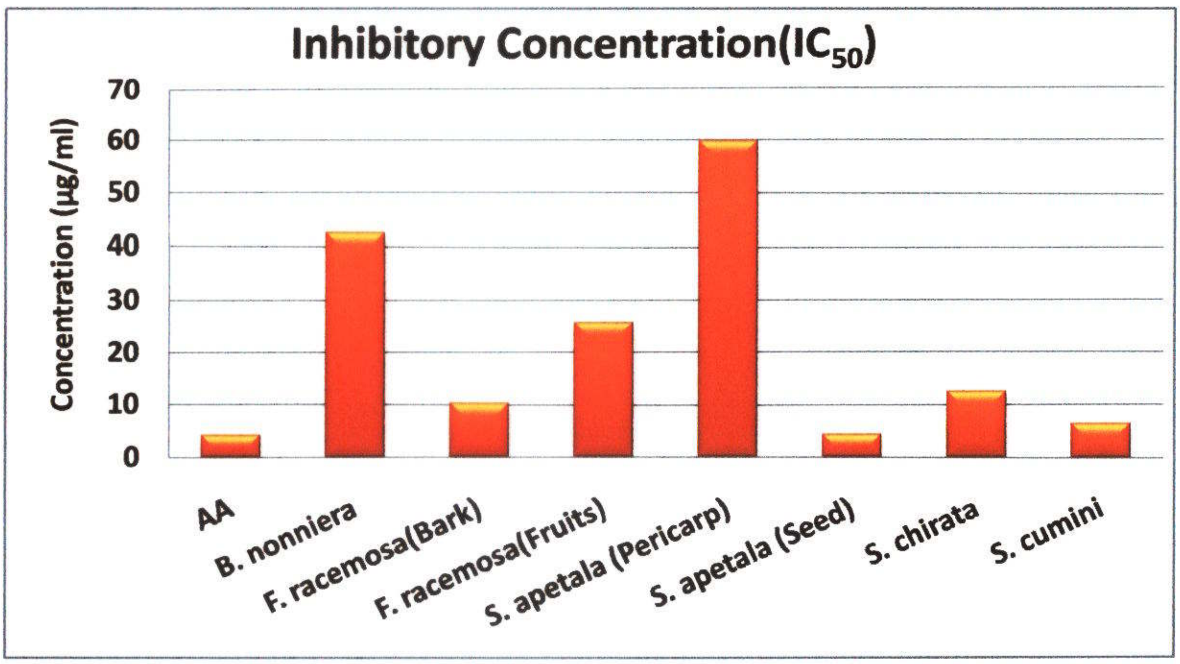


Fig.22: Comparison of IC_{50} values of DPPH free radical scavenging activity. The lowest value shows the highest activity.

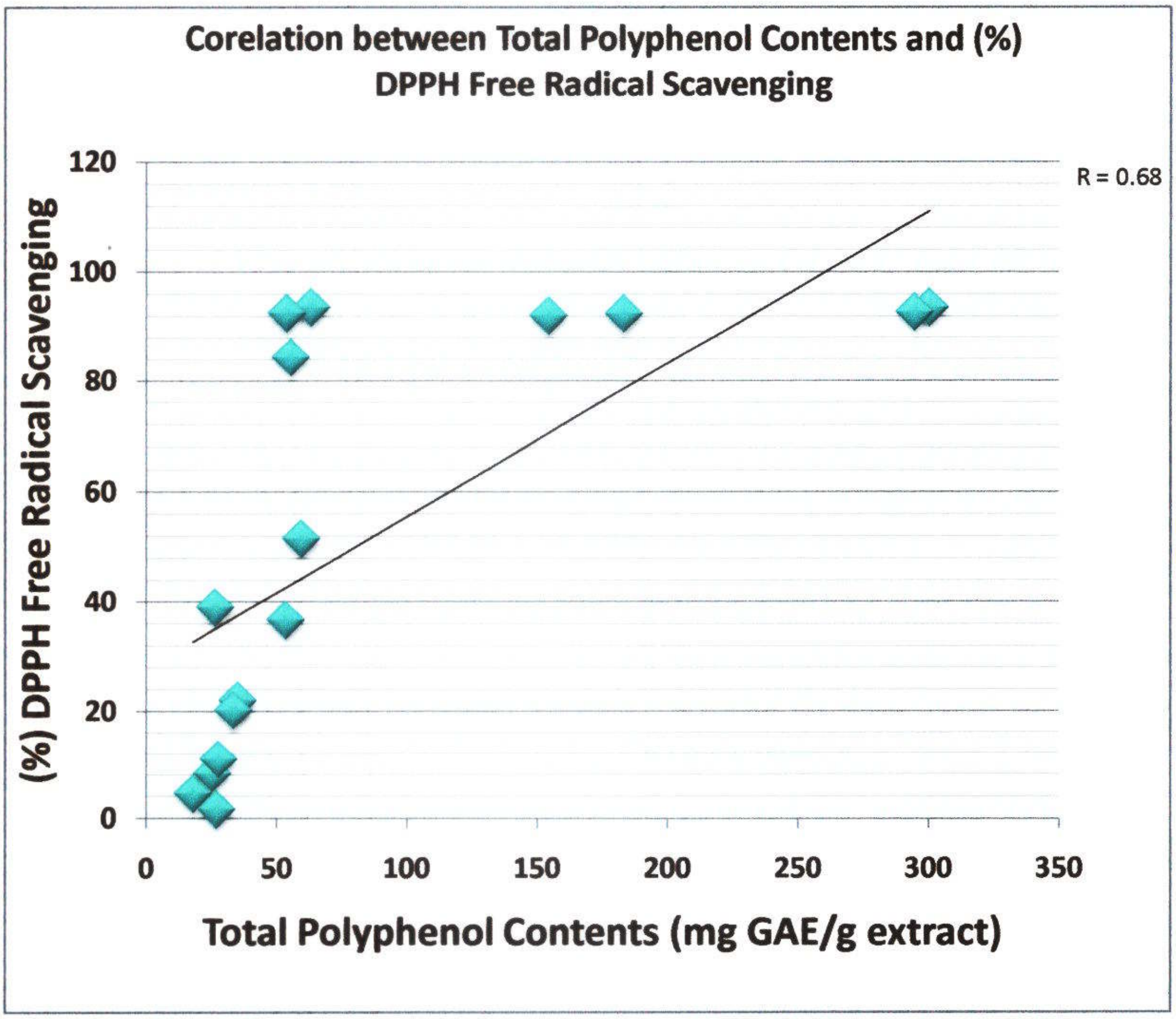


Fig.23: Correlation between total polyphenols content and DPPH free radical scavenging activity at 100 µg/ml of extracts of fourteen medicinal plants commonly used to treat diabetes. (r= ±0.68).

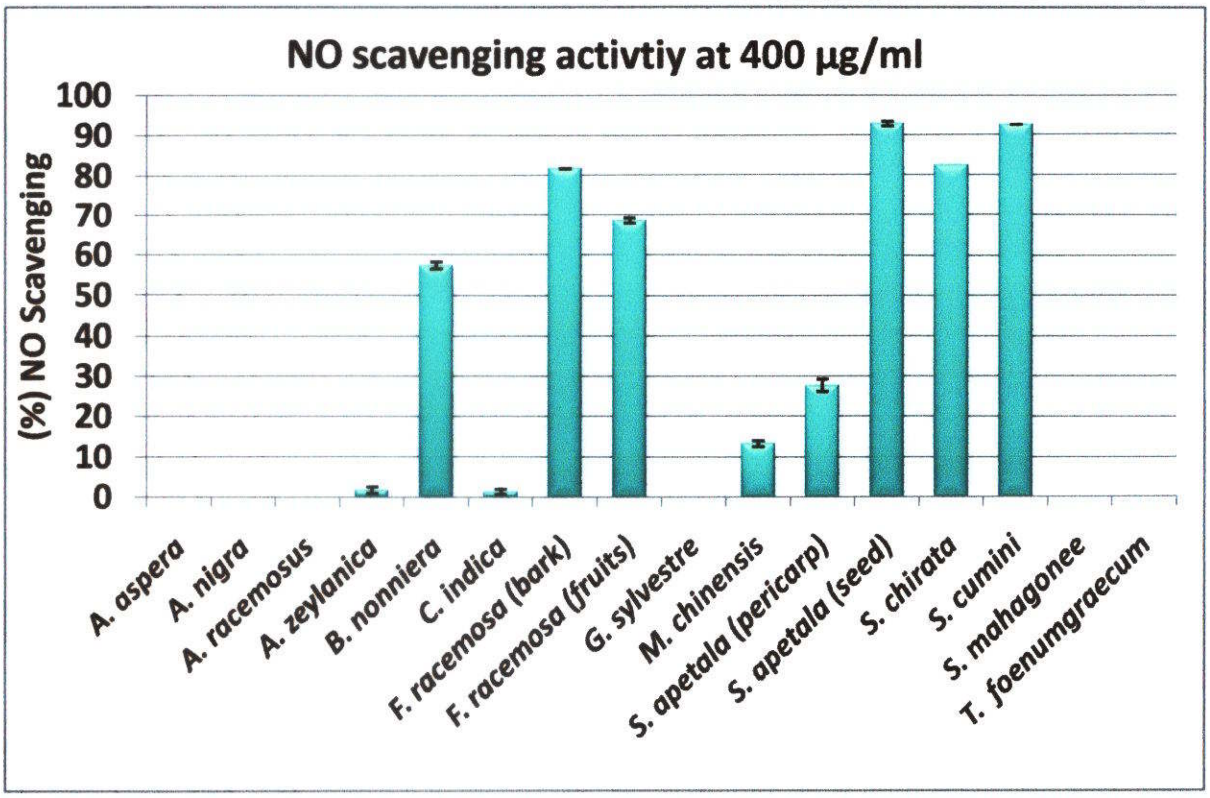


Fig.24: NO scavenging ability of fourteen antidiabetic plants. The data are presented as mean ± SDs of at least three experiments.

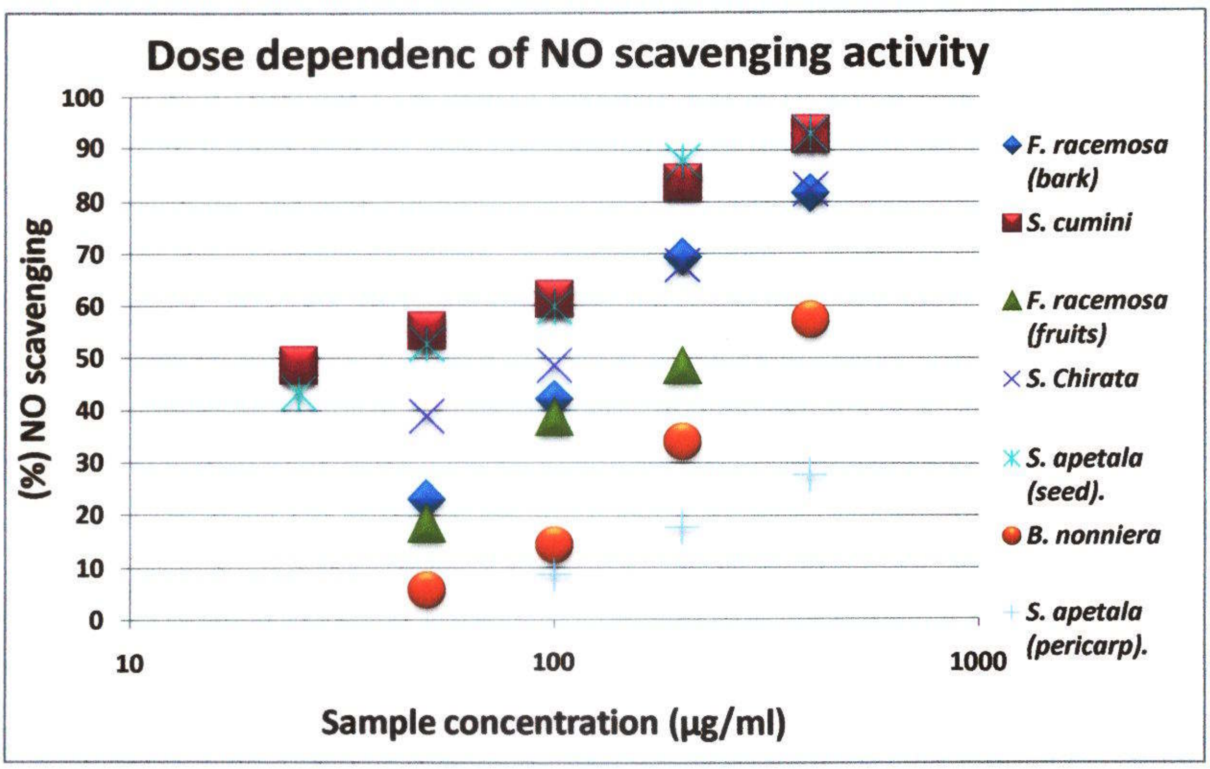


Fig.25: Dose dependence of NO scavenging activity.

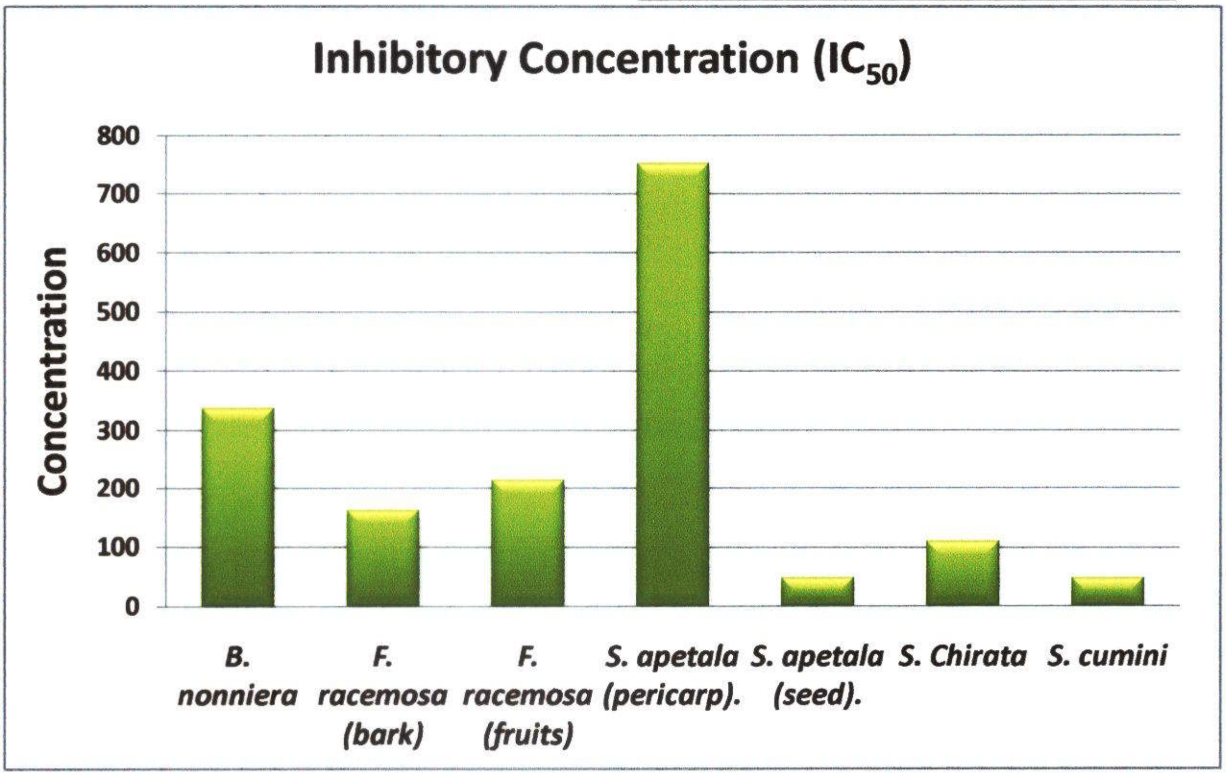


Fig.26: Comparison of IC_{50} values of NO free radical scavenging activity. The lowest value shows the highest activity.

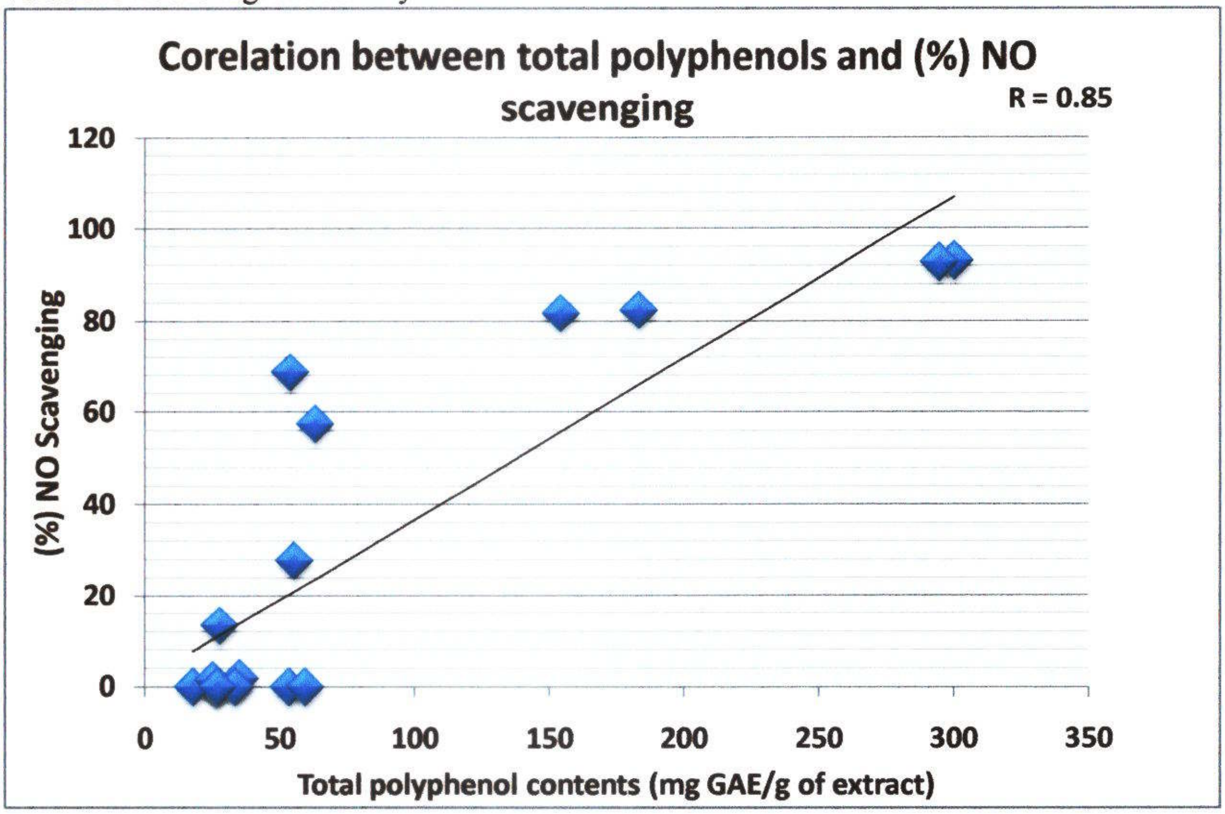


Fig.27: Correlation between total polyphenols content and NO scavenging activity at 400 μg/ml of extracts of fourteen medicinal plants. (r= ± 0.85).

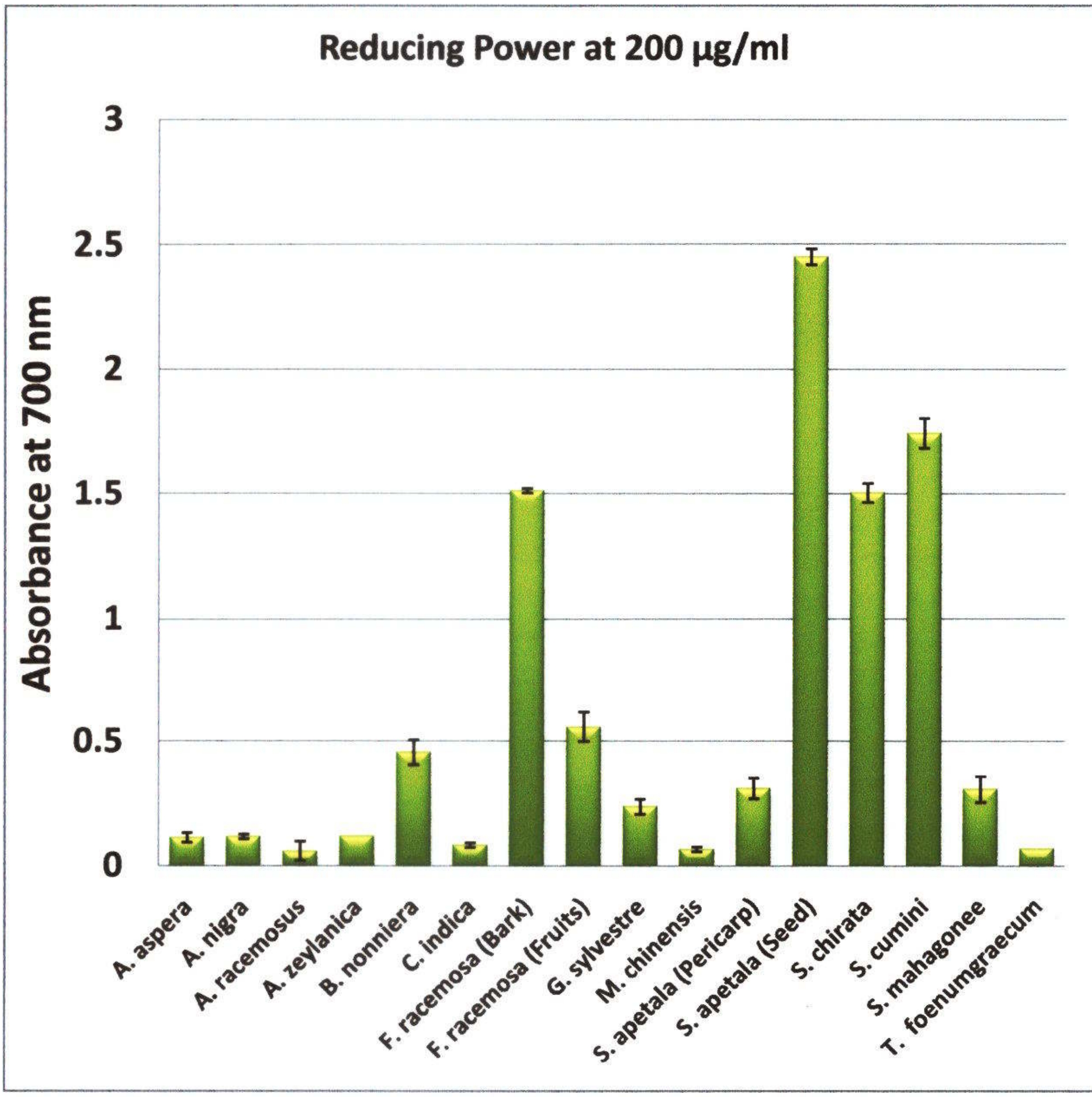


Fig.28: Reducing power of the methanolic extracts of fourteen medicinal plants at 200 µg/ml. The data are presented as mean ± SDs (bars) of at least three experiments, n=3 or 5.

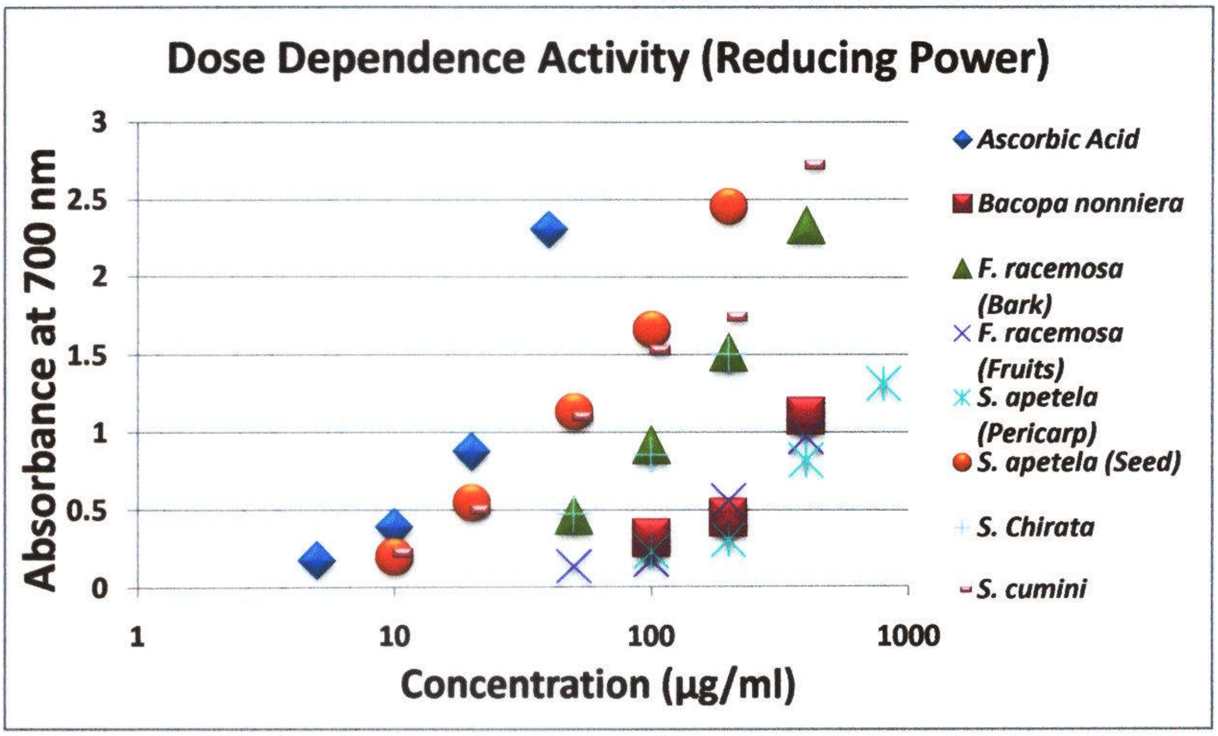


Fig.29: Dose-dependence of reducing power.

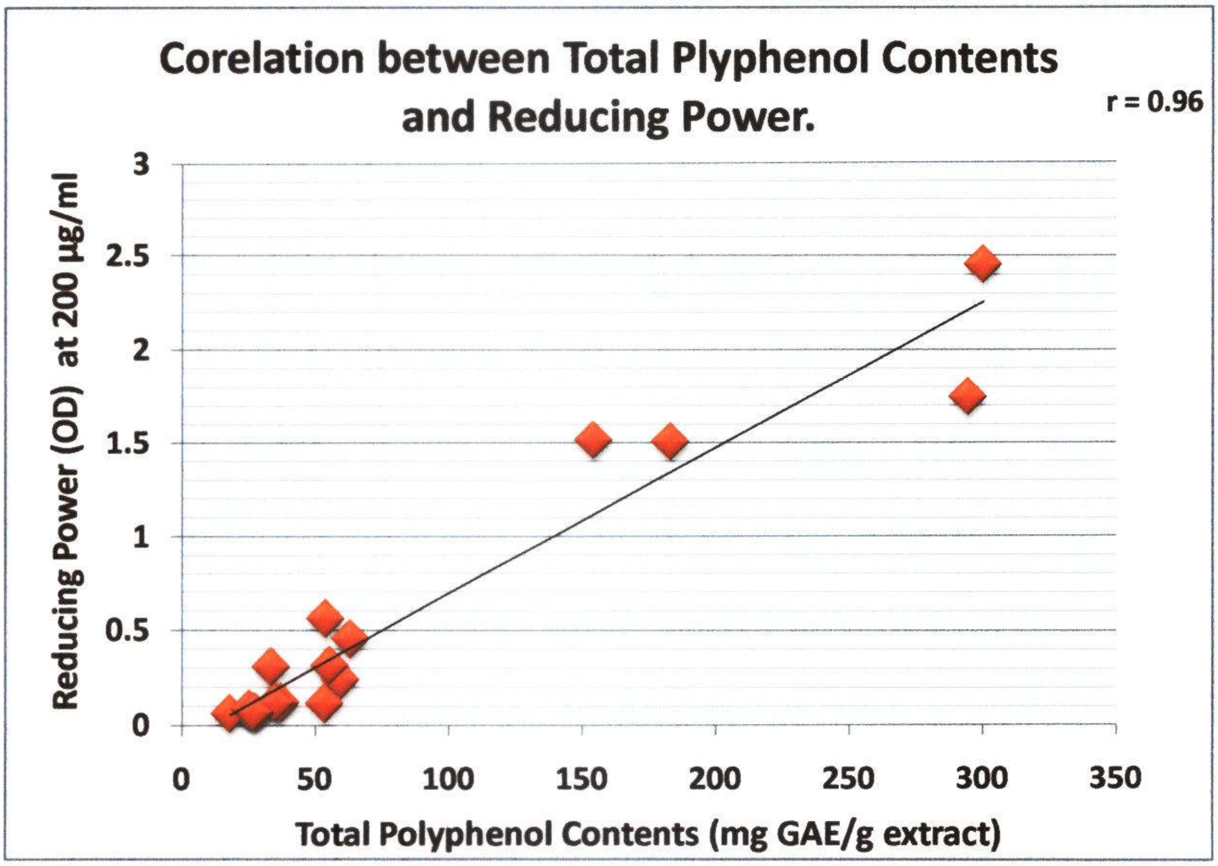


Fig.30: Correlation between total polyphenols and reducing power of fourteen medicinal plants commonly used to treat diabetes.

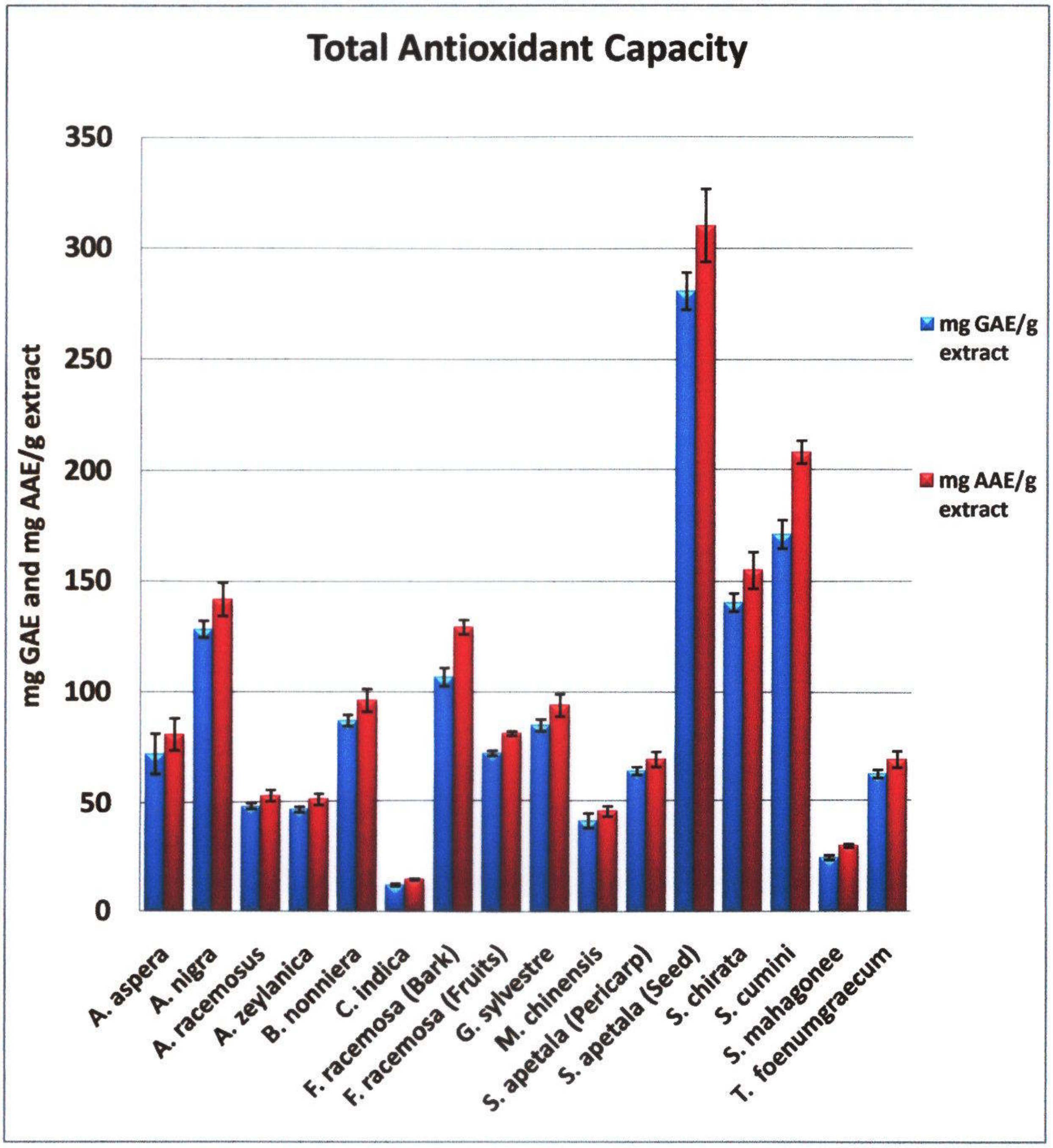
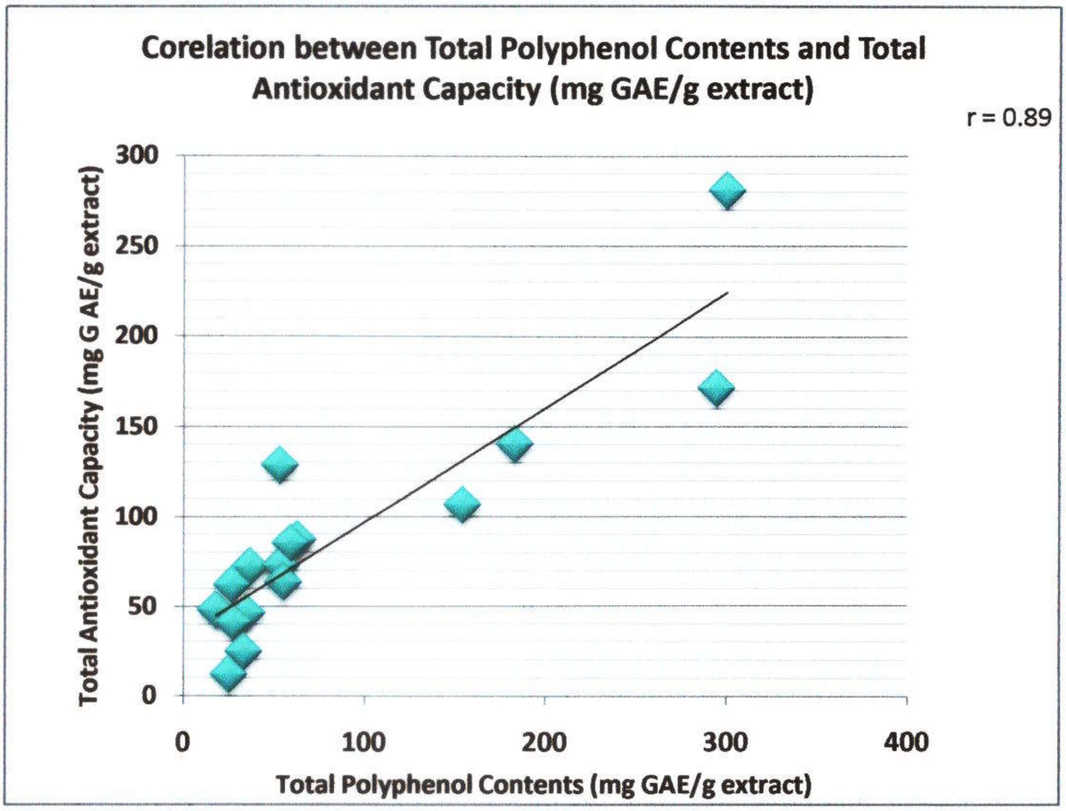


Fig.31: Total antioxidant capacity (mg/g extracts) of fourteen medicinal plants. (GAE: gallic acid equivalents; AAE: ascorbic acid equivalents). Each data represents the mean ± SDs (bars), n=3 or 5.

a.



b.

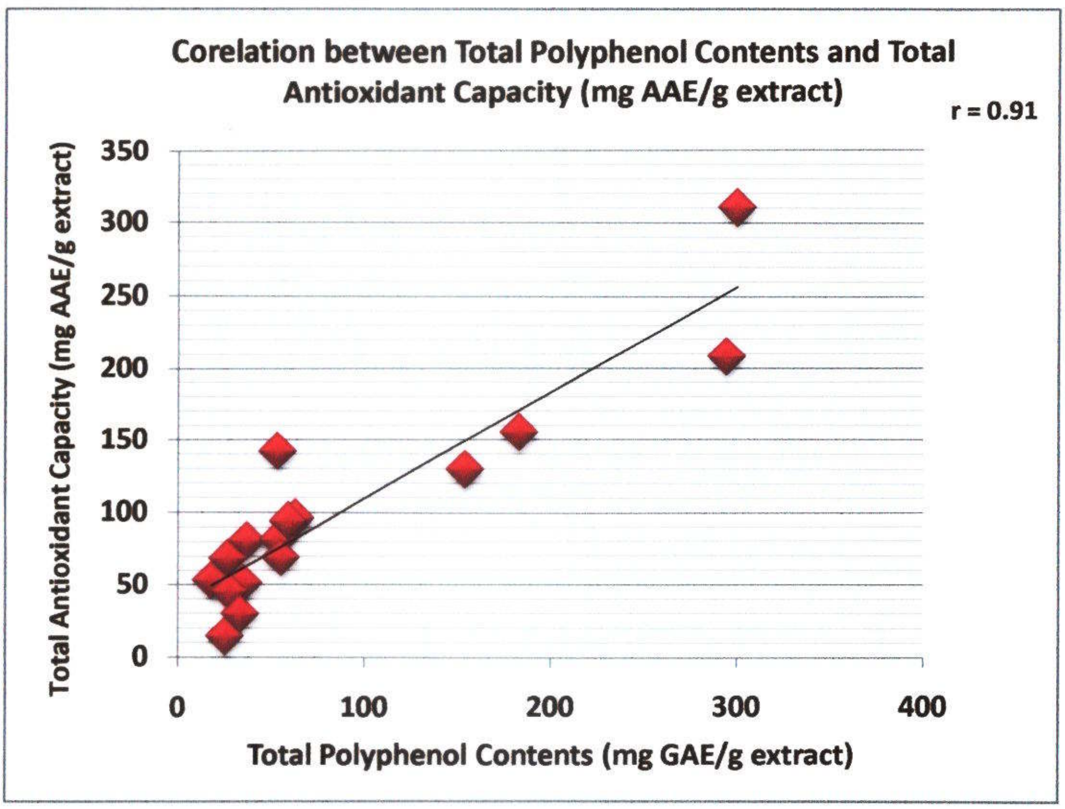


Fig.32: Correlation between total polyphenols and total anti-oxidative capacity of fourteen medicinal plants where r = 0.89 (for a) and 0.91(for b).

CHAPTER: SIX
DISCUSSION

DISCUSSION

Fruits, vegetables as well as other plants part that has a medicinal property also act as natural protective agents against the oxidative stress generated in the human body due to the production of by-products (Reactive Oxygen Species; ROS; RNS etc) of normal metabolic process, pollution and poor environment. Fruits, vegetables as well as other plants that has a medicinal activity containing bioactive compound are known to possess a variety of antioxidant properties (Reşat et al., 2007). Antioxidants intercept the free radical chain of oxidation and donate hydrogen from their phenolic hydroxyl groups, thereby forming a stable end product, that does not initiate or propagate further oxidation.

Polyphenols, flavonoids and anthocyanins etc found in various kinds of plant that may be fruity plants, medicinal plants or vegetables are beneficial functional bioactive compounds. Polyphenols constitute one of the most numerous and ubiquitous groups of plant metabolites, and are an integral part of the human diet. Significant antioxidant, antitumoral, antiviral and antibiotic activities are frequently reported for plant phenols. Flavonols (such as quercetin, anthocyanins, myricetin, kaempherol) and flavones (e.g. apigenin, luteolin) in plant materials are closely associated with their antioxidant function mainly due to their redox properties exerted by various possible mechanisms: free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity (Lotito et al., 2006; Shan et al., 2005). Among the medicinal plants specially this 14 anti-diabetic plants are intake by the patient who has diabetes. In our experiment, it showed that among 14 anti-diabetic plant or plants part, the amount of polyphenol was highest in the extract of *S. apetala* (seed- 300.10 mg GAE/g extract) followed by *S. cumini* (294.46 mg GAE/g extract) and *S. chirata* (183.14 mg GAE/g extract) whereas it was lowest in the extract of *A. racemosus* (17.94 mg GAE/g extract) followed by *C. indica* (25.30 mg GAE/g extract) and *T. foenumgraecum* (25.94 mg GAE/g extract).

It is generally observed from the present study that anti-diabetic plants with higher polyphenols and flavonoids contents exhibited higher anti-oxidative properties e.g. DPPH free radical scavenging activity, reducing power, NO scavenging activity and total antioxidant capacity. DPPH is a stable free radical which has maximum absorption at 517 nm. In the presence of antioxidants, DPPH is reduced to a non radical form and the maximum absorbance at 517 nm is decreased.

DPPH assay is a simple and effective assay to evaluate antioxidant capacity of plant extracts or juices of vegetable, fruits or other plants that having medicinal value. However, the common notion that antioxidant activity strictly correlates with the total phenolic content does not always hold true (Chinnici et al., 2004). Hossain et al., (2009) conducted an experiment on Bangladeshi fruits and correlation between total polyphenol and DPPH radical scavenging activity, reducing power or total antioxidant capacity was 0.73, 0.65 or 0.79 respectively. In our study, correlation between total polyphenol and DPPH radical scavenging activity, NO scavenging activity, reducing power and total antioxidant capacity was 0.68, 0.85, 0.96 and 0.89 or 0.91 (GAE or AAE) respectively. Probably unknown components other than polyphenols present in the extracts of anti-diabetic plants might contribute or interrupt in part to their antioxidant activity. Besides, due to complexity of oxidation-anti-oxidation process, no single testing method is capable of providing a complete anti-oxidative profile (Parejo et al., 2002). Therefore a multi-method approach is required to asses a complete anti-oxidative activity.

As plant polyphenols are aromatic hydroxylated compounds, they are good source of electron donors and show reducing power (Ruch et al., 1984). Various oxidase enzymes, such as superoxide dismutase produce toxic H_2O_2 *in vivo.* It has detrimental effect on cells for the production of hydroxyl radical ($OH^\cdot$), which oxidizes a number of molecules including DNA, RNA. Thus, removing H_2O_2 is crucial to protect cell components from oxidation (Hossain et al., 2009). From our experimental observation, it was revealed that *S. apetala* (seed and pericarp), *S. cumini* , *F. racemosus* (bark and fruits) and *S. chirata* have very high reducing power, DPPH radical scavenging activity as well as NO scavenging activity. Their TAC (total anti-oxidant activity) was also high. So these above mentioned medicinal plant among the experimental anti-diabetic plant can be potential natural sources for alleviating the deleterious effect of H_2O_2, NO, HOCL, O_2^-, OH^- or other free radicals e.g. ROS, RNS by reducing it to H_2O.

Evidence is mounting for a role of dietary phytochemicals, including flavonoids, ascorbic acid, a tocopherol, and carotenoids, in the maintenance of health and protection from diseases. The epidemiological evidence for a protective effect of natural food and herb antioxidants against cancer, coronary heart disease, age-related macular degeneration, respiratory disease, cystic fibrosis, immune-disease, and Alzheimer's disease is impressive. Numerous surveys have shown an inverse relationship between the intake of vegetables, fruits or other plants that have medicinal value with high polyphenols and the incidence of those disorders (Ing-chien chen et al, 2004).

Patient having diabetes if consumes the anti-diabetic plant or plant part containing high polyphenol, flavonoids and anthocyanin in our country as well as the people of all over the world in lieu of allopathic drug as their diabetes treatment, with reducing blood glucose level it also may halt the intensity of the commencement of various diseases such as cancer, inflammation, atherosclerosis, hepatitis, aphrodisiac, Alzheimer's diseases, Parkinson's diseases, ageing, stroke, heart diseases etc.

CHAPTER: SEVEN
CONCLUSION

CONCLUSSION

Reportedly, with increasing the consumption/utilization of health promoting compound like polyphenol, flavonoids and anthocyanins, increased anti-oxidative activity *in vitro* or *in vivo* and thereby decreasing the intensity of cancer, aging, neurological diseases, inflammation, allergic problem, diabetes and bacterial infections. Besides, A polyphenol may affect cell-to-cell signaling, receptor sensitivity, inflammatory enzyme activity or gene regulation (Virgili and Marino, 2008). In our research work, fourteen most common Bangladeshi medicinal plants which have a common use to treat the diabetes were investigated to know the existence of beneficial functional bioactive components and antioxidant activity. The results of the experiments revealed that *S. apetala* (seed), *S. cumini, S. chirata* and *F. racemosa* (bark) are potential sources of bioactive compounds-polyphenols, flavonoids, anthocyanins, antioxidants and these medicinal plants therefore have activities beneficial to health. However, further *in vivo* and *in vitro* studies are necessary to know their mechanism of action and potential active compound(s). For knowing well efficiency of anti-diabetic plant, anti-diabetic test is also necessary. It is also required to elucidate potential cytotoxic effects when they are used for the preparation of dietary supplements to the diabetic patient, as some polyphenols perturb the membrane structure (Hossain et al., 2002; Aoshima et al., 2005). Moreover, fractionation of potential extracts is essential to know the phenolic or nonphenolic compounds responsible for the anti-oxidative activities.

CHAPTER: EIGHT
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APPENDIX

1. Arithmetic Mean ($\overline{X}$) = $\frac{\Sigma X}{n}$

Where, ΣX = Summation of Observed Value

n = No. of Observation

2. Standard Deviation (SD) = $\sqrt{\frac{\Sigma(X-\overline{X})^2}{n}}$

Where, X = individual Value

$\overline{X}$ = Mean Value

n = No. of Observation

3. Standard Error (SE) = $\frac{SD}{\sqrt{(n-1)}}$

Where, SD = Std. Deviation

n = No. of Observation

Table 1: Dietary antioxidants: source, bioavailability, and concentrations in human plasma.

Dietary Antioxidants	Dietary source	Bioavailability	Concentration	Comment
Ascorbic acid (vitamin C)	Fruits and vegetables, particularly strawberries, citrus, kiwi, Brussels sprouts, and cauliflower	100% at low doses (<100 mg) decreasing to <15% at >10 g	25–80 mmol l^{-1}	Unstable at neutral pH, concentrated in cells and the eye
Vitamin E (in humans mainly α-tocopherol)	Green leafy vegetables, e.g., spinach, nuts, seeds, especially wheat germ, vegetable oils, especially sunflower	10–95%, but limited hepatic uptake of absorbed tocopherol	15–40 mmol l^{-1} (depending on vitamin supply and lipid levels)	Major tocopherol in diet is γ form, but α form is preferentially taken up by human liver
Carotenoids (hundreds)	Orange/red fruits and vegetables (carrot, tomato, apricot, melon, yam), green leafy vegetables	Unclear, dose and form dependent, probably <15%	Very low (<1 mmol l^{-1})	Lutein and zeaxanthin are concentrated in macula region of the eye
Flavonoids (enormous range of different types)	Berries, apples, onions, tea, red wine, some herbs (parsley, thyme), citrus fruits, grapes, cherries.	Most poorly absorbed, quercetin absorption 20 50%, catechins <2%, dependent on form and dose	No data for most, likely <3 mmol l^{-1} in Total	Quercetin and catechins may be most relevant to humans health as intake is relatively high, there is some absorption, possible gastrointestinal-tract protection by unabsorbed flavonoids

Source: The Encyclopedia of Human Nutrition, 2005.

Table 2: Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids.

Antioxidant	**RDA (adults)**	**Upper Level (adults)**	**Comment**
Vitamin E	15 mg	1070 mg natural vitamin E 785 mg synthetic vitamin E	Higher amounts impair blood clotting, increasing likelihood of hemorrhage.
Vitamin C	Women: 75 mg Men: 90 mg	2,000 mg	Higher amounts could lead to diarrhea and other GI disturbances. Extremely high levels may lead to cancer, atherosclerosis, and kidney stones.
Beta-carotene	None	None	Chronic high doses turn your skin yellow-orange, but it is not toxic. However, research indicates it is unwise to consume doses of beta-carotene beyond what is in a multivitamin and your regular diet.
Selenium	55 micrograms	400 micrograms	Higher amounts could cause hair loss, skin rashes, fatigue, GI disturbances, and nervous system abnormalities.

Source: The Food and Nutrition Board of the Institute of Medicine, 2000; America.

Table 3: Application of Antioxidants.

Antioxidant	Strengthens	Helps Prevent	Other	Found In
Lipoic Acid	Memory, energy production, controls adult diabetes	Heart disease, cancer, stroke, cataracts	Recycles all five of the network antioxidants	Potatoes, spinach
Vitamin E	Increases life span, helps clear clogged arteries, protects skin, relieves arthritis	Heart disease, stroke, Alzheimer's, skin, prostate and breast cancer	Need all 4 tocopherols and 4 tocotrienols. "d-alpha" is natural, "dl-alpha" is synthetic	Vegetable oils, nuts, grain, tocotrienols in bran
Vitamin C	Immune system, recycles vitamin E	Cancer, heart disease, scurvy	More than 300 mg per dose is excreted	Red peppers, broccoli, cranberries, citrus
CoQ10	Rejuvenates brain cells	Heart disease, gum disease	Has been used for 20 yrs in Japan to treat and prevent heart disease	Seafood and organ meat
Glutathione	Detoxifies body, strengthens immune system, prolongs life	Prevents bronchitis and pneumonia	Takes 100 mg of lipoic acid for body to make it. It is the cell's primary antioxidant	Fresh fruits and vegetables
Flavonoids	Improve memory and concentration, lowers blood pressure	Blood clots, LDL cholesterol in arteries, reduces inflammation	Boosts effectiveness of vitamin C, found with it in nature	Citrus fruits, apples, onions, red grapes, berries, pine bark
Carotenoids	Converts to Vitamin A if needed, good for eyes and vision	Cancer, lycopene for prostate cancer, lutein for cataracts, heart attack	Give the color to yellow, orange, red, purple and dark green vegetables	The colorful fruits and vegetables: Alpha carotene is orange and in carrots, lutein is the green in spinach, lycopene in red tomatoes.
Selenium	Synergistic effect with vitamin E	Lung, prostate and colon cancer, stroke and heart disease	Trace mineral needed for formation of two antioxidants	Garlic, onion, broccoli, whole grains, red grapes, egg yolks

Source: The Antioxidant Miracle; Packer and Carol Colman, 1999.