Phytochemical determination and antibacterial activity of *Trichosanthes dioica Roxb* (Patal), *Cucurbita Maxima* (pumpkin) and *Abelmoschus esculentus Moench* (Okra) plant seeds

Dissertation submitted in partial fulfillment of the requirements for the Degree of

Master of Science in Life Science

By

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CERTIFICATE

This is to certify that the project report titled **"**Phytochemicals determination and antibacterial activity of *Trichosanthes dioica Roxb* (patal), *Cucurbita maxima* (pumpkin) and *Abelmoschus esculentus Moench* (okra) plant seeds" submitted by Mr. Karmjit Singh to the department of Life Sciences, National Institute of Technology, Rourkela in partial fulfillment of the requirements for the degree of Masters of Science in LIFE SCIENCES is a bonafide record of work carried out by him under my supervision. The contents of this report in full or parts have not been submitted to any other Institute or University for the award of any degree or diploma.

Dr. Bismita Nayak

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DECLARATION OF CANDIDATE

The work embodied in this report is an original investigation carried out by me, on the topic entitled, "Phytochemicals determination and antibacterial activity of *Trichosanthes dioica Roxb* (patal), *Cucurbita maxima* (pumpkin) and *Abelmoschus esculentus Moench* (okra) plant seeds", for partial fulfillment of degree in Master of Life Science, NIT Rourkela. To the best of my knowledge and belief, this work has not been submitted to any other University or Institution to confer any Degree or Diploma.

Date:

Mr. Karmjit Singh
Master of Life Science

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KARMJIT SINGH

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C. ABSTRACT

Phytochemical is non nutritive chemical constituents of plants which occur naturally in it *i.e.* termed as Phytochemical, or the chemical which is derived from plants are called Phytochemical. The herbal products today symbolize shelter in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been used for their medicinal, flavoring and aromatic character, over three quarters of the world population believes mainly on plants and plant extracts for health trouble. As population is increasing to a higher side day by day, there are not enough supply of drugs, there are excessive cost of treatments or curing, side effects of several allopathic drugs and development of resistance to at present used drugs for infectious diseases have led to increased importance on the use of plant materials as a source of medicines for a wide variety of human ailments. *Trichosanthes dioica Roxb* (Parwal) is a summer vegetable crop belonging to the family Cucurbitaceae. *Cucurbita Maxima* (Pumpkin) belongs to the genus of *Cucurbita* and the family Cucurbitaceae and it is monoecious. Okra its botanical name is *Abelmoschus esculentus Moench*. It is a flowering plant and belongs from family mallow Okra valued for its edible green seed pods. Various phytochemical methods were used for extract the medicinal compounds present in *Trichosanthes dioica Roxb*, *Cucurbita Maxima* and *Abelmoschus esculentus Moench*. Plants phytochemical components like Tannins, Saponins, Phlobatannins, Terpenoids, Flavonoids, Glycosides, steroids etc are used in many pharmaceutical and drugs fields. These are the either drugs from any plants material that destroy or inhibit the growth of bacteria act as chemotherapeutic agents. It has also the ability to prevent or treat bacterial infections. In my study, we had chosen the *Bacillus subtilis, Pseudomonas fluorescens, Escherichia coli, Staphylococcus aureus* and *Klebsiella Pneumonia* bacteria for testing the antibacterial activity by the plants seed of *Trichosanthes dioica Roxb, Cucurbita Maxima* and *Abelmoschus esculentus Moench* in aqueous medium

Keywords- *Trichosanthes dioica Roxb* (Parwal), *Cucurbita Maxima* (pumpkin), *Abelmoschus esculentus Moench* (Okra), pharmaceutical drug

1. INTRODUCTION

In nature many plants and plants seed provided source of medicine at the earlier times. Plants have proven to be the most useful in curing diseases and provide an important source of pharma and medicine. Plants have great significance to the health of individuals. The medicinal importance of these plants lies in some chemical substances that produce a distinct physiological action on the body of human. The major importance of these bioactive constitute of plants are Steroid, Terpenoids, Tannins, Carotenoids, Flavonoids, Alkaloids and Glycosides. Plants in all aspect of life have served as important material for drug development. Antibiotic and Antimicrobial components like Saponins, Glycosides, Flavonoids and alkaloids are found in plants. Medicinal plants are the foundation of many important drugs of the modern world. Plants are now playing an important role in many medicines like allopathic medicine, herbal medicine, homoeopathy and aromatherapy. Many of these local medicinal plants are used as spices and food items. Many plants are cheaper and more simply to get to most people especially in the developing countries and these plants have lower incidence of side effect after use. Due to this reason they are used worldwide. The medicinal properties and other properties of some plants have been recognized by various researchers. In the current study, we have chosen three plant's seeds such as *Trichosanthes dioica Roxb* (patal), *Cucurbita Maxima* (pumpkin) and *Abelmoschus esculentus Moench* (Okra) taken for phytochemical analysis study as well as antimicrobial activities.

Pumpkin is gourd-like squash belongs to genus Cucurbita and the family Cucurbitaceae. It normally belongs to the species *Cucurbita pepo, Cucurbita mixta, Cucurbita maxima*, and *Cucurbita moschata* and its native is North America. They naturally have a thick, orange or yellow shell. Pumpkins are broadly grown for commercial use, and are used both in food and recreation. In India, it is most consuming vegetables. Pumpkins are considered to be a fruit and it contains 90 percent water. Pumpkins have antioxidant beta-carotene, which help to improve the immune function and can reduce the risk of diseases like heart disease and cancer. In Australia 'pumpkin' is generally called as winter squash, and its botanical name is *Cucurbita Maxima*. The term pumpkin derived from the word Greek word *pepon* that means "large melon" and later American changed it to the word, "pumpkin". Pumpkins are a squash-like fruit that range in between 9-18 lbs (4-8kg) to 75 lbs (34kg). Pumpkin stems are rigid, spiky, and angular than

squash stems, which are generally softer, more rounded where joined to the fruit. Pumpkins are generally orange or yellow, some pumpkin fruits are dark green, pale green, orange-yellow, white and gray. It is monoecious plant and it has both male and female flowers on the same plant. Pepita is the term use in Spanish word for the pumpkin seed. The seeds are characteristically flat and asymmetrically oval, and light green in color inside pumpkin.

Trichosanthes dioica Roxb is called as the pointed gourd, in Hindi Parwal; in Bengali it is called Patol. Pointed guard is commonly cultivated in the eastern part of India, mostly in Bihar Orissa, Bengal, Assam, and Uttar Pradesh. It is a good source of carbohydrates, vitamin A, and vitamin C. Pointed guard is perennial. It is a dioecious plant with heart-shaped leaves and is grown on a lattice. The fruits of pointed guard are green. The size of patal fruits is of 2 to 6 inches. Patal is grown through cuttings shoot tips, seeds don't give the impression to do well. Lady's Finger or Okra its botanical name is *Abelmoschus esculentus Moench*, lady's finger is a flowering plant belong from mallow family. The geographical distribution of okra is South Asian, Ethiopian and West African origins. The lady's finger plant is grown in tropical, subtropical and warm regions around the world. The plant is an annual perennial, rising to 2 m tall. The leaves of lady's finger are 10–20 cm long and wide. The flowers of this plant are 4–8 cm in diameter. The fruit is pod up to 18 cm long, containing many seeds.

Phytochemical are non nutritive chemical compounds which occur naturally in plants are called phytochemicals, or the chemical which is derived from plants called phytochemical. Phytochemical words came from Greek word *Phyto*–plant and chemicals. The term phytochemical is generally used to those chemicals that may have biological importance but are not established as important nutrients. In a narrower sense the terms phytochemical describe the number of secondary metabolic compounds found in plants. Scientists estimate that about 10,000 different phytochemicals having the capability to have an effect on diseases like cancer and metabolic syndrome etc.

Antibacterial activity is method to destroying or suppressing the growth or reproduction of bacteria. The term antibacterial terms derives from Greek word "anti" that means against. The compound which destroys or suppresses the growth or reproduction of bacteria, and that type of compound or agent having such properties is called antibacterial agent or antibacterial compounds. These are the either drugs or any plants material that destroy or inhibit the growth of

bacteria, chemotherapeutic agents also having ability to prevent or treat bacterial infections. The main purpose of my study is to screen secondry metabolites that are phytochemical analysis and to estimate the antibacterial activity of plants seed of *Trichosanthes dioica Roxb*, *Cucurbita Maxima* and *Abelmoschus esculents Moench*. The phytochemical is done to screen Tannin, Alkaloids, flavonoids, Saponins phlobatannins, Terpenoids, cardiac glycosides, anthraquinones, Carotenoids, Reducing sugar. Antibacterial activity is done *in vitro* by agar cup diffusion method by using distilled water as solvent and aqueous extract are use against bacteria eg., *Escherichia coli, Klebsiella Pneumonia*

2. REVIEW AND LITERATURE

In estimation, the plant were used to cure the diseases and infections during ancient time. The World Health Organization supports the use conventional medicine provided they are established to be effective and safe (WHO, 1985). In developing countries, a large number of people lives in extreme lower condition and some are suffering and dying for want of protected water and medicine, they have no option for primary health care (WHO, 1985). Consequently, they require using medicinal plants as alternatives to traditional medicines in the provision of primary health care can be overemphasizing. Herbal medicines have recognized much attention as sources of lead compounds since they are measured as time tested and relatively safe for both human use and environment friendly (Fazly Bazzaz et al., 2005). Medicinal plants are cheap, easily available and affordable. Many medicinal plants are maintained to be useful for wound healing in the conventional system of medicine. These medicinal plant remedies are used since ancient times even if the mechanisms of action, toxicity and competence of very few of them have been evaluated scientifically. Wound healing is the method of repair the injury of the skin and other tissues. Subsequent injury, an inflammatory response occurs and the cells under the dermis begin to increase protein (Collagen) production. Afterward, the epithelial tissue is regenerated (Wilmore D, 1999).

Medicinal plants have great significance in health of individuals and communities. The medicinal importance of plants lies in some chemical substances that produce a specific physiological action on the human body. The most essential of these bioactive constituents of plants are Alkaloids, saponins, Tannins, Flavonoids and Phenolic compounds and many more components. Most of the important medicinal plants are used as spices and food plants. They also sometimes additional to foods meant for pregnant women and nursing mothers for medicinal purposes (Okwu, 1999). Pumpkin belongs to the genus of Cucurbita and family Cucurbitaceae (Itis.gov. 2009). It frequently refers to cultivars of any one of the species *Cucurbita pepo, Cucurbita mixta, Cucurbita maxima,* and *Cucurbita moschata,* and its came from North America. They characteristically have a thick, orange or yellow shell, wrinkled from the stem to the bottom, it contain the seeds and pulp. Pumpkins are broadly grown for commercial use. In Australian English, the name 'pumpkin' generally refers to the broader category called winter squash. The word pumpkin derive from the word pepon, this is Greek word that means “large

melon". The French modified this word to pompon, that is changed by the British to pumpion and later Americans changed that to the word which we use today, "pumpkin" (The Pumpkin Patch, 2007). The source of pumpkins is not accurately known, although they are considered to have originated in North America. From the oldest information, pumpkin-related seeds dating between 7000 and 5500 BC, they found in Mexico (The Pumpkin Patch, 2007; The Columbia Encyclopedia, 2004) Pumpkins are a squash-like fruit that size range in between 1 pound (0.45 kg) to 1,000 pounds (450 kg) (Michael and Orsolek, 2000).

Some squash distribute the same botanical classifications as pumpkins. The names are commonly used interchangeably. In general, pumpkins stem are more rigid, prickly, and angular than squash stems, which is commonly softer, more rounded, and more flare where it join to the fruit (Reinhold., 1995, pumpkin. 1992).

Pumpkins are generally weigh 9–18 lbs (4–8 kg) with the largest species is *Cucurbita. Maxima* able to reaching a weight of more than 75 lbs (34 kg) (pumpkin,2007). Pumpkins vary greatly in shape, ranging from oblate to tetragon. The skin is smooth and usually lightly grooved (pumpkin, 2007). Although pumpkins are generally orange or yellow (pumpkin, 1992) and the pumpkin fruits are dark green, pale green, orange-yellow, white, red and gray in colour (Pumpkin Nook: Color Me Pumpkin).

Pumpkins are monoecious, and it having both male and female flowers on the same plant. The female flower of pumpkin is distinguished by the small ovary at the base of the petals. These bright and colorful flowers have particularly short life spans and may only open for as short time in a day. Pumpkin color is derived from the orange pigments abundant in it. The main nutrients are Lutein and alpha and beta both Carotene, which is later it produces or generated the vitamin A in the body (Arnum, 1998).

Pumpkins are grown all over the world in different types and for a variety of reasons ranging from agricultural purposes like animal feed to commercial and decorative sales (Wolford, 2008) of the seven continents, In Antarctica only it is unable to produce pumpkins. The biggest international producers of pumpkins are the United States, Canada, India, and China (The Pumpkin Patch', 2007), pumpkins are the healthiest Food of the world.

Pointed gourd its botanical name is *Trichosanthes dioica Roxb* and belongs to the family from Cucurbitaceae is a dioecious perennial herbaceous vegetable. The Pointed guard plant is of Indo–Malayan origin and distribution and is widely grown in eastern India (Chakravarthy, 1982) and other parts of South Asia. Fruits of pointed guard form the edible portion and are a resource of vitamin C and minerals (Gopalan et al., 1982). The fruits and other parts of plant such as the leaves and shoots are used as medicine since early times (Sharma et al., 1989; Singh, 1989).

In recent times, definite medicinal property have been recognized which include hypocholesterolemic, hypoglyceridemic and hypophospholipedemic effects on normal and diabetic patients (Sharma et al., 1989; Mukherjee, 1996). Seeds of the pointed guard plant are also found to have antifungal and antibacterial activity and are broadly used in the treatment of acid dyspeptic disease (Harit and Rathee, 1996).

The plant is generally propagated by stem cuttings using 60–90 cm long segments from the basal portion of the vine. These cuttings stem are cultivated under 15 cm deep, coiled shape ring exposing both the ends (Tindall, 1983). Seed propagation is not use due to the cross pollinated nature of the crop, reduced germination, slow growth of seedlings and segregation of male and female plants. Late fruiting and low yield of seedling plantations are also connected with seed propagation.

The perennial nature of the plant gives a chance for cost effective micropropagation of better clones of the plant. There are only a few enhanced cultivars of this difficult to breed crop. There are two such more productive female types “Swarna Alaukik” and “Swarna Rekha” released from the Central Horticultural Experiment Station (CHES), Ranchi (North India) are in great demand and there is no adequate supply of the planting material. To our information, there has been no report on in vitro propagation of this crop.

Okra its botanical name is *Abelmoschus esculentus Moench,* in many English-speaking countries it is called as lady's fingers or gumbo. It is a flowering plant and belongs from family mallow Okra valued for its edible green seed pods. The biological origin of okra which is disputed, with supporters of many countries South Asian, Ethiopian and West African origins countries. The plant *Abelmoschus esculentus Moench* cultivated in tropical, subtropical and warm temperate regions all over the world (National Research Council, 2006-10-27).

The name "okra" is most often used in the United States, with differences of the pronunciation– English Caribbean "okra"– used primarily around the Philippines. Okra is mainly called as "lady's fingers" outside of the United States, include with Singapore one of the countries and regions (curing-colds.com, accessed 3 June 2009).

In various languages, okra is mainly called kingombo or an alternative Thereof, and this is the origin of its name in Portuguese quiabo, In Dutch and French, and also perhaps of the name is "*gumbo*", used in parts of the United States and English-speaking Caribbean for both the vegetable, and a stew based on it (tamu.edu).

In India, Pakistan, and in the United Kingdom it is mainly called as bhindi or bhendi or Bendai. In southern India, it is known as "vendaykka" in Malayalam, vendaikkai in Tamil, Telugu, bandakka in Sinhala, and bende kayi in Kannada. The species of okra is an annual or perennial, the plant *Abelmoschus esculentus* has large bisexual flowers (Mc Gregor, 1976). Its flower arrangement combines hermaphroditism and self-compatibility. Hence in okra, as the conditions for selfing become favourable, it also has an opportunity for cross-pollination (Al Ghzawi et al., 2003) growing to 2 m tall. It is related to such species as cotton, cocoa, and hibiscus. The leaves of plant is 10–20 cm long and wide, palmately lobed with 5–7 lobes. The flowers of okra are 4–8 cm in diameter and five white to yellow petals in flowers, often with a red or purple spot at the base of each petal. The plant fruit is a capsule shaped and about 18 cm long, containing numerous seeds.

Abelmoschus esculentus is grown throughout the tropical and warm temperate regions of the world for its fibrous fruits or pods having round, white seeds. It is among the all most heat- and drought-tolerant vegetable species in the world—but high frost can damage the pods and it will stand poor soils with heavy clay and intermittent moisture.

In cultivation of plant, the seeds are soaked overnight to planting to a depth of 1–2 cm. Germination are shown between six days (soaked seeds) and three weeks. Seedlings require sufficient water (citation needed). The seed pods quickly become fibrous and woody and must be harvested within a week of the fruit being pollinated to be edible (tamu.edu). The fruits are harvest when it immature and eaten as a vegetable.

Medicinal plants have great significance to the health of individuals and communities. The medicinal importance of plants lies in some chemical substances that produce a specific physiological action on the human body. The most essential of these bioactive constituents of plants are Alkaloids, saponins, Tannins, Flavonoids and Phenolic compounds and many more components. Most of the important medicinal plants are used as spices and food plants. They also sometimes additional to foods meant for pregnant women and nursing mothers for medicinal purposes (Okwu, 1999, 2001 and Hill, 1952).

Before the discovery of the existence of microbes, the idea about some plant had healing potentials, definitely that they have what we would currently characterize as antimicrobial principle was well accepted (Doughari et al., 2008). As we know that prevention is a better strategy than treatment for chronic diseases, a steady supply of phytochemical containing plants with desirable health benefits beyond basic nutrition is necessary to provide the defense mechanism to reduce the risk of chronic diseases in humans (De-marino et al., 2008).The researcher has discovered of new effective drugs from plants is based on the use of plant extracts and crucial oils to treat infection or infectious diseases. Phytochemicals simply called as plant chemicals; these components are the naturally occurring in fruits, vegetable, herbs, spices, grains and legumes. These components give plants color, flavor, smell and parts of a plant which naturally give defense system for the plant (disease resistance) and the consumer.

Anderson define phytochemical in (2004) a plants derived chemicals which are useful to human health and disease prevention. Some plants have essential nutritional importance by their components of protein, carbohydrates, oils, fats, minerals, vitamins, and water which are responsible for growth and development in man, animals and plants. In addition to vitamins and provitamins in fruits and vegetables the occurrence of bioactive plant components mainly called as phytochemical has been considered of fundamental nutritional importance in the prevention of many disease such as cancer, cardiovascular disease and diabetes (Aruoma, 2003). Plants has been discovered that regular utilization of fruits, vegetables, herbs and spices have been associated with health benefits of human and animals, but its action has become understandable only in recent years. These valuable plants contain a wide range of biologically active, non nutritive components known as phytochemicals (Sheetal and Jamuna, 2009). These phytochemical components help in health benefits beyond basic nutrition (Oomah and Mazza, 2008). A lot of researchers have come up with the information that some plants chemicals which

have been used as anti nutritional or antioxidants have ability to help in decrease the risk of many deadly diseases in man (Agte et al., 2000). Information showed that the maximum sources of these chemicals are fruits and vegetables obtained from plants (Liu, 2004). This is a great require to find out the great potentials of these chemical, and this will help to give fundamental information for consumers for selection of these plants. Many Researchers have estimated that each serving increase in fruits, vegetables, herbs and spices decreases the risk of cancer by 15%, heart disease by 39% and death by any causes 20%. This idea has been established by epidemiology study (Steimez, 1996). This is often attributed to different phytochemicals compounds like antioxidants components such as ascorbic acid, caroteniods, tocopherol, lycopenes, and anti-nutrients such as tannins, alkaliods , saponins and steroids (Prior and Cao, 2009).

Phytochemical Studies have shown the components of plant to minimize the risk of several diseases. Up to present time 3,000 phytochemicals are discovered in plants (Oliveri, 2003). On the other hand, the levels of these components in plants depend on their species and varieties of the plants. In my present work, wehave selected three plants seed and aimed to identify their phytochemical components in distilled water extracts.

2.1. Tannins

Tannin sometimes it is called as tannoid, it is a type of biomolecule, it is present in plant and it is assign by phytochemical process. The term tannin came from a German word tannenbaum that means fire trees or oak. The term tannin use of wood tannins from oak in tanning animal hides into leather, therefore the words “tan” and “tanning” for the treatment of leather(Fig 2.1) The tannin compounds which is present in many species of lants, here tannin play a role in protection from predation, and maybe also as pesticides, and also in plant growth regulation (Richard W, 2006).The tannins is causes the dryness and puckery inside the mouth when it use by consumers in the form of red wine or unripened fruits (Harold, 2004). Similarly, the destruction or alteration of tannins with time tannin plays an important role in the fruit ripening and the production of wine. Tannins have molecular weights range in between 500 to over 3,000 (Bate-Smith and Swain, 1962) (gallic acid esters) and about 20,000 (proanthocyanidins).

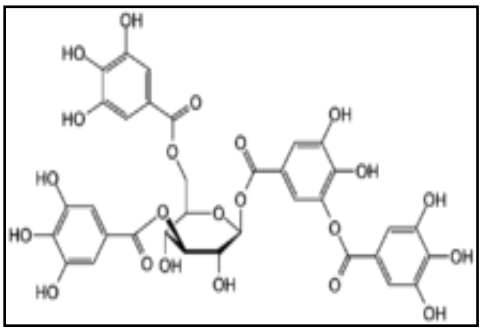

Fig 2.1 Tannin

2.2. Phlobaphenes

Phlobaphenes also called as phlobaphens, Phlobaphenes can be defined as either reddish color phenolic substances which is extracted from plants that are soluble in alcohol and insoluble in water and the reddish colored, which are insoluble in water products that result from action on tannin extracts along with mineral acids (Tanner's red) (J. Branham, 1992). The term phlobaphen come from the Greek words its meaning is bark and baphe (Rompp, 2006).Dr. Duke's conducted the Phytochemicals but there is no any biological behavior are reported for Phlobaphenes (Dr. Duke's Phytochemical and Ethnobotanical database) Phlobaphenes are converted into humins in soils (Sollmann, 1996)

2.3. Terpenoids

The terpenoids, sometimes it is called as isoprenoids, it have large and different class of naturally occurring organic chemicals which is similar to terpenes, it derived from five-carbon isoprene units assembled and arranged in thousands of ways. Most of them having multi cyclic structure which differs from one another, not only by functional groups but also in their basic carbon skeletons or structure. These lipids can be found in all classes of living beings, and are the largest group of natural products (fig 2.2).

Plant components terpenoids are used widely for their aromatic qualities. They play a role in conventional herbal medicine and are under study for antibacterial, antineoplastic, and many other pharmaceutical functions. Terpenoids components contribute to the odor of eucalyptus, the

flavors of cinnamon and ginger, the yellow color provided in sunflowers, and the red color given in tomatoes (Michael Specter, 2009).

Some known terpenoids like citral, menthol, camphor, salvinorin-A which is present in the many plant. In animals the steroids and sterols are naturally produced from terpenoid precursors. Sometimes plant component terpenoids are add with proteins, which enhance their affection to the cell membrane; it is called as isoprenylation.

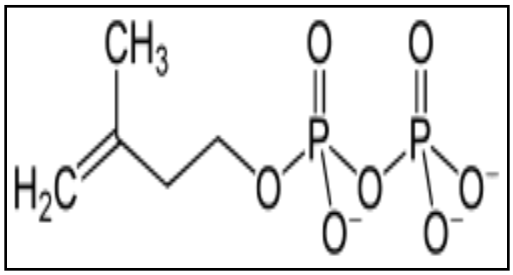

Fig: 2.2 Terpenoids

2.4. Saponins

Saponins are the class of chemical compounds which is found naturally in plants, saponins are the secondary metabolites those found in natural sources, saponins found particularly in large quantity in various plant species. More specifically saponin are present in amphipathic glycosides grouped, it is called as phenomenology, saponin form soap-like foaming when it shaken hardly in aqueous solutions. The structure of saponins is defined in terms of their composition of one or more hydrophilic glycosides which combined with a lipophilic triterpene derivative (Marston, 1995).

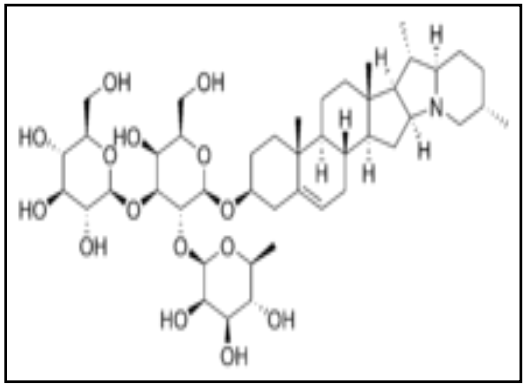

Fig: 2.3 Saponins

2.5. Glycosides

Glycoside is a type of sugar which is bound to a non-carbohydrate moiety, generally glycosides is a small organic molecule. Glycosides play various important roles in living organisms. Many plants species store chemicals in the form of inactive glycosides. These chemicals can be activated by enzyme hydrolysis (Brito and Marco 2007), which is cause the sugar fraction to be broken down and make the chemical which available for use of living things. Many plant having glycosides which are used as medication. In human and animals, poisons are always bound to sugar molecules for their elimination from the body.

In normal terms a glycoside may be define as, any molecule in which a sugar group is attached through its anomeric carbon to another group through a glycosidic bond. Glycosides can be bonded by an O-, N-, S- or C- glycosidic bond. The given explanation is the used by IUPAC, which recommend by the Haworth projection to correctly assign as stereochemical configurations (Lindhorst, 2007). The sugar group is present in glycosides is known as the glycone and the non-sugar group present in it is the aglycone or genin. The glycone is consisting of a single sugar group which is monosaccharide or many sugar groups called oligosaccharide. The first glycoside was identified by French scientist Pierre Robiquet and Antoine Char land as Amygdalin in 1830. (Physique, 1830).

2.6. Steroid

Steroid it is type of organic compound and component of plant which having a characteristic arrangement of four cycloalkane rings that are attached to each other. The examples of steroids are the dietary fat cholesterol, sex hormones estradiol or testosterone, and the anti-inflammatory drug like dexamethasone. Steroids core is composed of twenty carbon atoms joined together which form four fused rings, all these four, three are cyclohexane rings and remaining one cyclopentane ring. Steroids having a special type of component called Sterol, which having hydroxyl group at position-3 and a structure derived from cholestane (Moss, 1989). Hundreds types of different steroids are found in living beings like plants, animals, and fungi. All steroids are completed in cells either in from of sterols lanosterol or cycloartenol presents in plants. Both steroid lanosterol and cycloartenol are obtained from the cyclization of the triterpene squalene (International Union Of Biochemistry and Molecular Biology).

2.7. Cardiac glycosides

Cardiac glycosides, cardiac term came from heart so it is defined as cardiac glycosides are the type of drugs which is mainly used in the treatment of heart disease or cardiac disease. Glycosides compounds are found as secondary metabolites in many plants species, glycosides are also found in some animals, like the milkweed butterflies.

Therapeutic uses of cardiac glycosides mainly involve in the treatment of cardiac failure. Their function is to increase the cardiac output by increasing the force of contraction. By increasing the intracellular calcium, the cardiac glycosides help to increase calcium-induced calcium release and thus contraction. Drugs like ouabain and digoxin are the example of cardiac glycosides. Digoxin are found in the foxglove plant is used clinically, while ouabain is used only experimentally due to its extremely high effectiveness (. Diplock, 1998).

2.8. Carotenoid

Carotenoids are the tetra terpenoid organic component that are naturally occurring in the chloroplasts and chromoplasts of plants species and few other photosynthetic organisms like algae, bacteria, and some types of fungus. Carotenoids can be produce fats and other basic organic metabolic building blocks of many organisms. Carotenoids usually cannot be manufactured by species in the animal kingdom (Jarvik, et al., 2010). Animals take carotenoids in their diets, and may employ them in various ways in metabolism.

There are more than 600 known carotenoids are found, these are in two classes, xanthophylls (contain oxygen) and carotenes (which having purely hydrocarbons, and no oxygen). Carotenoids in commonly absorb blue light. Carotenoid serve as two key roles in plants and algae which absorb light energy for which is use in photosynthesis, and they are protect chlorophyll from photodamage (Hearst, 1996).

In humans, four type of carotenoids such as beta-carotene, alpha-carotene, gamma-carotene, and beta-cryptoxanthin which have vitamin A activity and it also act as antioxidants. In the eye of organism, certain other carotenoids like lutein and zeaxanthin are present which acts as directly to absorb damaging blue light and near-ultraviolet light, which is protect the macula lutea. People intake diets rich in carotenoids from natural foods, like fruits and vegetables, are healthier and have lesser mortality from a number of chronic illnesses (Diplock, 1998), although this

conclusion may be due to the inclusion of studies connecting to smokers.(Unlu et al., 2005) With the prominent exclusion of Vietnam Gac and crude palm oil, most carotenoid-rich in fruits and vegetables but low in lipids. While dietary lipids have been hypothesized to be an significant factor for carotenoid bioavailability. The study establishes that the addition of both avocado fruit and oil considerably improved the subjects' absorption of all carotenoids tested such as α-carotene, β-carotene, lycopene, and lutein (Unlu et al., 2005).

In some photosynthetic organisms, especially flora, carotenoids play a fundamental role in the photosynthetic reaction centre. Carotenoids either participate in the energy-transfer process, or defend the reaction center from auto-oxidation. In non-photosynthetic organisms, like humans, carotenoids have been connected to oxidation-preventing mechanisms. Carotenoids have a lot of physiological functions.

Animals and human are generally incapable of synthesizing carotenoids, and it obtains through their diet. The prominent exception is the red pea aphid, which have the genes which synthesizing carotenoids, (Buchanan et al., 2005). Carotenoids are a generally and normally ornamental feature in animals.

2.9. Flavonoid

Flavonoids are widely spread in plants which play in many functions. Flavonoids are the most significant for plant which provided pigments for flower coloration that producing yellow or red or blue pigmentation in petals that attract to pollinator animals. In higher plants flavonoids are help in UV filtration, nitrogen fixation and also floral pigmentation. Flavonoids may act as a chemical messenger or physiological regulator; it is also act as cell cycle inhibitor.

Flavonoids secreted through the root of their host plants help in the formation of a root nodule. Some flavonoids contain inhibitory activity against organisms that cause plant disease e.g. Fusarium oxysporum (Lanzotti, 2008). Beginning research indicate that flavonoids may modify allergens, viruses, carcinogens, and so it may be called as biological "response modifiers". In vitro studies show that flavonoids also have anti-allergic, anti-inflammatory activity (Jeremy, 2008), anti-microbial (Lamb, 2005), anti-cancer (Aoyama, 2007), and anti-diarrheal activities (Fischer, 2005).

Saponins are the type of chemical compounds, saponins are secondary metabolites found in natural sources, and saponins are found in particular abundance in various plant species. Saponins are amphipathic glycosides, it form the soap-like foaming when it shaken in aqueous solutions. Structure of saponins is defined by their composition of one or more hydrophilic glycoside structure is combined with a lipophilic triterpene derivative (Marston, 1995). The aglycone it is glycoside-free portion present in the saponins are called as sapogenins. In saponins the number of saccharide chains attached to the sapogenins or aglycone core can vary which give rise to another dimension of arrangement which are monodesmosidic, bidesmosidic, etc (Marston, 1995). Dietary monosaccharides like D-glucose and D-galactose are the most common components of the attached chains.

Saponins are mainly derived from plants, but they are also present in marine organisms (Marston, 1995). Saponins are definitely found in many plants (Marston et al., 1995) and their name came from the soapwort plant, the root of that plant is used as a soap from ancient time (Marston, 1995). Saponins are the chemical compounds which are found in various parts of the plant like- leaves, stems, roots, bulbs, blossom and fruit. Commercial produce saponins from plant derivatives – e.g., soap bark tree formed (Quillaja saponaria), and also other sources are available by means of controlled developing processes, which compose them of use as chemical and biomedical reagents.(Irvin, 1980).

There is great, commercially driven promotion of saponins as dietary supplements and nutritional substance. There are facts of the presence of saponins in conventional medicine preparations (Qin, 1996). Antibacterial activity means the substance which kills or inhibits the growth of bacteria (Webster Online Dictionary, 2009-05-02) such as E. coli, Pseudomonas, bacillus etc. Antibacterial drugs either kill microbes is known as Bacteriocidal or stop the growth of microbes is called micro biostatic. Disinfectants are antimicrobial or antibacterial substances used on non-living objects or outside the body.

The history of antimicrobials begins after the observations of Pasteur. Experimentally, antibiotics are only those substances that are formed by one microorganism that kill, or prevent the growth, of a different microorganism. The discovery of antimicrobials by Flemming like penicillin and tetracycline covered the way for better health for millions of people around the world. Before

these thousands of years ago, human has known about the advantage of drugs from nature. Plant extracts, for the treatment of various diseases, were extremely regarded by the ancient civilizations (Grabley and Thiericke, 1999).

Even today, many plant materials remain play an important role for treating illnesses, including infectious diseases, and many of these plants material have been investigated for novel drugs or templates for the production of new therapeutic agents (Konig, 1992). Plants are produce about 7000 different pharmaceutically and important compounds and a number of top selling drugs of present time which is help in treat many infectious disease, e.g. quinine, artemisinin, taxol etc (Tshibangu et al., 2002). There are more than 120 plant derived drugs are approved worldwide, and they come from only 95 plant species. Out of the 250 000 species of flowering plants, only 5000 plants have pharmaceutical activity (Lewington, 1990). The treatment of infectious diseases by antimicrobial agents produced through the plants component. Following type of much investigation shows an important role of plants to produce several antibacterial compounds and antibiotics (Kunin, 1993and Finch, 1998).

There are many published information showed that effectiveness of traditional herbs against Gram-positive and Gram-negative bacteria, and as a result, plants are produced many modern medicine to treat infectious diseases (Evans et al., 2002). During the last century, the search for new anti-microbial or antibacterial agents from plants had mainly been focused on tropical or sub-tropical plants and less concentration on temperate plant species. However, it does not mean that there is no any activity of temperate plants in the treatment of infectious diseases or produce pharmaceutical components. Some of the earliest investigation it is proved that translated medical manuscripts in Britain were the Gaelic medical manuscripts which belonged to two families who produced medicine in the Scotland, and there is sufficient evidence to show that they used various Scottish plants for treating wounds and infections and infectious disease. (Comrie, 1860 and Florae, 2000). Nature has produces allot of medicinal agents for thousands of years and an impressive number of modern drugs contain isolated from natural resources. Conventional medicine is an important source of many useful new compounds for the development of chemotherapeutic agents (Racio et al., 1989). Appearance of pathogenic microorganisms that are resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetics.

Antimicrobial drugs (Karaman et al., 2003), In addition, high cost and adverse side effects are usually associated with popular synthetic antibiotics (such as hypersensitivity, allergic reactions, immuno suppression etc.) and are major burning global issues in treating infectious diseases (Schinor et al., 2007).

Even though pharmacological industries had produced significant number of commercial antibiotics time to time but resistance in pathogens towards these drugs too has increased at high rate and multi drug resistant microorganisms have exacerbated the situation (Nino et al., 2006). In the present situation, there is an urgent and continuous need of exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines (Essawi and Srour, 2000). Antimicrobials of plant origin have enormous therapeutic potential and have been used since the past time. They have been proved useful in the treatment of infectious diseases at the same time which have fewer side effects which are often linked with synthetic antibiotics (Iwu et al., 1999). Positive response of plant based drugs (no side effects) might lies in the structure of the natural products which reacts with toxin and pathogens in such a way that less harm is done to other important molecules or functioning of host. It is because of this reason that drug designing studies now a day have as new field of research.

The use of plants in the organization and treatment of diseases started with life. In recent years, with substantial research, it has been found that many plants do indeed have medicinal values (Racio et al., 1989). Some medicinal plants used in the treatment of asthma, hypertension, typhoid fever, pile. Various species of the plant are used by traditional medicine practitioners in Nigeria in the management and treatment of several disorders which include rheumatism, hypertension, cancer, and inflammatory diseases (Schinor et al., 2007).

3. MATERIALS AND METHODS

Distilled water, ferric chloride, hydrochloric acid, chloroform, sulphuric acid, sodium hydroxide, glacial acetic acid, ammonia solution, Grinder or blender, conical flask, test tubes, petri plates, laminar air flow, Incubator, autoclave, Freeze, hot air oven, water bath and heater

3.1. Plant collection

In my project wehad done phytochemical determination and antibacterial activity of three plants seed, these plants are -

• *Trichosanthes dioica Roxb.* (or) patol,

Family- Cucurbitaceae

• *Cucurbita Maxima* (or) Pumpkin,

Family- Cucurbitaceae

• *Abelmoschus esculents* (or) Bhindi (or) Lady's finger (or) Okra

Family- Mallow (Malvaceae)

These plants seed were identified and collected from field of Rourkela market. The seeds were removed from fruit, washed properly with distilled water and air dried at room temperature until dried. The dried plants seeds blended using a blender or grinder and stared in a clean glass were container until for analysis. The plant seed extracts were filtered using Whatmann filtered paper (125mm)

3.2. Phytochemical screening

Phytochemical tests were carried out in the aqueous condition with the help of distilled water, when plants seed powdered were mixed with water using standard procedures to identify the constituents of selected plants seed. In identifying the some components some chemicals were used other than distilled water.

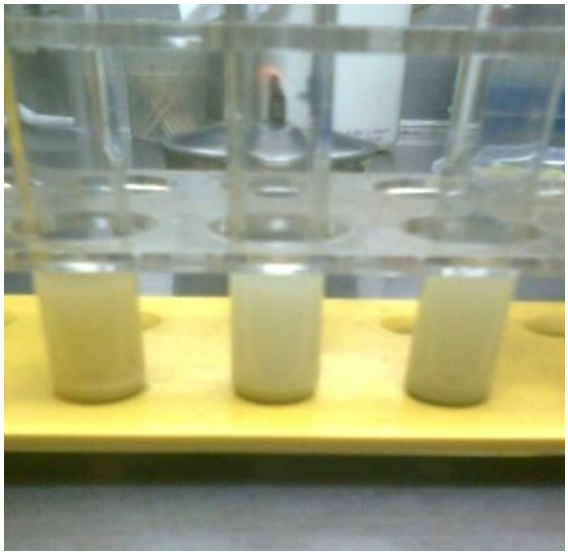

Fig 3.1 Plants seed extract.

3.3. For the test of tannins

Each powder sample had taken separately 1 g in separate flask and it boiled with 20 ml distilled water for five minutes in a water bath or on heater and it filtered while hot with the help of filter paper and then 1 ml of cool filtrate is again distilled with 5 ml of distilled water and a few drops of 10% ferric chloride solution are added, then we observed the formation of precipitates in solution and also it show the colour are change as bluish or brownish-green precipitate it indicate the presence of tannins.

3.4. For the test of saponins

Each seed powder sample takes 1 g separately and it separately boiled with 10ml of distilled water in a bottle bath for 10 minutes on a heater. Then the mixture was filtered while hot with Whatmann filter paper and it allows cooling. The following tests are then carried out.

Test for frothing- 2.5 ml of seed extract sample are filter then it diluted with 10ml distilled water and shaken vigoursly for 2 minutes, in solution frothing are present this indicate the presence of saponin in the filtrate sample.

3.5. For the test of phlobatannins

Extract of each seed powder were boiled separately with 1% aqueous hydrochloric acid and then it was showed deposition of a red precipitate in flask that determined as confirmation for the phlobatannins.

3.6. For the test of terpenoids

We had take 5 ml of each seed extract and it mixed in 2 ml of chloroform and 3 ml of concentrated sulphuric acid (H2SO4) was added in to it then sample form a layer. These experiments give a reddish brown colour precipitate at the border or interfere of chloroform and H2SO4 it determine the conformation of terpenoids.

3.7. For the test of flavonoids

1 g of the each seed powder sample were boiled separately in flask with 10 ml of distilled water for the time of 5 minutes and filtered it while extract solution are hot with whatmann filter paper. Few drops of 20% sodium hydroxide solution were added to 1 ml of the cooled seed filtrate sample. It shows change to colour like yellow colour to colourless when sodium hydroxide were added in seed extract. Theses colour change indicated that the presence of flavonoids.

3.8. For the test of glycosides

Each plant seed extract of 5 ml were treated with 2 ml of glacial acetic acid which contained one drop of ferric chloride solution. Then in this solution 1 ml of concentrated sulphuric acid were added, after adding the acid a brown ring are show at the interface which indicated the present of deoxysugar and also a violet ring were appear below the ring of the acetic acid layer.

3.9. For the test of anthraquinones

1 g of powdered sample of each plant seed samples were boiled separately with 2 ml of 10% hydrochloric acid for 5 mins in a flask. The mixture was filtered through Whatmann paper at the same time as it is hot and filtrate was allowed to cool. The cooled filtrate was separated against equal volume of chloroform and the chloroform layer was separate into a clean dry test tube with the help of clean pipette. Same volume of 10 % ammonia solution were added into the

chloroform layer and then it shake hardly and it separated from the mixture, after separation of the aqueous layer were show for any colour change like rose pink colour were indicate the presence of an anthraquinone.

3.10. For the test of free anthraquinones

1st take 0.5 g of powder seed extract and then in plant seed powder 5 ml of chloroform was added in separate flask. Then the mixture was shaken hardly for 5 mins after shaken it were filter. The filtrate solution were again shaken with equal volume of 10% ammonia solution; these experiment show the colour of bright pink colour in the aqueous layer it determined the presence of free anthraquinones.

3.11. For the test of carotenoids

1 g of each seed extract sample was mix with 10 ml of chloroform in a separate test tube with vigorously shaking. After that mixture the seed extract filtered with filter paper and 85 % sulphuric acid was added. Blue colour at the interface was present which indicates the presence of carotenoids.

3.12. For the test of alkaloids

1 g of seed extract powder sample of each plant were separately boiled with water on heater and then 10 ml hydrochloric acid were added in the solution after that it filtered with filter paper. The pH of the filtrate was maintained with the help of ammonia to about 6-7. Very small quantities of the following reagents were added separately to about 0.5 ml of the filtrate sample in a different test tube and it observed. The chemicals like picric acid solution, 10% tannic solution, Mayer's reagent are added in solution. Then the test tubes were observed for coloured precipitates it indicates the presence of alkaloids.

3.13. For the test of reducing sugar

Seed extract powder sample were prepared with distilled water and make 0.5 ml of seed sample extract solution, after that 1 ml of water and 5 to 8 drops of Fehling's solution were added at hot condition and then it observed brick red precipitate form which shows the presence of reducing sugar.

3.14. Antibacterial activity

3.14.1. Seed Extract

Vigorously washed dried seed of three plants of *Trichosanthes dioica Roxb, Cucurbita Maxima* and *Abelmoschus esculentus Moench* then plants seed were dried in shade for five days and then these seeds turned into powdered form with the help of grinder and blender. 25 gm of seeds powder were filled in the test tube and make extracted sequentially with the help of distilled water solvent after it Soxhlet extractor for 48h. The seed solvent extracts were concentrated under compact pressure and conserved it at 5°C in airtight bottle for use.

3.15. Growth and preservation of Microorganism for Antibacterial Studies

3.15.1. Preparation of broth

I have selected five types of microorganism (bacteria) for further studies; these are *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus* and *Klebsiella Pneumonia* bacteria were obtained from the life science lab, Department of Life science National Institute of Technology, Rourkela, Odisha. The bacteria were incubated in triptic soya broth (casein digestive enzyme) with the help of sterile inoculation loop in laminar air flow. Then pre culture triptic soya broth stand overnight in a rotary shaker at 37°C, after it the culture maintained on triptic soya broth in freeze for further use at the low temperature.

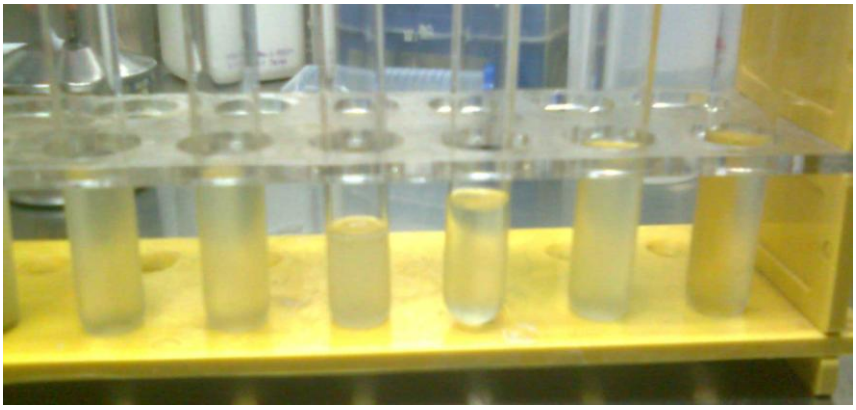

Fig 3.2 Microorganism culture in TSB

3.15.2. Preparation of growth media:

Mueller Hinton Agar (Hiveg infusion-2g, Hiveg acid hydrolysate-17.50g, Starch-1.50g, Agar-17g, pH-7.3±0.2, 1000mL Distilled Water) are used in preparation of medium for growth of following organisms. Mueller Hinton agar were taken 5.7g with 150ml of distilled water for preparing of 150ml MHA medium, after preparing media a tight cotton plug prepare and settled in flask.

Prepared MHA were autoclave at 120°C and 15lb pressure, and then MHA poured in petriplates under the laminar air flow with suitable sterile condition after solidifying it kept in incubator at 24 hrs for the checking of contamination in media, after cheeked Petriplates it used for testing the antibiotic susceptibility of the isolated strains.

3.16 Antimicrobial Activity study of seed extracts by Disc diffusion method

The water seed extracts of Patal, pumpkin and Okra were tested by disc diffusion methods. Muller Hinton Agar (Himedia) and sterile discs as well as streptomycin discs (High media) were used in antibiotic sensitivity testing. Firstly the entire agar surface was streaked with the swab for 3 times for *Bacillus subtilis, Staphylococcus aureus, Klebsiella Pneumoniae, Escherichia coli* and *Pseudomonas fluorescens* using sterile cotton swab First swab the prepared culture evenly at straight line then turn the plate at 90o angle from the left side swab evenly after it swabbing is done between each streaking at angle of 45o. The inoculums were allowed to dry for 5 mins. Three sterile discs of 6 mm diameter were placed on the medium with the help of disc dispenser and were numbered properly. After completion of streaking, the aqueous extract solutions 50 mg/ml of each *Trichosanthes dioica* seed, *Cucurbita Maxima* seed and *Abelmoschus esculentus* seeds were poured on the discs with the help of sterilized micropipette. Discs were left for some time till the extract diffuses in them. The effects were compared with that of the standard antibiotic streptomycin loaded sterile disc at a concentration of 1 mg/ml (Khan and Omotoso, 2003). Discs were observed for zone of inhibition by measuring diameter of inhibition zone (DIZ) using scale. After completion of the following work petriplates are incubated for overnight at 37 °C and examined it after 18-24 hours. The zones showing complete inhibition were measured and the diameters of the zones were measured to the nearest millimeter. By the antibiotic zone scale, the area of inhibition was measured for each seed sample. Sensitivity of the isolates to each seed extract was determined according to the chart provided by Himedia.

4. RESULT

4.1. Tannins

From the following experiment, we found the brown-greenish precipitation in *Trichosanthes dioica Roxb* (patal). This precipitation shows the presence of tannins in the plant seed of *Trichosanthes dioica Roxb* (patal) but such component was absent in *Cucurbita Maxima* (pumpkin) and *Abelmoschus esculentus Moench* (okra), because their plant seed does not show any precipitation and any such colour.

Fig 4.1. Brown green pricipitate

4.2. Saponins

Through the following experiment, we observed that when solution is shaken for two minutes then frothing appeared in the extract. This shows the presence of saponins in *Trichosanthes dioica Roxb* plant seed, and its absence in *Cucurbita Maxima* and *Abelmoschus esculentus Moench* because their extract not shows frothing.

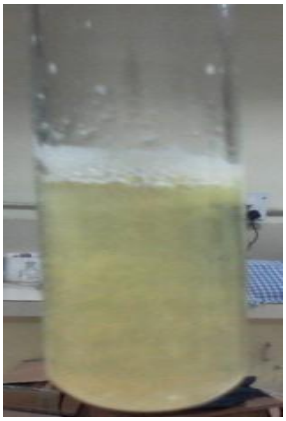

Fig 4.2. Frothing appears in seed extract.

4.3. Flavonoids

We observed the colourless compound from the yellowish colored seed sample by the following methods, This colourless compound are shows the presence of flavonoids in the plant seed of *Abelmoschus esculentus Moench* (okra) but such component was absent in *Cucurbita Maxima* (pumpkin) and *Trichosanthes dioica Roxb* (patal), because the plant seed does not show any such colourless compound after test.

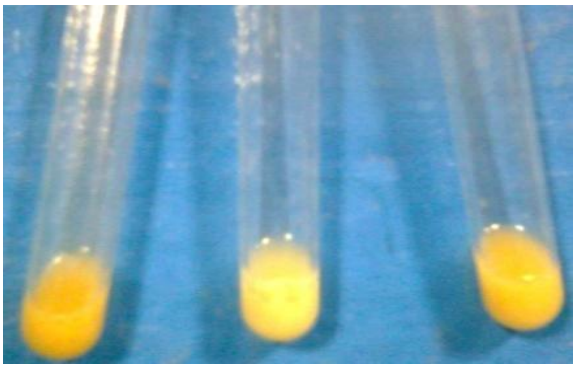

Fig 4.3.Colourless seed extract.

4.4. Terpenoids

From the following experiment we observed the reddish brown colour precipitate at the border or interfere of chloroform and H2SO4 (sulphuric acid). It determines the conformation of terpenoids; terpenoids were present in the *Trichosanthes dioica Roxb* (patal) and in *Cucurbita Maxima* (pumpkin) but absent in the *Abelmoschus esculentus Moench* (okra) because in my experiment it does not shows any precipitation or coloration.

Fig 4.4. Reddish brown rings at interfere.

4.5. Cardiac glycosides

Through the following experiment, we observed that brown ring are show at the interface which indicated the present of deoxysugar and also a violet ring are appear below the ring of the acetic acid layer. This observation appeared in *Trichosanthes dioica Roxb* (patal) and *Abelmoschus esculentus Moench* (okra) but such component was absent in *Cucurbita Maxima* (pumpkin).

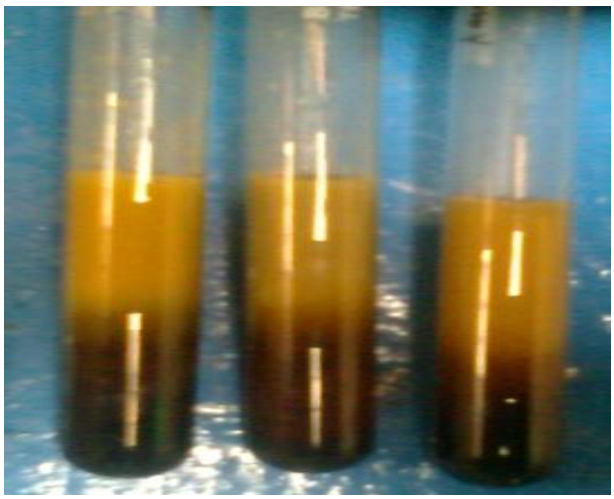

Fig 4.5. Brown rings at interface.

4.6. Phlobatannins

We found that the deposition of a red precipitate through following experiment in plant seed extract, it indicate the presence of phlobatannins in *Trichosanthes dioica Roxb*. Plant seed and remaining plant did not show red precipitate.

Fig 4.6. Deposition of red precipitate

4.7. Carotenoids

Through the following experiment, we observed in following plants seed extract does not show blue colour at the interface this indicates the absence of carotenoids in *Trichosanthes dioica Roxb*, *Abelmoschus esculentus Moench* (okra) and *Cucurbita Maxima* (pumpkin).

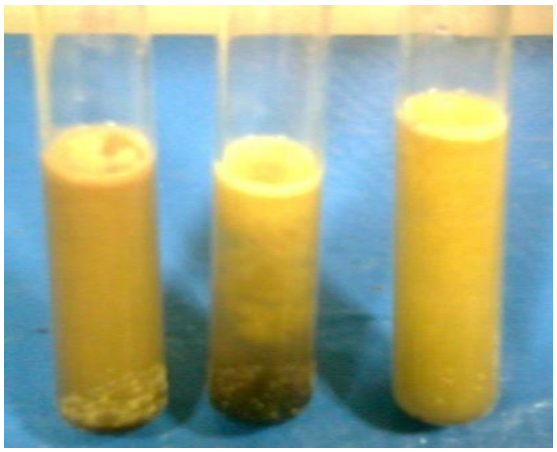

Fig 4.7 Blue colour does not show.

4.8. Glycosides

From the following experiment, we found brown ring at the interface which indicated the present of deoxysugar and also a violet ring, this brown ring were indicated the presence of glycosides in following plant seed. *Trichosanthes dioica Roxb* shows the brown ring at interfaces but *Abelmoschus esculentus Moench* (okra) and *Cucurbita Maxima* (pumpkin) does not show so we concluded that glycosides absent in these plants seeds.

Fig 4.8 Brown ring at interface.

Table 1:Phytochemical Analysis

PARAMETERS	PATAL	PUMPKIN	OKRA
Saponins	+	-	-
Alkaloids	+	-	-
Tannins	-	-	+
Flavonoids	+	+	-
Terpenoids	+	-	+
Cardiac glycosides	-	-	-
Glycosides	+	-	+
Steroids	Not identify	Not identify	Not identify

4.9. Result of antibacterial activity

The seeds extract of the *Trichosanthes dioica* seed, *Cucurbita Maxima* seed and *Abelmoschus esculentus* seeds have been tested for their antimicrobial activities and an interesting antimicrobial profile has been observed against *Bacillus subtilis, Staphylococcus aureus, Klebsiella Pneumoniae, Escherichia coli*, and Pseudomonas *fluorescens*. All the three seed extracts showed sustained activity against all five bacteria tested (fig 1-5). The zones of inhibitions (mm) are mentioned in table 1. The diameter of inhibition zones (DIZ) against Bacillus subtilis was 14, 16, 22 mm for *Trichosanthes dioica*, *Cucurbita Maxima* and *Abelmoschus esculentus* seed extracts respectively. Whereas, the lowest activity was shown with streptomycin of 10 mm DIZ (fig.4.9.1). The diameter of inhibition zones (DIZ) against *Staphylococcus aureus* was 24, 28, 30 mm for *Trichosanthes dioica, Cucurbita Maxima* and *Abelmoschus esculentus* seed extracts respectively. However, the standard drug streptomycin did not show any activity (fig4.9.2).

The diameter of inhibition zones (DIZ) against *Klebsiella Pneumoniae* was 12, 10, 12 mm for the *Trichosanthes dioica*, *Cucurbita Maxima* and *Abelmoschus esculentus* seed extracts respectively. But, the standard drug streptomycin did not show any activity (fig. 4.9.3). The diameter of inhibition zones (DIZ) against *Escherichia coli* were 18, 20 1nd 22 mm for *Trichosanthes dioica*, *Cucurbita Maxima* and *Abelmoschus esculentus* seed extracts respectively. Whereas, the lowest activity was shown with streptomycin of 10 mm DIZ (fig.4.9.4). The diameter of inhibition zones (DIZ) against *Pseudomonas flurescences* was 20, 24, 25 mm for *Trichosanthes dioica*, *Cucurbita Maxima* and *Abelmoschus esculentus* seed extracts respectively. Whereas, the standard drug streptomycin of showed the DIZ 12 mm against *Pseudomonas fluorescens* (fig. 4.9.5).

Table.2: Zone of inhibition seed extracts against different bacteria

Plant seed extract	Zone of inhibition				
	Bacillus subtilis	***Staphylococcus aureus***	***Klebsiella Pneumoniae***	***Escherichia coli***	***Pseudomonas fluorescens***
Trichosanthes dioica	14 mm	24 mm	12 mm	18 mm	20 mm
Cucurbita maxima	16 mm	28 mm	10 mm	20 mm	24 mm
Abelmoschus esculentus	22 mm	30 mm	12 mm	22 mm	25 mm

Fig. 4.9.1: Inhibition zone of *Trichosanthes dioica* (14 mm), *Cucurbita Maxima* (16 mm), *Abelmoschus esculentus* (22 mm) seed extracts (50 mg/ml) and streptomycin against *Bacillus subtilis*.

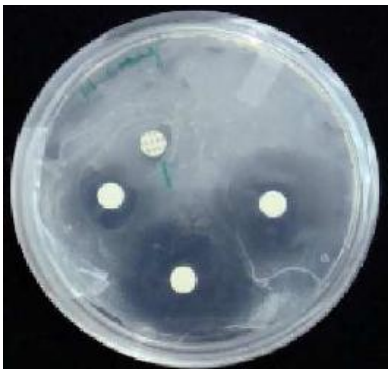

Fig. 4.9.2: Inhibition zone of *Trichosanthes dioica* (24 mm), *Cucurbita Maxima* (28 mm), *Abelmoschus esculentus* (30 mm) seed extracts (50 mg/ml) and streptomycin against *Staphylococcus aureus*.

Fig. 4.9.3: Inhibition zone of *Trichosanthes dioica* (12 mm), *Cucurbita Maxima* (10 mm), *Abelmoschus esculentus* (12 mm) seed extracts (50 mg/ml) and streptomycin against *Klebsiella Pneumoniae.*

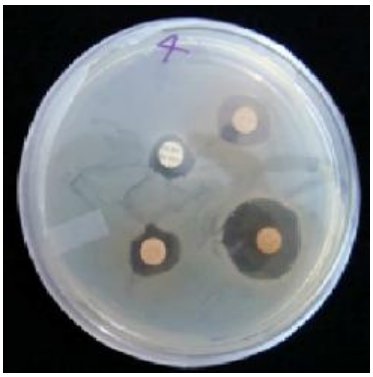

Fig. 4.9.4: Inhibition zone of *Trichosanthes dioica* (18 mm), *Cucurbita Maxima* (20 mm), *Abelmoschus esculentus* (22 mm) seed extracts (50 mg/ml) and streptomycin against *Escherichia coli.*

Fig. 4.9.5: Inhibition zone of *Trichosanthes dioica* (20 mm), *Cucurbita Maxima* (24 mm), *Abelmoschus esculentus* (25 mm) seed extracts (50 mg/ml) and streptomycin against *Pseudomonas fluorescens*

5. DISCUSSION

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, tannins and steroids in different extracts of *Trichosanthes dioica Roxb*, *Cucurbita Maxima* and *Abelmoschus esculents Moench* seeds. These results revealed that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions. Although their specific roles were not investigated in this study, it has been reported that most active components in plants are mostly flavonoids, steroids, glycosides and alkaloids

Further work could also be possible to investigate the specific phytoconstituents responsible for these activities. Phytochemicals can serve as a valuable source of information and provide appropriate standards to establish the quality of this plant seed material in future study or application.

Resistance in microorganisms to many antibiotics has resulted in morbidity and mortality from treatment failure and increased health care costs. Though a number of antibiotics are available but increasing ability of microbes to develop multidrug resistance has forced scientists for search of new, protective and efficient bioactive agents of herbal origin.

Results of the my project study showed that *Trichosanthes dioica Roxb*, *Cucurbita Maxima* and *Abelmoschus esculents Moench* plant seeds extract are tested for the inhibition the growth of selected bacteria *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Klebsiella Pneumonia*, indicating broad spectrum bioactive nature of selected plants.

6. CONCLUSION

In this research we observed the result for phytochemicals in my study, it is inference that *Trichosanthes dioica Roxb* (patal), *Cucurbita Maxima* (pumpkin) and *Abelmoschus esculentus Moench* (Okra) seed powder contains the chemical constituents like Alkaloids, saponins, Tannins, Flavonoids, Terpenoids, Glycosides, Steroids and Phenolic compounds in these plants seed. However, it is recommended that further work be carried out to isolate the bioactive constituents in *Trichosanthes dioica Roxb, Cucurbita Maxima* and *Abelmoschus esculentus Moench* seed powder using various extraction solvents with a view to characterize the presence of chemicals in such plants seed. These plants seed play very important role in the fields of medicine and pharmaceutical and also treat many infectious disease. Among these three plant seeds, *Trichosanthes dioica Roxb, Abelmoschus esculentus Moench* and *Cucurbita Maxima* gave the best result respectively.

In my study, the antibacterial study of plants seed extract of *Trichosanthes dioica Roxb, Cucurbita Maxima* and *Abelmoschus esculentus Moench* in aqueous medium by cup diffusion methods against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus* and *Klebsiella Pneumonia* was observed which showed the antibacterial activity, so we concluded that these plants seeds are having antibacterial substance. Among these three plant seeds, *Abelmoschus esculentus Moench, Cucurbita Maxima* and *Trichosanthes dioica Roxb* shows more zone of inhibition, respectively.

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