Evaluation of phytochemicals as antimicrobial agents on *Aspergillus terreus* and *Aspergillus niger*

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CERTIFICATE

This is to certify that the work entitled, "**Evaluation of phytochemicals as antimicrobial agents on *Aspergillus terreus* and *Aspergillus niger*".**

Submitted by **Ms Poonam Kumari (121563) and Anushruti Mehta (121569)** impartial fulfilment for the award of degree of **Bachelor of Technology in Biotechnology** of **Jaypee University of Information Technology, Waknaghat, Solan** has been carried out under my supervision.

Signature of the Supervisor:

Dr. Jata Shankar

Assistant Professor

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DATE:

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

µl	Microlitre
mL	MilliLitre
µg	Microgram
mm	Millimeter
DMSO	Dimethyl sulfoxide
Conc.	Concetration
A.terreus	*Aspergillus terreus*
A.niger	*Aspergillus niger*

1. INTRODUCTION

Background

Fungi are ubiquitous to the environment and primarily saprophytic, using non living organic material as a nutrient source for growth and reproduction. During the digestion process fungi secrete enzymes into the nutrient source to break down complex compounds into simpler compounds, which are taken up by the fungi and digested. The digested nutrients are classified into two categories: primary and secondary metabolites. The primary metabolites consist of cellulose and other compounds that are used for energy to grow and reproduce (Mohamed Refai *et al*., 2014)

Mycotoxins are secondary metabolites produced by filamentous fungi that have adverse effects on human and animal (Maggon *et al*., 1977). They are structurally diverse, deriving from a number of biosynthetic pathways and their effect upon consumers is equally diverse ranging from acutely toxic to immunosuppressive or carcinogenic. The production of a particular mycotoxin is restricted to a limited number of fungal species and, in some instance may be limited to particular strains within a species.

Mycotoxin contamination is an economic problem for live stock and feed industries. The presence of mycotoxins in feedstuffs reduces the feed quality in terms of both energy and protein value. High moisture content (>12%) and grain damage favour mould growth. Mycotoxins may get concentrated from 30-500 times in broken grain as compared to whole grain (Atef A.Hassan *et al.*, 2013) Mycotoxins can cause a variety of short term as well as long-term health effects. Symptoms due to exposure to mycotoxins are: dermatitis, cold, flu, sore throat, headache, fatigue and diarrhea.

Some of the important mycotoxins are:

- **Aflatoxins** produced mainly by *Aspergillus flavus* and *Aspergillius parasiticus.*
- **Ochratoxins** The predominant member of the group is ochratoxin A which is a potent nephrotoxin and hepatotoxin. Ochratoxin A is produced by *Aspergillus niger.*
- **Citrinin and citreoviridin** produced by A. *terreus* and other species of *Aspergillus. A. terreus* also produces aspterric acid, terrain, terreic acid, patulin and gliotoxin.

Chronic Pulmonary Aspergillosis and Aspergillomas are caused by *Aspergillus* infecting the body and growing in cavities in the lungs. These cavities in the lungs would usually have to be created by a previous health problem such as tuberculosis. Once the *Aspergillus* mold has infected the lungs it begins to grow into a fungal ball which then makes the person sick because of the allergens or toxin

it puts out into the person's body. Aspergillomas or the fungal balls in lungs can be detected through x-rays or performing blood tests. Drugs such as itraconazole or voriconazole can be given to the patients once aspergilloma is treated. Surgery is recommended in some severe cases where the size of aspergillomas gets bigger (E. Dannaoui e*t al.*, 2006).

Antifungal compounds have been overshadowed by antibacterials in research interest and application due to the greater impact bacterial infections have had on health. Resistance to antibacterial drugs and the resultant clinical impact is of widespread concern regarding public health. However, resistance by pathogenic fungal infections to drug treatment has become more common in the last 20 years as well. Some mechanisms of the development of fungal resistance have similarities to those of the development of drug resistance in bacteria, and knowledge of those bacterial mechanisms is being applied to understanding fungal drug resistance (Malcolm D. Richardson, 2005).

Several key antibiotic compounds function by targeting the integrity of the cell. Many compounds increase the porosity of the cell wall or membrane, or interfere with key steps in the synthesis of cell walls. While prokaryotic bacteria and eukaryotic fungi do not have identical cell wall and membrane components, there are corresponding lipids and key structural molecules. As a result, similar to antibacterials, most antifungal compounds work because they directly or indirectly damage the cell wall or cell membrane (Patrick Vandeputte *et al*., 2012).

The fungal cell wall is composed of multiple layers, with mannoproteins being predominantly expressed at the external surface (Figure 1). An underlayer of β-glucan creates a supporting matrix for the mannoproteins and provides structural rigidity to the cell wall. The glucan structure is strengthened by frequent β(1→3) and additional β(1→6) linkages and by chitin interspersed with the β-glucan. Mannoproteins and glucan make up more than 80% of the cell wall composition, while chitin represents less than 2%. The plasma membranes of fungi are primarily composed of ergosterol, analogous to cholesterol in animal cells. Since ergosterol and cholesterol have sufficient structural differences, the majority of chemicals found to act as fungicides target ergosterol biosynthesis or cell membrane porosity and do not cross react with host cells (Ghannoum *et al*., 1999).

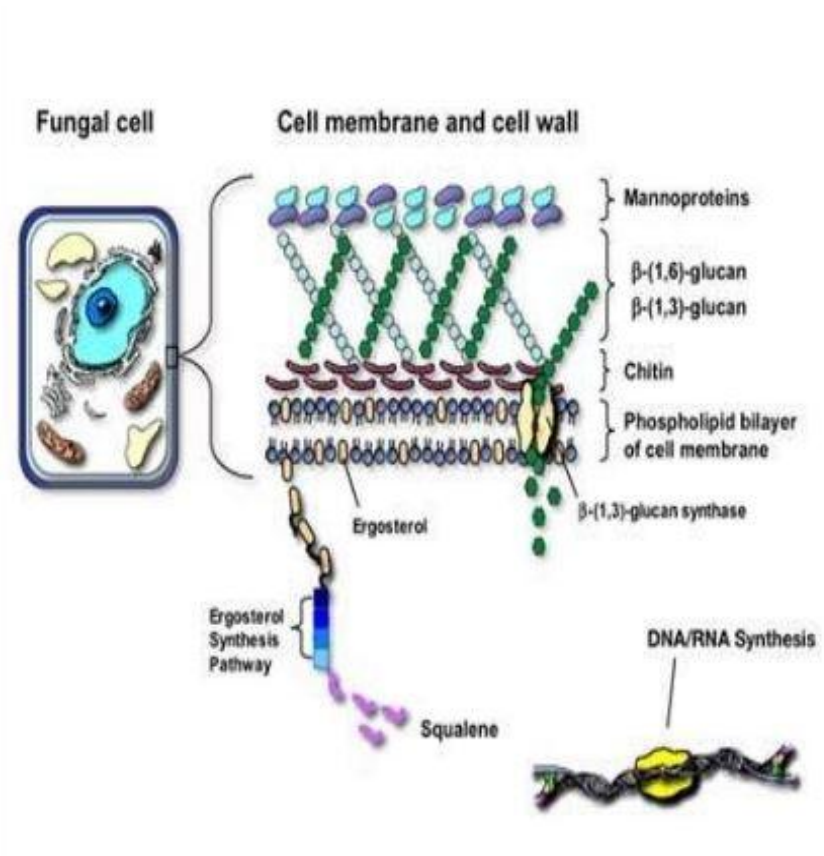


FIGURE 1- Structure of the fungal cell wall. The wall is primarily composed of mannoproteins and β-glucan that is linked (1→3) and (1→6). Ergosterol is the major lipid component of the underlying plasma membrane.

Plant sources of antifungal compounds, though studied extensively, still haven't been able to be of any commercial use, for example, as a drug or as a potential antimicrobial compound to control *A. flavus* disease incidence in commercially important crops. Medicinal plants have been associated with the prevention of degenerative diseases such as cancer and cardiovascular diseases. The presence of wide range of phytochemicals such as phenolics, thiols, carotenoids, anthocyanins and tocopherol have been suggested to exert chemopreventive and cardio protective effects as well as protecting the human body against oxidative damage by free radicals (Naz *et al*., 2013) . Natural phytochemicals derived from fruits, vegetables and herbs have been reported to possess a wide range of biological effects, including antioxidant, antimicrobial and anti-inflammatory actions.

OBJECTIVE

Different strategies have been established for preventing *Aspergillus* species contamination on susceptible plants and crops, by physical and biological methods. These methods often require sophisticated equipment and expensive chemicals and reagents. The application of chemicals compounds has led to a number of environmental and health problems due to their residual toxicity, carcinogenicity, hormonal imbalance and spermatotoxicity. There is a need to design new and environmentally safe methods of reducing infection by aflatoxigenic *Aspergilli* and to inhibit aflatoxin biosynthesis. Plants are considered as sources of useful metabolites. Use of plant products in the form of plant extracts and essential oils provide an opportunity to avoid synthetic chemical preservatives and fungicide risks. A plant contains a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, reported to have in vitro antifungal properties.

The objective of this study is to evaluate various phytochemicals as antimicrobial agents on different *Aspergillus* species that produce mycotoxin.

2. REVIEW OF LITERATURE

Aspergillus flavus is a contaminating agent, which has the ability to colonize on common crop species such as corn, cotton, peanuts, and many other crops. Economic losses due to the infection of grain crops such as maize (Zea mays L.) by *A. flavus* is not primarily due to the expression of symptoms known as *Aspergillus* earrot but, is due to the subsequent contamination of the grain with the fungal metabolite- mycotoxins called aflatoxin (Jake C. Fountain *et al*.,2014)

Mycotoxins are a group of structurally diverse secondary metabolites produced by various fungal species. These toxic compounds can contaminate foodstuffs, crops or human foods. The ingestion of these contaminated materials may be pathogenic in animals and humans as they may lead to serious health problems, such as liver, kidney or nervous system damage, immune suppression. They comprise a group of several hundreds of chemically different toxic compounds. Aflatoxins (AFs) are a group of mycotoxins produced by the spoilage of fungi *Aspergillus,* particularly *A. flavus* and *A. parasiticus* (Mohamed E. Zain, 2011).

Aspergillus flavus is complex in its morphology and can be classified into two groups:

Group I: Consists of L strains which greater than 400 μm in diameter.

Group II: Consists of S strains which are less than 400 μm in diameter. Both L and S strains can produce the two most common aflatoxins (B1 and B2). S strains can also produce aflatoxin G1 and G2.

The four major Aflatoxins are B1, B2, G1and G2, which can be distinguished by the colour of their fluorescence under ultraviolet light (B, blue; G, green). The International Agency for Research on Cancer (IARC) has clarified B1, B2, G1 and G2 in the group I as human carcinogen. Aflatoxin B1 is the most toxic. It also produces other toxic compounds: cyclopiazonic acid , β-nitropropionic acid and aspergillic acid (Zohra Mohammedi *et al*.,2013)

Aspergillus parasiticus is also known to produce aflatoxins. It is closely related to *Aspergillus flavus* but it will be distinguishable based on DNA sequencing and Amplified fragment length polymorphism fingerprinting.

Aspergillus niger was discovered by van Tieghem in 1967. It produces *gliotoxin* which has been identified in the sera of humans and mice. It is one of the most common species of the

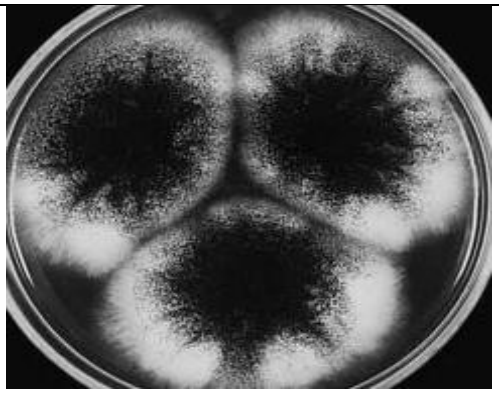
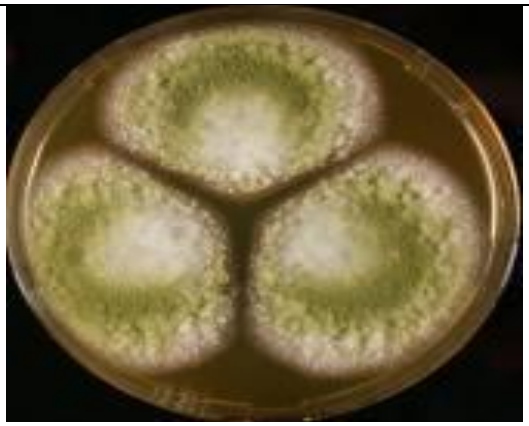
genus *Aspergillus*. It causes a disease called black mould on certain fruits and vegetables such as grapes, apricots, onions, peanuts and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments. Some strains of *A. niger* have been reported to produce potent mycotoxin called ochratoxins. It is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss, and damage to the ear canal (Mohamed K. Refai *et al*., 2013).

Various strains of *Aspergillus niger* are used in the industrial preparation of citric acid and gluconic acid by the World Health Organisation. *A. niger* fermentation is "generally recognized as safe" (GRAS) by the United States Food and Drug Administration. By using A.niger as industrial fermentation the useful enzymes which are produced such as: *A. niger* glucoamylase , pectinases , Alpha-galactosidase and proteases .

Aspergillus terreus, also known as *A. terrestrius*, is a fungus found worldwide in soil. It is a multinational fungus which is primarily isolated from compost, plant material, and from soil. It is more common in tropical or sub – tropical areas. It is isolated occasionally from outer ear canal colonizations. *A. terreus* can cause opportunistic infection in people with deficient immune systems. *A. terreus* is commonly used in industry to produce important organic acids, such as itaconic acid and *cis*-aconitic acid. It is relatively resistant to amphotericin B drug. *A. terreus* produces citrinin and citreoviridin (Mohamed K. Refai *et al*., 2013).

Citrinin is a nephrotoxin produced by *Penicillium* and *Aspergillus* species. Renal damage, vasodilatation, and bronchial constriction are some of the health effects associated with this toxin. Citreoviridin, a mycotoxin, displays the ability to potently inhibit mitochondrial ATPases via uncompetitive inhibition of ATP hydrolysis. Additional research shows that Citreoviridin can inhibit ATP-driven reduction of NAD^+ by succinate and ATP driven NAD transhydrogenase in ox hearts (Paul E. Linnett *et al*., 1978).

TABLE 1: Different *Aspergillus* groups (Mohamed K. Refai et al., 2013)

Group	Morphology	Colonies
Aspergillus niger		Black
Aspergillus flavus		Yellow or green
Aspergillus parasiticus		Yellow, Green
Aspergillus terreus		White& Brown colony coloration

Phytochemical effect on different fungal groups

Natural phytochemicals derived from fruits, vegetables and herbs have been reported to possess a wide range of biological effects, including antioxidant, antimicrobial and anti-inflammatory actions.

In this, eight phytochemicals are tested for anti-fungal activity.

- Caffeine
- Gallic Acid
- Ascorbic Acid
- Quercetin
- Piperine
- Ellagic acid
- Tannic acid
- Cinnamic acid

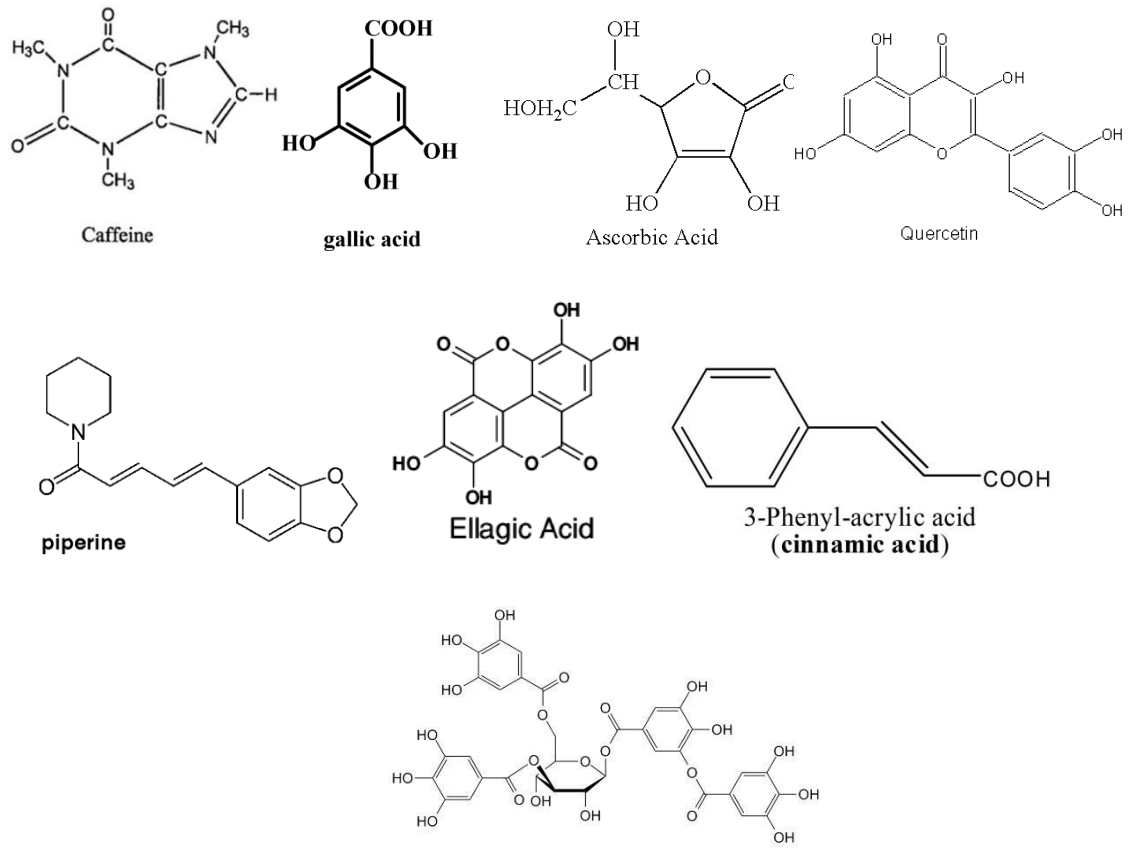


FIGURE 2: Structures of various Phytochemicals used.

Caffeine is a commercially important purine alkaloid synthesized by plants. It is rich in carbohydrates, protein, minerals. It is a water-soluble alkaloid. It is closely related to other alkaloids such as theophylline (mainly found in tea) and theobromine (mainly found in cacao beans). It acts on the nervous system by blocking adenosine receptor thus slowing down nerve cell acitivity. It stimulates the central nervous system, respiration and blood circulation. It also increases the circulation and oxidation of fatty acids. It is used in combination with aspirin to treat headaches. It also relaxes smooth muscle, stimulates cardiac muscle, stimulates diuresis, and appears to be useful in the treatment of some types of headache. Several cellular actions of caffeine have been observed, but it is not entirely clear how each contributes to its pharmacological profile.

Gallic acid is found in almost all plants. It exists in plant material in the form of free acids, esters, catechin derivatives and hydrolysable tannins. The antioxidant activity of gallic acid and its derivatives has been reported in several studies. Pure gallic acid is a colourless crystalline organic powder. Plants which are known for their high gallic acid content are: gallnuts, grapes, tea, hops and oak bark. Gallic acid has been shown to possess antimicrobial activity against human pathogens, a plant pathogen and human pathogenic yeast .Gallic acid is known to exhibit good antifungal activity against *M. grisea* and *Erysiphe graminis.* Gallic acid acts as an antioxidant and helps to protect our cells against oxidative damage. Gallic acid was shows cytotoxicity against cancer cells, without harming healthy cells (Magdalena Karama *et al*., 2006)

Ascorbic Acid is a six carbon compound. Ascorbic acid is an essential nutrient in human diets, and necessary to maintain connective tissue and bone. Its biologically active form, vitamin C, functions as a reducing agent and coenzyme in several metabolic pathways. Vitamin C is considered an antioxidant. It is a well-known anti-microbial compound. The antimicrobial action of ascorbic acid may probably be due to its ability to promote the cyclic reduction of cupric ions which then combine with ion-containing sites within the cell and/or the oxidative products. Vitamin C plays an important role in the body. It is needed to maintain the health of skin cartilage, bone, and blood vessels. It is known as an antioxidant (Naz *et al*., 2013). It is known to show 14.3% inhibition against *A. flavus* at 1% concentration according to Obaleye *et al*. in a study of fungitoxic effects of ascorbic acid and its acid-metal complexes on four standard strains of bacterial species - *Staphylococcus aureus, Escherichia coli, Bacillus subtilis*and *Klebsiella pneumonia* and four fungal species - *Trichophyton* sp., *Penicillium* sp., *Aspergillus flavus* and *Aspergillus niger*.

Quercetin belongs to a group of plant pigments called flavonoids that give many fruits, flowers, and vegetables their color. Flavonoids are ubiquitous in photosynthesising cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. The antimicrobial activity of quercetin has been at least partially attributed to inhibition of DNA gyrase. Quercetin shows anti-inflammatory activity by its direct antioxidant action. It also inhibits the release of histamine, which causes congestion, by basophils and mast cells (Syed Ibrahim Rizvi *et al*., 2006).

Ellagic acid has antioxidant, anti-mutagen and anti-cancer properties. Ellagic acid is present in many red fruits and berries, including raspberries, strawberries, blackberries, cranberries, pomegranate and some nuts including pecans and walnuts. The highest levels of ellagic acid are found in raspberries. It is a fused four-ring polyphenol. Pure ellagic acid is a cream to light yellow crystalline solid. In plants ellagic acid is present in the form of ellagitannin, which is ellagic acid bound to a sugar molecule. It is a natural phenol antioxidant. It has been shown to have antibacterial and anti-inflammatory activities .Medicinally, ellagic acid is used to prevent cancer and treat viral and bacterial infections. The mode of antifungal action of ellagic acid could be associated with the inhibition of fungal cell wall (Mahmoud A. Ghannoum *et al*., 1999).

Cinnamic acid: It is an organic acid occurring naturally in plants that has low toxicity and a broad spectrum of biological activities. Many cinnamic acid derivatives, especially those with the phenolic hydroxyl group, are well-known antioxidants. It is also well known that cinnamic acid has antimicrobial activity. Its derivatives, both isolated from plant material and synthesized, have been reported to have antibacterial, antiviral and antifungal properties. Cinnamic acid is also a kind of self-inhibitor produced by fungal spores to prevent germination (Sova M. *et al*., 2012).

Piperine was the first natural product isolated from *Piper* species back in 1819. Piperine was evaluated for its antimicrobial activity against *Aspergillus niger, Aspergillus flavus* and Fusarium oxysporum. It is the major chemical constituent responsible for the bitter taste of the black pepper (Joaquim V. Marques *et al*., 2010). Piperine and several other amides have shown a variety of biological activity, *example* anti-tumoral, efflux-pump inhibitor, insecticidal and antifungal activity and anti-inflammatory (S.K. Shiva Rani *et al*., 2013). At low concentration, it activated the fenton reaction but at high conc. there is increased generation of hydroxyl radicals. Piperine showed maximum antifungal activity towards *Fusarium oxysporum* (14mm) and very least effect against Aspergillus niger (38mm) (Savita Saini *et al.*, 2016).

Tannic acid is toxic to fungi, bacteria and viruses and inhibits their growth. Tannic Acid is a water-soluble polyphenol containing sugar esters, mainly glucose, phenol carboxylic acid. It is found in a wide variety of plants produced from secondary metabolism of plants. Antibacterial properties of vegetable tannins have been exploited in various fields, particularly in medicine. TA, exhibits antioxidant, antimutagenic and anticarcinogenic properties. It is well known in literature that compounds found in the structure of plant extracts, such as tannic acid, gallic acid, ellagic acid, catechin and essential oils have antimicrobial effect. TA exhibited antimicrobial activity against bacteria, moulds and yeasts (Ali Nail Yapici *et al.,* 2010) .Tannic acid was found to be fungistatic at concentrations of 10, 50, 300 and 500µg/ml, and produced fungicidal effect at 700µg/ml concentration.

MTT Assay

A method that was originally developed as a rapid assay for growth and survival of mammalian lymphoma cells is based on the transformation and colorimetric quantification of MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide]. The respiratory chain and other electron transport systems reduce MTT and other tetrazolium salts and thereby form non-water-soluble violet formazan crystals within the cell. The amount of these crystals can be determined spectrophotometrically and serves as an estimate for the number of mitochondria and hence the number of living cells in the sample. These features can be taken advantage of in cytotoxicity or cell proliferation assays, which are widely used in immunology, toxicology, and cellular biology (Florian M. Freimoser *et al.,* 1999).

MTT assay is fast, simple, cheap, and accurate method for the determination of cell densities of the fungi. In particular, the MTT method proved to be useful to estimate cell densities in small culture volumes and more accurate and reliable than hemocytometer counting. The cultivation in small culture volumes and the sensitive evaluation with the MTT assay allow the screening and testing of many different substances, fractions, and nutrients indispensable to the development of defined media for the cultivation fungi (Florian M. Freimoser *et al.,* 1999).

3. MATERIALS AND METHODS

Aspergillus niger and Aspergillus terreus are used for project work.

1. Identification of *Aspergillus niger* (770032) , *Aspergillus terreus* (860035) strains :

For the identification of *different s*trains in laboratory, Lactophenol Cotton Blue Staining was used. The Lactophenol Cotton Blue (LPCB) is the most widely used method of staining and observing fungi .The preparation has three components:

- Phenol: kills any live organisms;
- Lactic acid : It preserves fungal structures, and
- Cotton blue: It stains the chitin in the fungal cell walls.

The following protocol was used:

- Took a slide and wiped it with ethanol.
- Put a drop of Lactophenol Cotton blue.
- Took the fungal culture from the agar slants with the help of a loop.
- Mixed it well and teased it with the help of a needle.
- Observed it under the microscope at 40 X and 100X.

2. Counting of conidia through Hemocytometer

Hemocytometer is a device used for determining the number of cells per unit volume of a suspension is called a counting chamber. It is the most widely used type of chamber, since it was mainly designed for performing blood cell counts. It is now used to count other types of cells.

TABLE 2: Dimensions of Hemocytometer

Dimensions	Area	Volume at 0.1mm depth
1 x 1 mm	1 mm^2	100 ml
0.25 x 0.25 mm (1/16)	0.0625 mm^2	6.25 ml
0.25 x 0.20 mm (1/20)	0.05 mm^2	5 ml
0.20 x 0.20 mm (1/25)	0.04 mm^2	4 ml
0.05 x 0.05 mm (1/400)	0.0025 mm^2	0.25 ml

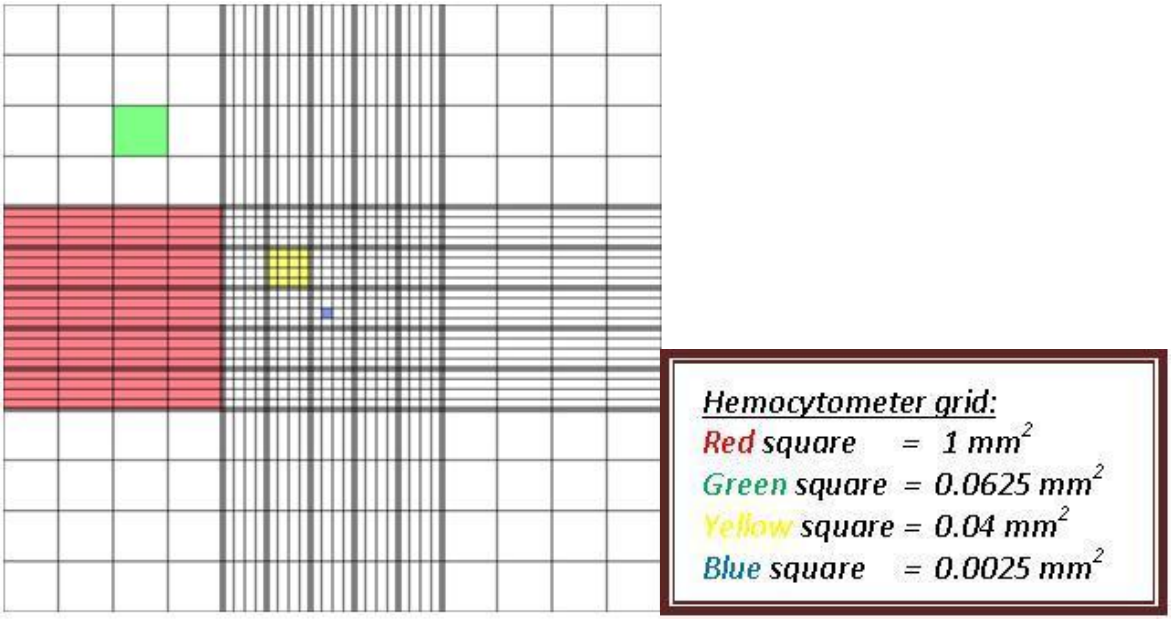


FIGURE 3: Divisions on a Hemocytometer and their dimensions.

The following protocol was used:

- Prepared a spore suspension in 1mL Phosphate Buffer Saline.
- Cleaned all surfaces of the hemocytometer and cover-slip.
- Pipetted approximately 10 µL of the cell suspension into one of the two counting chambers.
- Counted the conidia.

Table 3: Stock solution of different Phytochemicals

Samples	Dilution	Cell suspension	Cell suspension	Stock
AF67 ***(A. flavus)***	10^3	10µl=$0.72*10^5$	1µl=$0.72*10^4$	$1.33*10^8$
8189 ***(A. parasiticus)***	10^3	10µl=$1.09*10^5$	1µl=$1.09*10^4$	$9.17*10^8$
BT05 ***(A. flavus)***	10^3	10µl=$0.65*10^5$	1µl=$0.65*10^4$	$1.5*10^8$
770032 ***(A. niger)***	10^3	10µl=$1.2*10^5$	1µl=$1.2*10^4$	$8.33*10^8$
860035 ***(A. terreus)***	10^3	10µl=$1.49*10^5$	1µl=$1.49*10^4$	$6.71*10^7$

Formula used:

Cells/mL= (n) x 10^4 x dilution factor

Where, n = the average cell count per square of the four corner squares counted.

Phosphate Buffer Saline (PBS/PBST) was used for the maintenance of cell viability. Saline maintains osmotic balance between internal and external environment of cell. Phosphate buffer maintain physiological pH.

It was made using the following composition: Sodium Chloride, Potassium chloride, Disodium Hydrogen Phosphate, Potassium Dihydrogen phosphate and Tween-20.

3. **Colony forming unit (CFU) of *A. niger* (770032) and *A. terreus* (860035)**

Table 4: CFU of *A. niger* & *A. terreus*

Samples	Dilution factor	Total no. of cells	CFU	Inoculum taken
770032*(A. niger)*	10^4	276	$2.76*10^7$	100µl
860035*(A. terreus)*	10^4	165	$1.65*10^7$	100µl

Formula used:

CFU= no. of colonies* dilution factor/ Inoculum taken

4. MTT assay of different *Aspergillus* strains (AF67, BT05, 8189, 870035, 770032) with control

The MTT [3-(4, 5-Dimethylthiazol-2-yl)-2, 5-DiphenyltetrazoliumBromide] assay is a fast and reliable method for colorimetric determination of fungal cell densities. It is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. It is safe, sensitive, in vitro assay for the measurement of cell proliferation. It is best method because it is useful to estimate the cell density at small culture volume.

Reagents: Phosphate Buffered Saline (PBS), sterile Cell culture medium (RPMI), sterile 96-well plate, photochemical, pipettes, sterile tips

MTT solution: 5 mg/ml MTT in PBS.

MTT solvent: DMSO (Dimethyl sulfoxide)

Procedure:

1. **Day 1:** 10 µl of culture and 100 µl of RPMI media added into each well and incubated for 3-4 hours.
2. After 3-4 hours, 50 µl of drug added at different concentration and incubated overnight at 37°C.
3. **Day 2:** 20 µl of 5 mg/ml (stock solution) MTT added into each well.
4. Incubated for 4-6 hours at 37°C in culture hood.
5. After 4-6 hours, the violet colour appeared in 96-well plate.
6. 100 µl of MTT solvent (DMSO) added into each well. After that incubation in dark for 2 hours was done.
7. O.D taken at 570 nm.

The experiment was performed in triplicates for each *Aspergillus* strain (BT05, AF67, 8189, 770032, 870035) used.

Control:

- Methanol
- RPMI media
- Culture+ methanol+ RPMI media

5. MIC_{50} of *Aspergillus niger* (770035) *Aspergillus terreus* (870035)

The MIC_{50} represents the MIC value at which ≥50% of the isolates in a test population are inhibited; it is equivalent to the median MIC value. MIC_{50} values for different phytochemicals on *A. niger* and *A. terreus* was calculated from graph % inhibition VS concentration (µg/ml).

$$\text{Percentage inhibition} = \frac{O.D_{\text{test sample}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}} \times 100$$

Media Used:

Fungal culture was grown in Petri Plates containing Potato Dextrose Agar (PDA) and incubated 37ºC. PDA contains the essentials for bacterial and fungal species to grow and there for widely used. Potato Dextrose Agar is recommended by APHA and F.D.A. for plate counts of yeasts and moulds in the examination of foods and dairy products.

Composition:

Ingredients	**Gms / Litre**
Potato dextrose agar (PDA)	24.0000
Agar	15.000

Suspended 9.6 grams in 400ml of distilled water .Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Preparation of Stock Solutions: Stock solutions of various phytochemicals were made in methanol and water since not every phytochemical dissolves in water or methanol. 10mg of each phytochemical was dissolved in 1mL of methanol/water making a stock solution of 1mg/mL. Specific amount was substituted in 15mL PDA media used for making plates. The mixture was then mixed using a sterilized glass rod to achieve even consistency of the phytochemical throughout the media.

TABLE 5: Preparation of agar plates at different concentrations of phytochemical.

Concentration of Phytochemical (µg/mL)	**Amount pipetted from Stock solution of 1 mg/mL**	
	A. terreus	***A. niger***
1	900	825
100	349.5	274.5
200	126.3	138.75
400	696	129
600	663	900
800	900	987
1000	375	312
2000	354	550.5

Poisoned Food Technique: The antifungal activity of the phytochemicals was evaluated by using Poisoned food technique at different concentrations (1, 100, 200, 400, 600, 800, 1000 and 2000 µg/ml). *Aspergillus niger* MTCC770032 and *Aspergillus terreus* MTCC870035 was inoculated on Potato Dextrose Agar supplemented with phytochemicals extract at different concentrations via 5mm diameter disc and kept up-side down under aseptic conditions at 37ºC. PDA plate without any extract served as control and PDA plate substituted with 2ml of methanol served as methanol control. All the work was carried under aseptic conditions in Laminar Air Flow. Colony diameter was measured after 24, 48 and 36 hours. Data were statistically analysed and percent inhibition of each phytochemical was counted with respect to the control using the following formula.

$$\text{Inhibition Percentage:} \quad \frac{(\text{Control-Test})}{\text{Control}} \times 100$$

4. RESULTS AND DISCUSSIONS

1. Identification of different *Aspergillus* species strain :

The following was observed:

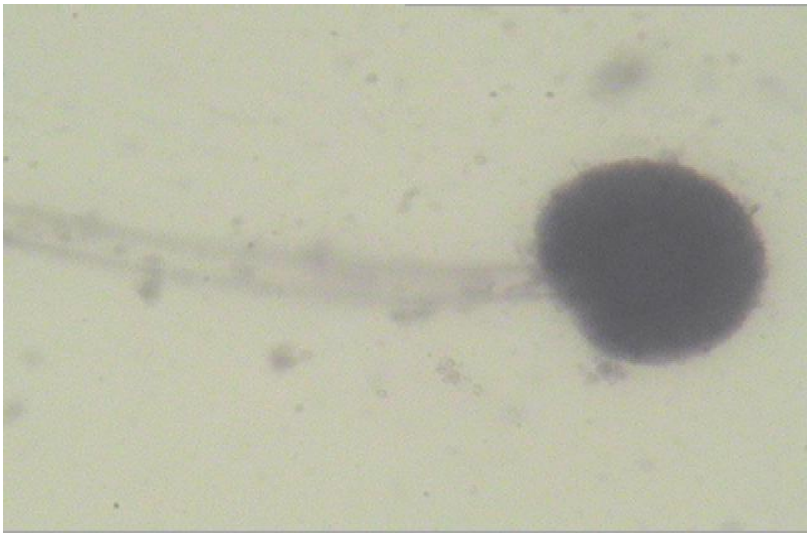


FIGURE 4: *Aspergillus niger under 40X*

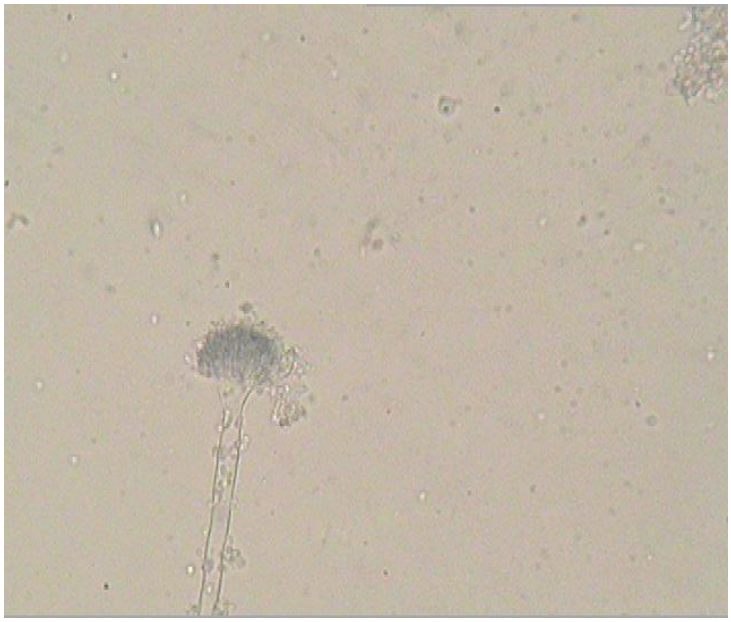


FIGURE 5: *Aspergillus terreus under 40X*

The following structures were observed under the microscope:

- Conidia
- Phialides
- Vesicles
- Conidiophore

Identification was done in order to ensure pure culture for further experimental use. The presence of above structures were a proof that the cultture used was pure.

2. Counting of conidia through Hemocytometer

Counting of conidia through hemocytometer was done to make working solutions of PBST containing $1x10^6$ cells per ml. The stock solution containing indefinite amount of conidia was serially diluted, mostly four times, and conidia count for each of the dilutions was done.

Table 6: Conidia count for *A. niger* and *A. terreus*

Samples	**Dilution Factor**	**Total no. of cells**	**Conidia count**	**Inoculum taken**
A.niger	10^4	223	$2.23*10^8$	10µl
A.terreus	10^4	150	$1.50*10^8$	10µl

3. Poisoned Food Technique: Diameter of each fungal inoculum containing $1x\ 10^6$cells were measured on 1^{st} and 2^{nd} day and % age inhibition was calculated.

MTT Assay:

		8189					
	conc. (µg/ml)	Avg	Control		conc. (µg/ml)	Avg	Control
Caffeine	1	0.325	0.79	Quercetin	1	0.412	1.052
	100	0.37	0.79		100	0.455	1.052
	200	0.3815	0.79		200	0.5	1.052
	400	0.383	0.79		400	0.548	1.052
	600	0.464	0.79		600	0.583	1.052
	800	0.493	0.79		800	0.6045	1.052
	1000	0.514	0.79		1000	0.646	1.052
	2000	0.543	0.79		2000	0.676	1.052
	conc. (µg/ml)	Avg	Control		conc. (µg/ml)	Avg	Control
Ascorbic Acid	1	0.307	1.224	Gallic Acid	1	0.345	0.748
	100	0.4215	1.224		100	0.371	0.748
	200	0.455	1.224		200	0.4138	0.748
	400	0.492	1.224		400	0.4185	0.748
	600	0.55	1.224		600	0.5087	0.748
	800	0.592	1.224		800	0.5305	0.748
	1000	0.61	1.224		1000	0.569	0.748
	2000	0.6413	1.224		2000	0.5875	0.748

	conc. (µg/ml)	Avg	Control		conc. (µg/ml)	Avg	Control
Cinammic Acid	1	0.378	0.895	Ellagic Acid	1	0.464	1.264
	100	0.392	0.895		100	0.4945	1.264
	200	0.4189	0.895		200	0.56	1.264
	400	0.4835	0.895		400	0.585	1.264
	600	0.5245	0.895		600	0.6474	1.264
	800	0.5725	0.895		800	0.69	1.264
	1000	0.6185	0.895		1000	0.7268	1.264
	2000	0.681	0.895		2000	0.736	1.264
	conc. (µg/ml)	Avg	Control		conc.(µg/ml)	Avg	Control
Piperine	1	0.371	1.04	Tannic Acid	1	0.344	1.119
	100	0.4245	1.04		100	0.415	1.119
	200	0.452	1.04		200	0.46	1.119
	400	0.56	1.04		400	0.4915	1.119
	600	0.5495	1.04		600	0.54	1.119
	800	0.618	1.04		800	0.585	1.119
	1000	0.648	1.04		1000	0.6181	1.119
	2000	0.685	1.04		2000	0.6311	1.119

Table 7: OD at different concentration (1µg/ml - 2000µg/ml) for each phytochemical used in experiment with control for *Aspergillus parasiticus* (8189)

		BT05					
	conc. (µg/ml)	Avg	Control		conc.(µg/ml)	Avg	Control
	1	0.4085	0.999		1	0.3965	1.167
Caffeine	100	0.4555	0.999	Quercetin	100	0.4055	1.167
	200	0.4635	0.999		200	0.4135	1.167
	400	0.472	0.999		400	0.4575	1.167
	600	0.4755	0.999		600	0.4815	1.167
	800	0.4885	0.999		800	0.514	1.167
	1000	0.5255	0.999		1000	0.5335	1.167
	2000	0.558	0.999		2000	0.57	1.167
	conc. (µg/ml)	Avg	Control		conc. (µg/ml	Avg	Control
	1	0.3085	1.308		1	0.3245	1.33
Ascorbic Acid	100	0.3505	1.308	Gallic Acid	100	0.3655	1.33
	200	0.4232	1.308		200	0.3721	1.33
	400	0.437	1.308		400	0.4145	1.33
	600	0.565	1.308		600	0.5295	1.33
	800	0.5685	1.308		800	0.5585	1.33
	1000	0.623	1.308		1000	0.6175	1.33
	2000	0.66	1.308		2000	0.6285	1.33

	conc.(µg/ml)	Avg	Control		conc. (µg/ml	Avg	Control
Cinnamic Acid	1	0.331	0.902	Ellagic Acid	1	0.4645	1.15
	100	0.3645	0.902		100	0.475	1.15
	200	0.4266	0.902		200	0.491	1.15
	400	0.4905	0.902		400	0.577	1.15
	600	0.529	0.902		600	0.5855	1.15
	800	0.586	0.902		800	0.589	1.15
	1000	0.6285	0.902		1000	0.6175	1.15
	2000	0.6584	0.902		2000	0.6855	1.15
	conc. (µg/ml)	Avg	Control		conc. (µg/ml	Avg	Control
Piperine	1	0.385	1.045	Tannic Acid	1	0.343	1.018
	100	0.403	1.045		100	0.4145	1.018
	200	0.431	1.045		200	0.4355	1.018
	400	0.48	1.045		400	0.482	1.018
	600	0.501	1.045		600	0.533	1.018
	800	0.545	1.045		800	0.5735	1.018
	1000	0.5805	1.045		1000	0.6155	1.018
	2000	0.6275	1.045		2000	0.6415	1.018

Table 8: OD at different concentration (1µg/ml - 2000µg/ml) for each phytochemical used in experiment with control for *Aspergillus flavus* (BT05)

		AF67					
	conc. (µg/ml)	Avg	Control		conc. (µg/ml	Avg	Control
	1	0.345	0.783		1	0.375	0.856
	100	0.35	0.783		100	0.4185	0.856
Caffeine	200	0.393	0.783	Quercetin	200	0.4725	0.856
	400	0.412	0.783		400	0.4925	0.856
	600	0.429	0.783		600	0.5268	0.856
	800	0.437	0.783		800	0.529	0.856
	1000	0.4545	0.783		1000	0.5375	0.856
	2000	0.4575	0.783		2000	0.585	0.856
	conc. (µg/ml)	Avg	Control		conc. (µg/ml	Avg	Control
	1	0.393	0.99		1	0.375	0.733
	100	0.4175	0.99		100	0.3827	0.733
Ascorbic acid	200	0.4275	0.99	Gallic Acid	200	0.4184	0.733
	400	0.479	0.99		400	0.4255	0.733
	600	0.535	0.99		600	0.4515	0.733
	800	0.54	0.99		800	0.514	0.733
	1000	0.5845	0.99		1000	0.557	0.733
	2000	0.6183	0.99		2000	0.583	0.733

	conc.(µg/ml)	Avg	Control		conc. (µg/ml	Avg	Control
	1	0.304	0.822		1	0.327	0.96
	100	0.3525	0.822		100	0.4105	0.96
Cinnamic Acid	200	0.3615	0.822	Ellagic Acid	200	0.497	0.96
	400	0.4121	0.822		400	0.5705	0.96
	600	0.465	0.822		600	0.6196	0.96
	800	0.5129	0.822		800	0.645	0.96
	1000	0.563	0.822		1000	0.675	0.96
	2000	0.6218	0.822		2000	0.738	0.96
	conc.(µg/ml)	Avg	Control		conc.(µg/ml)	Avg	Control
	1	0.307	0.835		1	0.348	0.854
	100	0.39	0.835		100	0.3905	0.854
Piperine	200	0.436	0.835	Tannic acid	200	0.4255	0.854
	400	0.4722	0.835		400	0.485	0.854
	600	0.5615	0.835		600	0.5108	0.854
	800	0.59	0.835		800	0.566	0.854
	1000	0.6284	0.835		1000	0.582	0.854
	2000	0.6405	0.835		2000	0.627	0.854

Table 9: OD at different concentration (1µg/ml - 2000µg/ml) for each phytochemical used in experiment with control for *Aspergillus flavus* (AF67)

			870035				
	conc. (µg/ml)	Avg	Control		conc.(µg/ml)	Avg	Control
	1	0.4075	0.837		1	0.407	0.851
	100	0.432	0.837		100	0.438	0.851
Caffeine	200	0.4425	0.837	Quercetin	200	0.47	0.851
	400	0.4455	0.837		400	0.522	0.851
	600	0.4705	0.837		600	0.5695	0.851
	800	0.5055	0.837		800	0.6055	0.851
	1000	0.476	0.837		1000	0.6615	0.851
	2000	0.504	0.837		2000	0.7485	0.851
	conc. (µg/ml)	Avg	Control		conc. (µg/ml	Avg	Control
	1	0.3925	0.915		1	0.3155	0.738
	100	0.437	0.915		100	0.348	0.738
Ascorbic Acid	200	0.456	0.915	Gallic Acid	200	0.3795	0.738
	400	0.475	0.915		400	0.404	0.738
	600	0.4865	0.915		600	0.48	0.738
	800	0.495	0.915		800	0.5375	0.738
	1000	0.561	0.915		1000	0.549	0.738
	2000	0.572	0.915		2000	0.6	0.738
	conc. (µg/ml)	Avg	Control		conc. (µg/ml	Avg	Control
	1	0.3275	0.82		1	0.455	0.999
	100	0.373	0.82		100	0.502	0.999
	200	0.386	0.82		200	0.5405	0.999
Cinnamic Acid	400	0.4315	0.82	Ellagic Acid	400	0.5855	0.999
	600	0.57	0.82		600	0.658	0.999
	800	0.637	0.82		800	0.662	0.999
	1000	0.647	0.82		1000	0.7115	0.999
	2000	0.684	0.82		2000	0.745	0.999
	conc. (µg/ml)	Avg	Control		conc.(µg/ml)	Avg	Control
	1	0.3705	0.849		1	0.379	0.856
	100	0.399	0.849		100	0.407	0.856
	200	0.407	0.849		200	0.454	0.856
Piperine	400	0.515	0.849	Tannic Acid	400	0.4715	0.856
	600	0.545	0.849		600	0.4835	0.856
	800	0.63	0.849		800	0.5365	0.856
	1000	0.6455	0.849		1000	0.5435	0.856
	2000	0.6635	0.849		2000	0.652	0.856

Table 10: OD at different concentration (1µg/ml - 2000µg/ml) for each phytochemical used in experiment with control for *Aspergillus terreus* (870035)

		770032					
	conc. (µg/ml)	Avg	Control		conc.(µg/ml)	Avg	Control
	1	0.3935	0.919		1	0.38	0.837
	100	0.411	0.919		100	0.409	0.837
Caffeine	200	0.4675	0.919	Quercetin	200	0.4895	0.837
	400	0.467	0.919		400	0.5215	0.837
	600	0.505	0.919		600	0.5455	0.837
	800	0.5315	0.919		800	0.5575	0.837
	1000	0.5515	0.919		1000	0.5675	0.837
	2000	0.6205	0.919		2000	0.632	0.837
	conc. (µg/ml)	Avg	Control		conc. (µg/ml)	Avg	Control
	1	0.311	1.082		1	0.3015	0.96
	100	0.3265	1.082		100	0.317	0.96
Ascorbic Acid	200	0.3345	1.082	Gallic Acid	200	0.46	0.96
	400	0.495	1.082		400	0.448	0.96
	600	0.505	1.082		600	0.4545	0.96
	800	0.575	1.082		800	0.507	0.96
	1000	0.612	1.082		1000	0.581	0.96
	2000	0.628	1.082		2000	0.596	0.96

	conc. (µg/ml)	Avg	Control		conc. (µg/ml)	Avg	Control
	1	0.379	0.905		1	0.47	1.096
	100	0.3925	0.905		100	0.556	1.096
	200	0.408	0.905		200	0.6185	1.096
Cinnamic Acid	400	0.463	0.905	Ellagic Acid	400	0.649	1.096
	600	0.501	0.905		600	0.651	1.096
	800	0.5045	0.905		800	0.715	1.096
	1000	0.6155	0.905		1000	0.7445	1.096
	2000	0.6675	0.905		2000	0.7835	1.096
	conc. (µg/ml)	Avg	Control		conc. (µg/ml)	Avg	Control
	1	0.419	0.978		1	0.3685	0.985
	100	0.4725	0.978		100	0.4265	0.985
	200	0.4965	0.978		200	0.453	0.985
Piperine	400	0.5535	0.978	Tannic Acid	400	0.5095	0.985
	600	0.6553	0.978		600	0.5315	0.985
	800	0.6565	0.978		800	0.572	0.985
	1000	0.6705	0.978		1000	0.6385	0.985
	2000	0.6865	0.978		2000	0.695	0.985

Table 11: OD at different concentration (1µg/ml - 2000µg/ml) for each phytochemical used in experiment with control for *Aspergillus niger* (770035)

Plotting values obtained by MTT Assay:

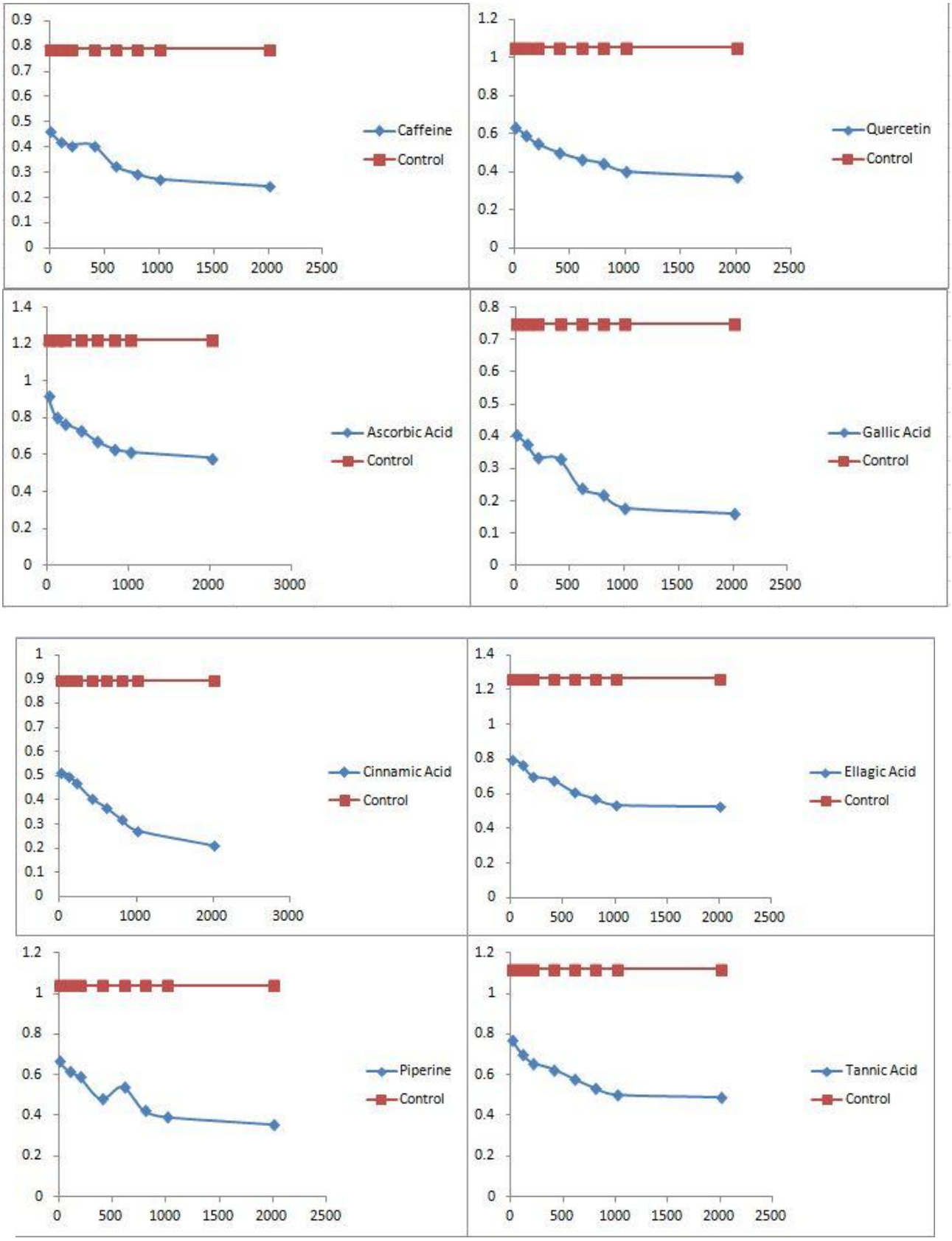


FIGURE 6: Graphs (OD VS Conc.) obtained by MTT Assay of *A. paraciticus* (8189)

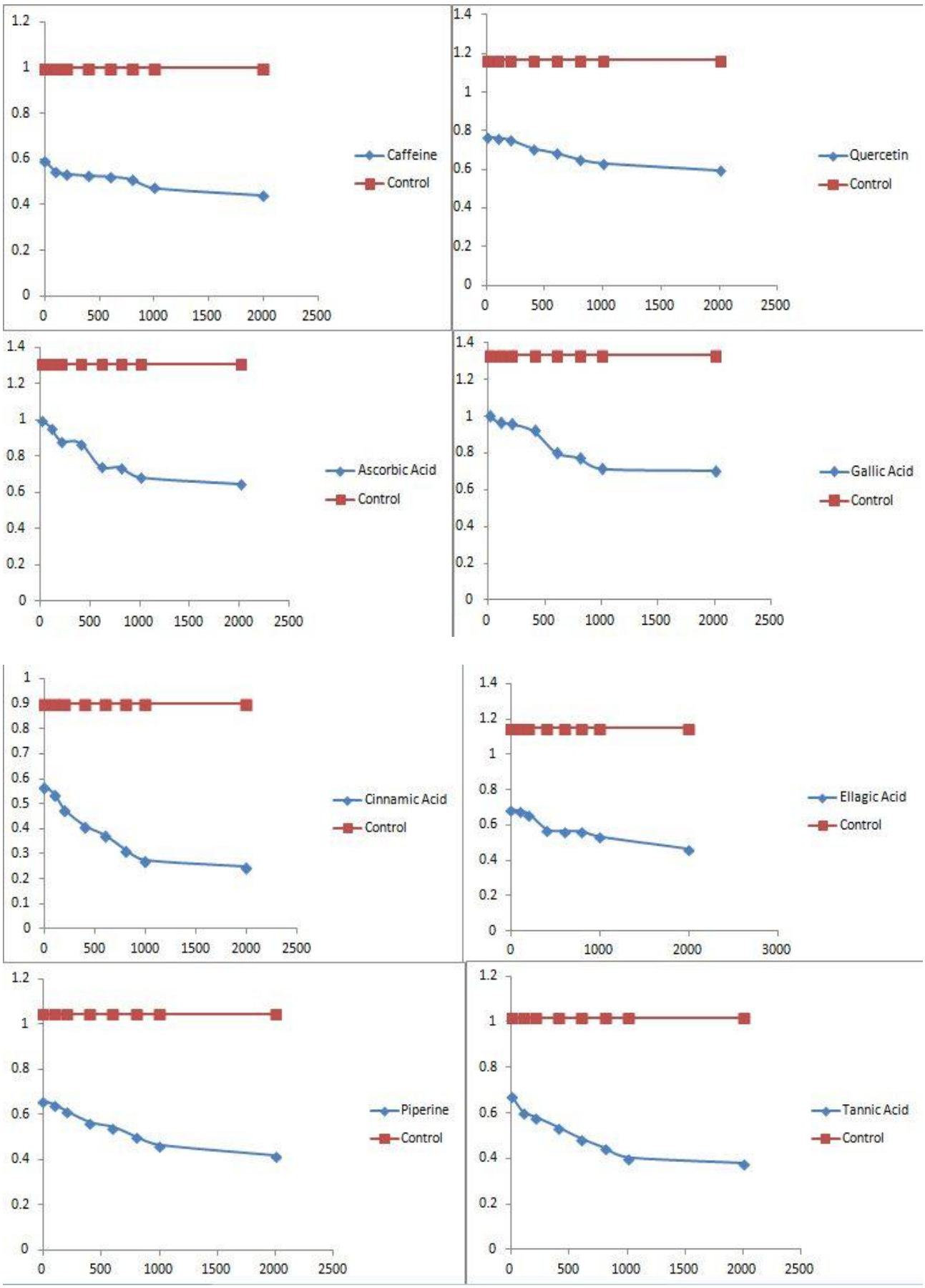


FIGURE 7: Graphs (OD VS Conc.) obtained by MTT Assay of *A. flavus* (BT05)

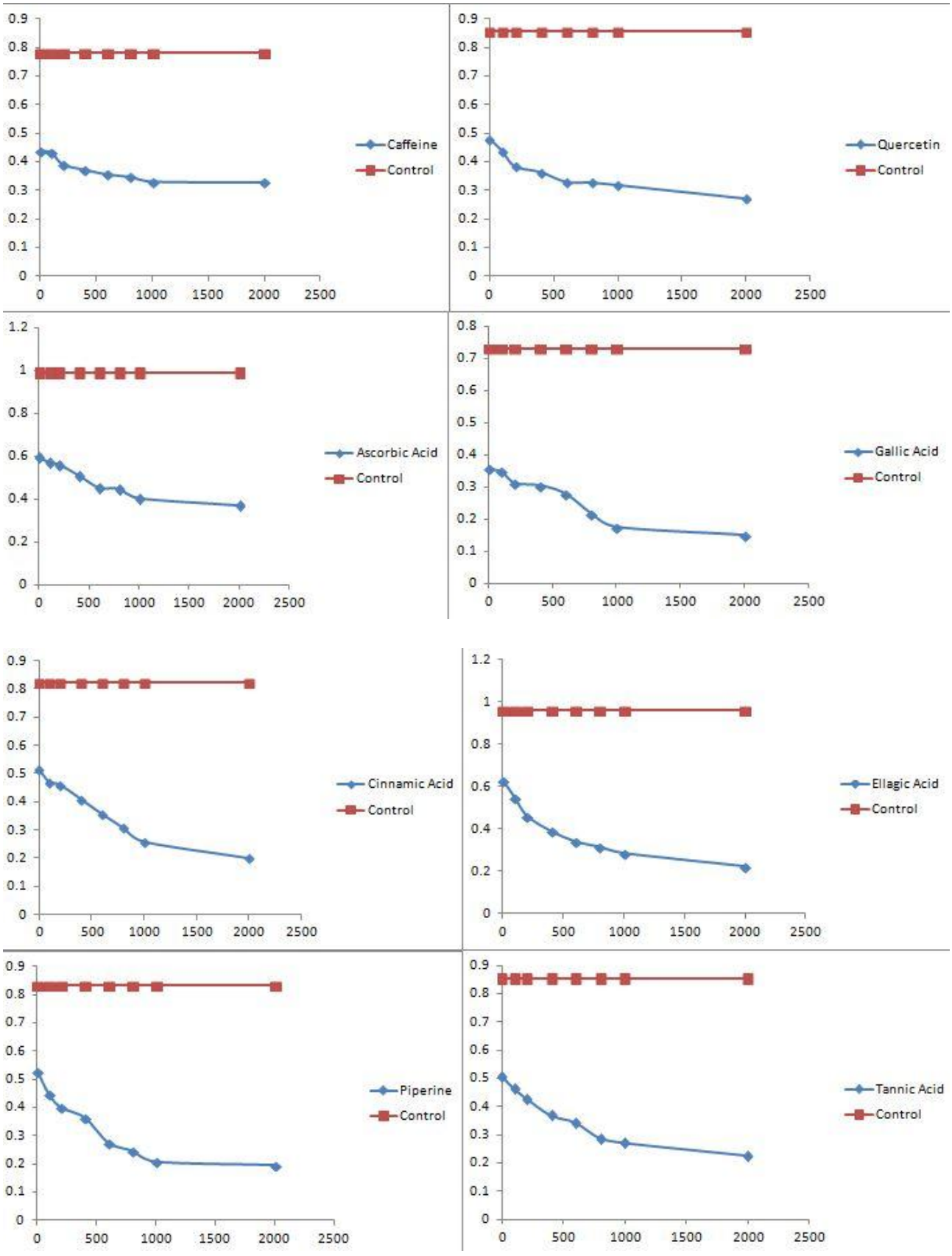


FIGURE 8: Graphs (OD VS Conc.) obtained by MTT Assay of *A. flavus* (AF67)

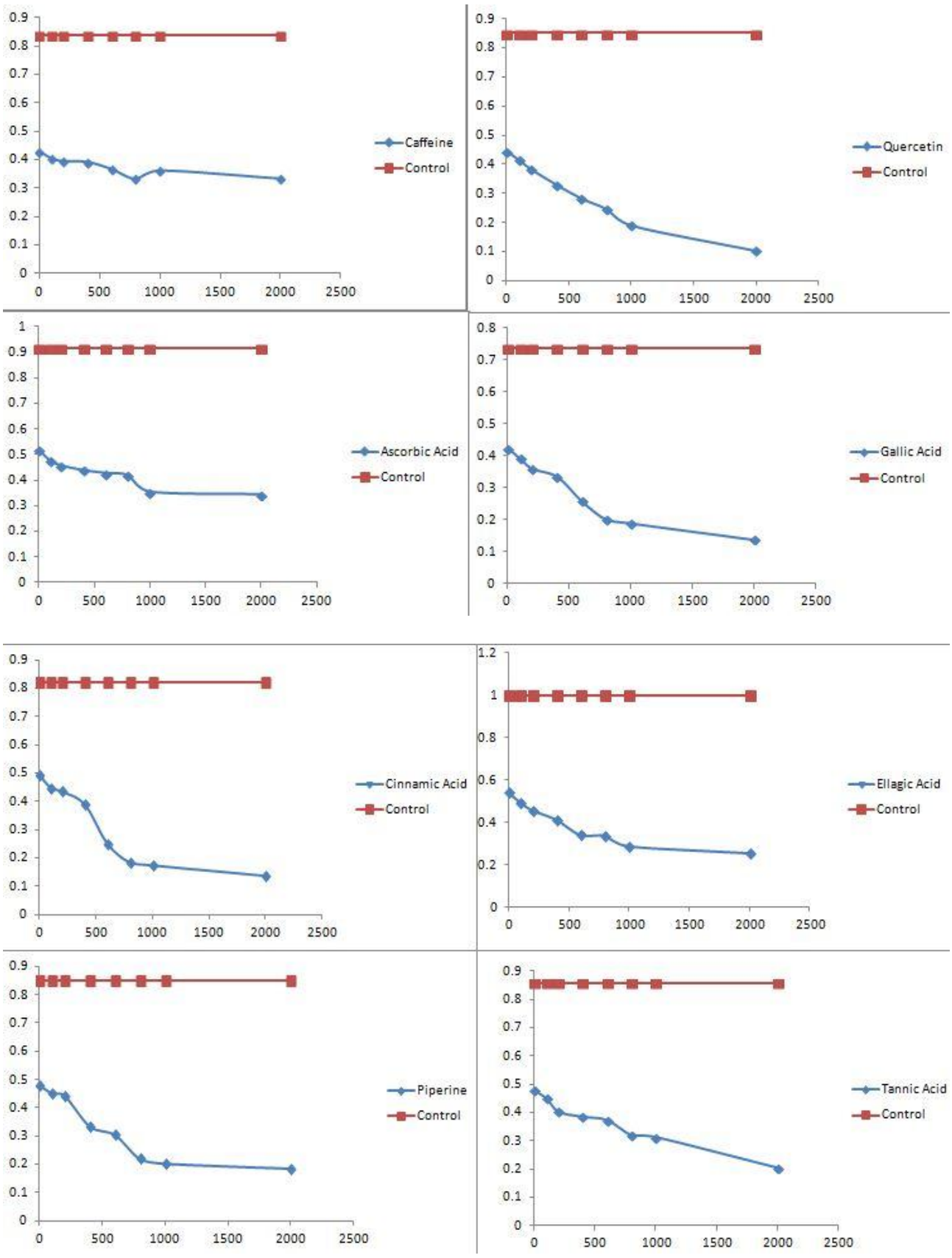


FIGURE 9: Graphs (OD VS Conc.) obtained by MTT Assay of *A. terreus* (870035)

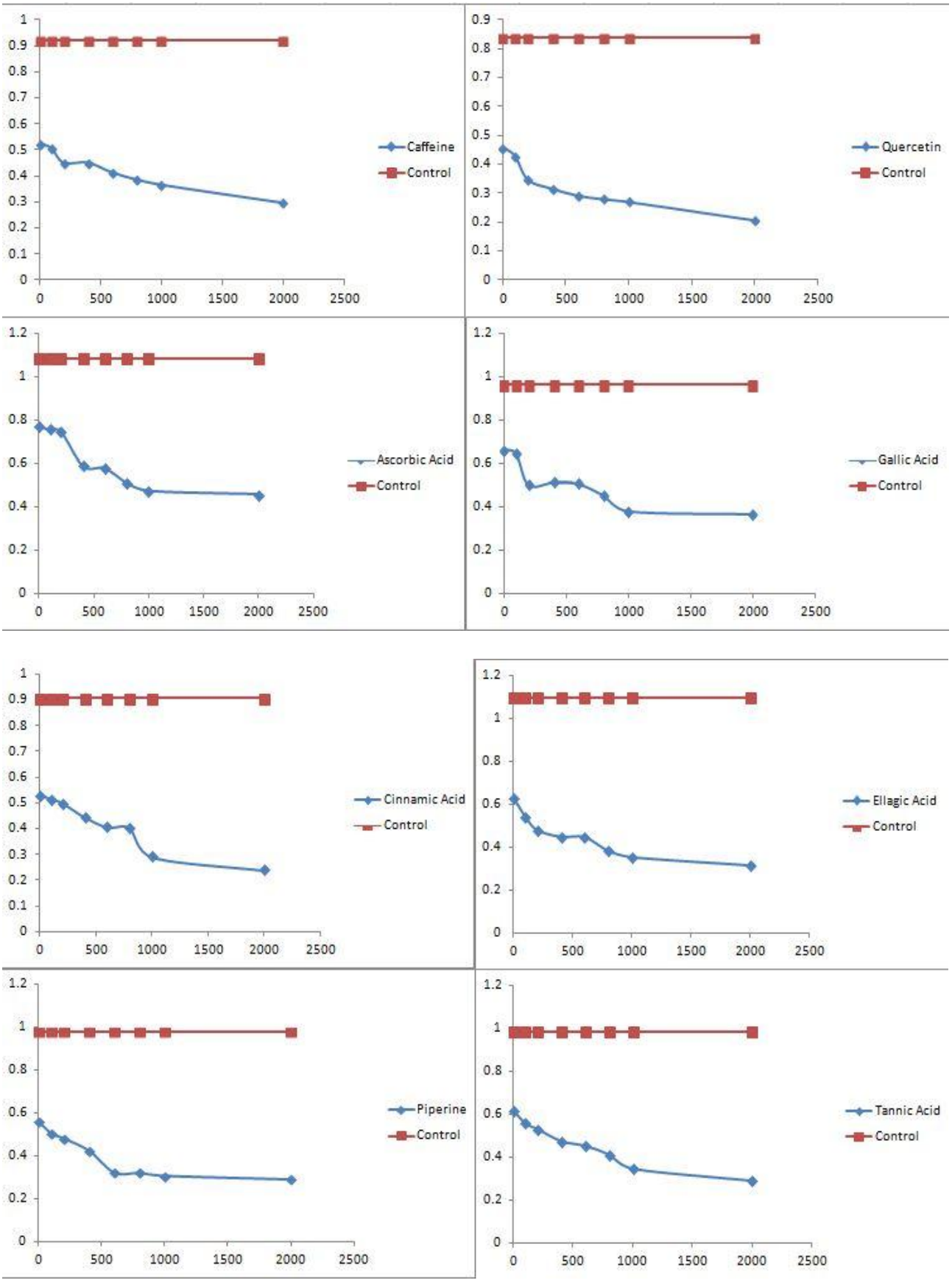


FIGURE 10: Graphs (OD VS Conc.) obtained by MTT Assay of *A. niger* (770032)

870035	% inhibition=OD(test sample)-OD(blank)\OD(control)-OD(blank)*100								
	Caffeine				Quercetin				
conc. (µg/ml)	% inhibition	Control	Avg		conc. (µg/ml)	% inhibition	Control	Avg	
1	41%	0.837	0.4075		1	40%	0.851	0.407	
100	44%	0.837	0.432		100	44%	0.851	0.438	
200	46%	0.837	0.4425		200	48%	0.851	0.47	
400	46%	0.837	0.4455		400	55%	0.851	0.522	
600	49%	0.837	0.4705		600	62%	0.851	0.5695	
800	54%	0.837	0.5055		800	67%	0.851	0.6055	
1000	50%	0.837	0.476		1000	74%	0.851	0.6615	
2000	54%	0.837	0.504		2000	86%	0.851	0.7485	
									OD(BLANK)
	Ascorbic Acid				Gallic Acid				0.105
conc. (µg/ml)	% inhibition	Control	Avg		conc. (µg/ml)	% inhibition	Control	Avg	
1	35%	0.915	0.3925		1	33%	0.738	0.3155	
100	40%	0.915	0.437		100	38%	0.738	0.348	
200	45%	0.915	0.475		200	43%	0.738	0.3795	
400	43%	0.915	0.456		400	47%	0.738	0.404	
600	43%	0.915	0.4565		600	59%	0.738	0.48	
800	48%	0.915	0.495		800	68%	0.738	0.5375	
1000	56%	0.915	0.561		1000	70%	0.738	0.549	
2000	57%	0.915	0.572		2000	78%	0.738	0.6	

	Cinnamic Acid				Tannic Acid			
conc.(µg/ml)	% inhibition	Control	Avg		conc. (µg/ml)	% inhibition	Control	Avg
1	31%	0.82	0.3275		1	36%	0.856	0.379
100	37%	0.82	0.373		100	40%	0.856	0.407
200	39%	0.82	0.386		200	46%	0.856	0.454
400	45%	0.82	0.4315		400	48%	0.856	0.4715
600	65%	0.82	0.57		600	50%	0.856	0.4835
800	74%	0.82	0.637		800	57%	0.856	0.5365
1000	75%	0.82	0.647		1000	58%	0.856	0.5435
2000	80%	0.82	0.684		2000	72%	0.856	0.652
	Ellagic Acid				Piperine			
conc. (µg/ml)	% inhibition	Control	Avg		concentration	% inhibition	Control	Avg
1	39%	0.999	0.455		1	35%	0.849	0.3705
100	44%	0.999	0.502		100	39%	0.849	0.399
200	48%	0.999	0.5405		200	40%	0.849	0.407
400	53%	0.999	0.5855		400	55%	0.849	0.515
600	61%	0.999	0.658		600	59%	0.849	0.545
800	62%	0.999	0.662		800	70%	0.849	0.63
1000	67%	0.999	0.7115		1000	72%	0.849	0.6455
2000	71%	0.999	0.745		2000	75%	0.849	0.6635

Table 12: % inhibition at different concentration of phytochemicals for *A. terreus* (870035)

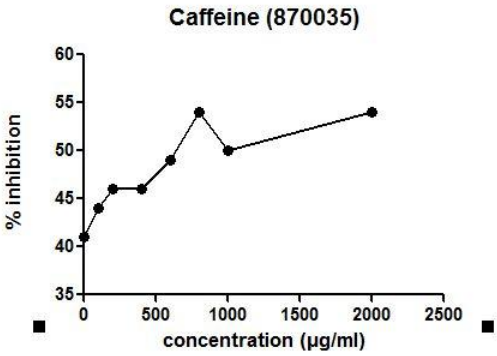
770032									
	Caffeine				Quercetin				
conc. (µg/ml)	% inhibition	Control	Avg		conc. (µg/ml)	% inhibition	Control	Avg	
1	36%	0.919	0.3935		1	37%	0.837	0.38	
100	39%	0.919	0.411		100	41%	0.837	0.409	
200	46%	0.919	0.4675		200	52%	0.837	0.4895	
400	46%	0.919	0.467		400	56%	0.837	0.5215	
600	51%	0.919	0.505		600	60%	0.837	0.5455	
800	54%	0.919	0.5315		800	61%	0.837	0.5575	
1000	56%	0.919	0.5515		1000	63%	0.837	0.5675	
2000	65%	0.919	0.6205		2000	71%	0.837	0.632	OD(BLANK)
									0.105
	Ascorbic Acid				Gallic Acid				
conc. (µg/ml)	% inhibition	Control	Avg		conc. (µg/ml)	% inhibition	Control	Avg	
1	21%	1.082	0.311		1	22%	0.96	0.3015	
100	22%	1.082	0.3265		100	24%	0.96	0.317	
200	23%	1.082	0.3345		200	41%	0.96	0.46	
400	39%	1.082	0.495		400	40%	0.96	0.448	
600	40%	1.082	0.505		600	40%	0.96	0.4545	
800	48%	1.082	0.575		800	47%	0.96	0.507	
1000	51%	1.082	0.612		1000	55%	0.96	0.581	
2000	53%	1.082	0.628		2000	57%	0.96	0.596	

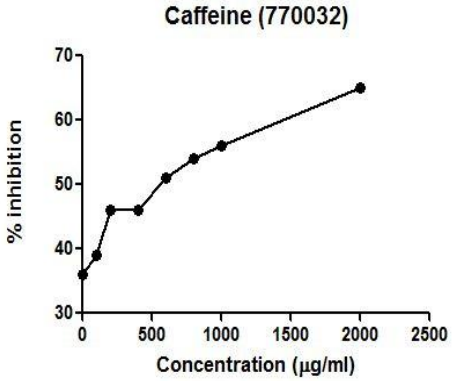
	Cinnamic Acid				Tannic Acid		
conc. (µg/ml)	% inhibition	Control	Avg	conc. (µg/ml)	% inhibition	Control	Avg
1	34%	0.905	0.379	1	29%	0.985	0.3685
100	35%	0.905	0.3925	100	36%	0.985	0.4265
200	37%	0.905	0.408	200	39%	0.985	0.453
400	44%	0.905	0.463	400	45%	0.985	0.5095
600	49%	0.905	0.501	600	48%	0.985	0.5315
800	49%	0.905	0.5045	800	53%	0.985	0.572
1000	63%	0.905	0.6155	1000	60%	0.985	0.6385
2000	70%	0.905	0.6675	2000	67%	0.985	0.695

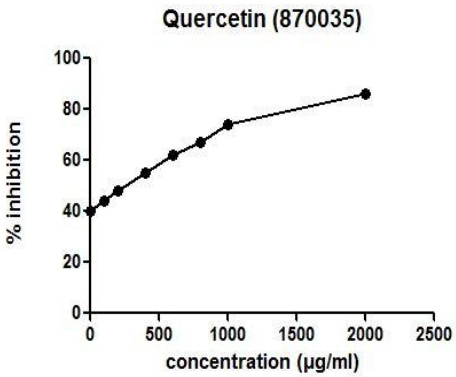
	Ellagic Acid				Piperine		
conc. (µg/ml)	% inhibition	Control	Avg	conc. (µg/ml)	% inhibition	Control	Avg
1	36%	1.096	0.47	1	35%	0.978	0.419
100	45%	1.096	0.556	100	42%	0.978	0.4725
200	51%	1.096	0.6185	200	44%	0.978	0.4965
400	54%	1.096	0.649	400	51%	0.978	0.5535
600	55%	1.096	0.651	600	63%	0.978	0.6553
800	61%	1.096	0.715	800	63%	0.978	0.6565
1000	64%	1.096	0.7445	1000	64%	0.978	0.6705
2000	68%	1.096	0.7835	2000	66%	0.978	0.6865

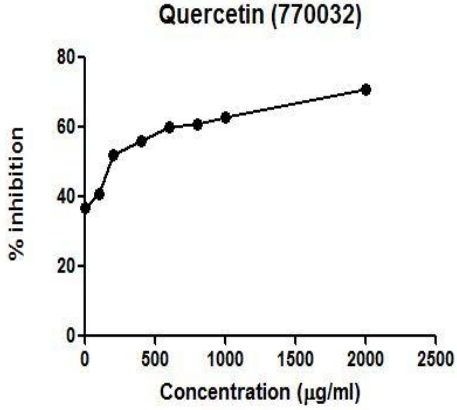
Table 13: % inhibion at different concentration of phytochemicals for *A. niger* (770032)

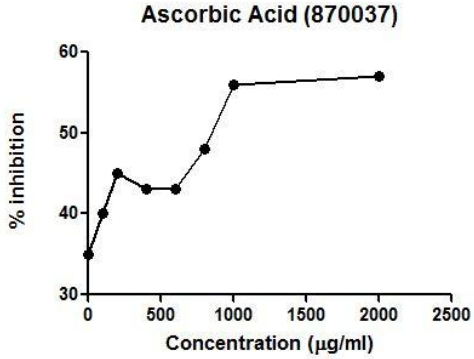
Graphs for calculating MIC_{50} of *Aspergillus terreus* (870035) and *Aspergillus niger* (770032)

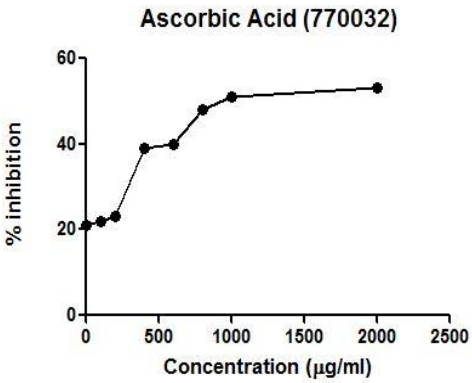


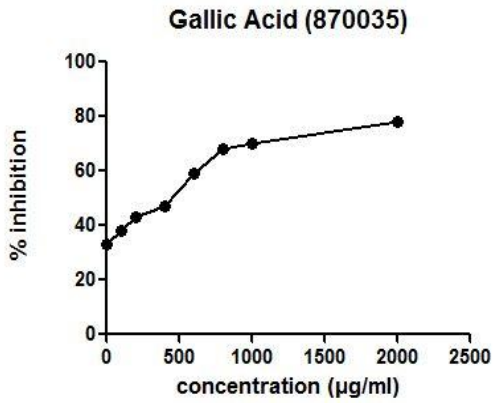


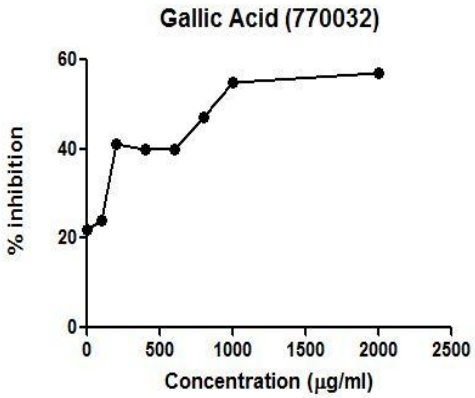


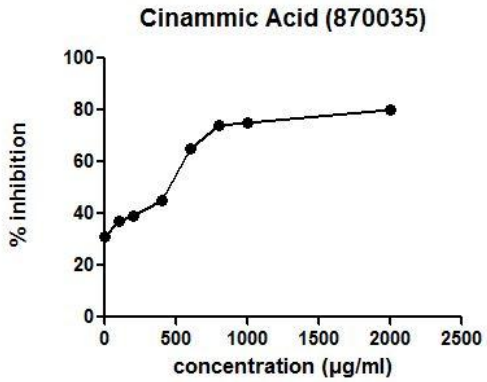


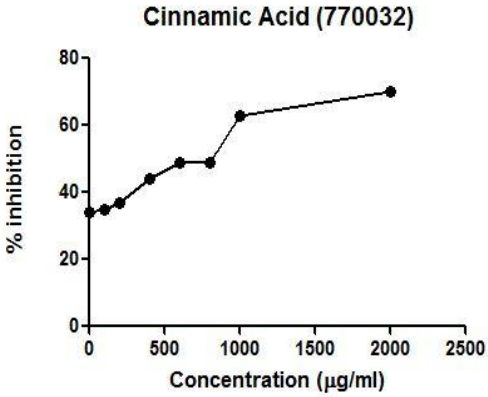


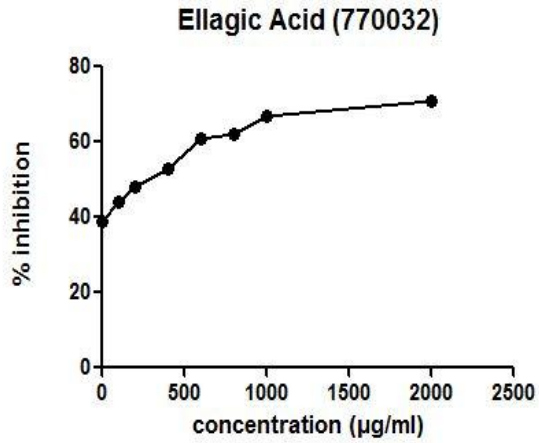


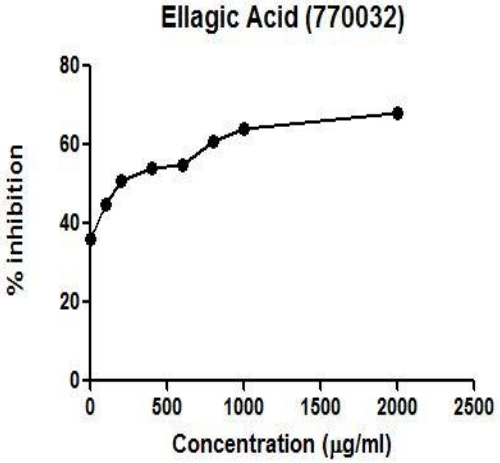

Gallic Acid (870035)
% inhibition
concentration (µg/ml)

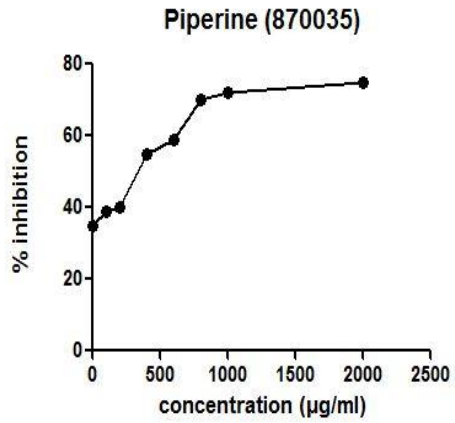

Gallic Acid (770032)
% inhibition
Concentration (µg/ml)

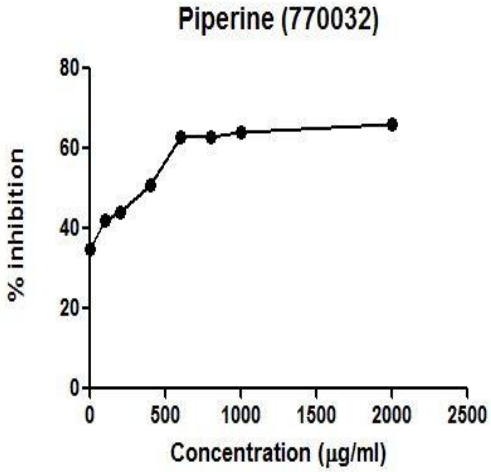

Cinammic Acid (870035)
% inhibition
concentration (µg/ml)

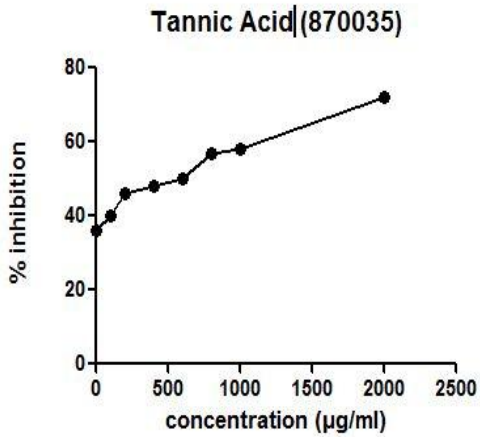

Cinnamic Acid (770032)
% inhibition
Concentration (µg/ml)


Ellagic Acid (770032)
% inhibition
concentration (µg/ml)


Ellagic Acid (770032)
% inhibition
Concentration (µg/ml)







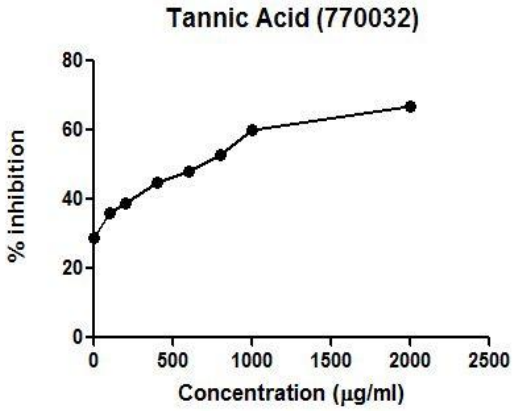


Table 14: Different values of MIC_{50} (µg/ml) obtained from above graphs for each phytochemical used in experiment for *A. terreus* (870035) and *A. niger* (770032)

	***A. terreus* (870035) (µg/ml)**	***A. niger* (770032) (µg/ml)**
Caffeine	600	550
Quercetin	233	183
Ascorbic Acid	842	925
Gallic Acid	464	860
Cinnamic Acid	442	600
Tannic Acid	600	658
Ellagic Acid	250	208
Piperine	236	367

CONCLUSION

MTT Assay: From this experiment it was observed that among eight phytochemical used (Caffeine, Quercetin, Ascorbic Acid, Gallic Acid, Cinnamic Acid, Ellagic Acid, Piperine and Tannic Acid) Quercetin showed maximum inhibition in all *Aspergillus* strains used (*A.flavus* MTCCBT05, *A. flavus* MTCCAF67, *A. parasiticus* MTCC8189, *A. terreus* MTCC870035 and *A. niger* MTCC770032). Further MIC_{50} was performed based on results obtained from MTT Assay for organism *Aspergillus terreus* MTCC870035 and *Aspergillus niger* MTCC770032.

MIC_{50}: It was observed that among all eight phytochemical used, Quercetin showed maximum inhibition in both A. terreus MTCC870035 and A. niger MTCC770032 followed by Piperine, Ellagic Acid, Cinammic Acid, Gallic Acid, Caffeine and Tannic Acid in *A. terreus* MTCC870035 while in case of *A. niger* MTCC770032 after Quercetin, Ellagic Acid and Piperine shows promising inhibitory effect followed by Caffeine, Cinnamic Acid, Tannic Acid and Gallic Acid. Ascorbic Acid shows minimum inhibition in both *A. niger* MTCC770032 and *A. terreus* MTCC870032.

Poisoned Food Technique: From this experiment it was observed that Quercetin, Piperine and Ellagic acid proved to be promising candidates as environment friendly antimicrobial compounds. Caffeine also showed promising results for *A. niger* MTCC770032 and *A. terreus* MTCC870032.

FUTURE PROSPECTS

This study, therefore, may form basis for future studies on combinations, derivatives, metal-complexes etc. of these phytochemicals to fight fungal infections better than conventional chemical pesticides. This study may also be utilized in developing novel therapeutic drugs from such phytochemicals in combination with pre-existing therapeutics in order to control, manage and cure deadly diseases like Invasive Aspergillosis. Likewise, identification of medicinal plant species which possess such antimicrobial compounds against plant pathogens may be useful in characterization of inhibitory fractions, their synthesis and development for management of plant diseases.

Natural products have evolved to encompass a broad spectrum of chemical and functional diversity. It is this diversity, along with their structural complexity, that enables nature's small molecules to target a nearly limitless number of biological macromolecules and too often do so in a highly selective fashion. Because of these characteristics, natural products such as phytochemicals have seen great success as antimicrobial agents.

The number of new phytochemicals being discovered yearly and the relatively few bioassays for potential fungicide activity of photochemical indicates that this area has a bright future. Chemical modification of these compounds and their use to discover new modes of action greatly expand the scope for future work. The success in this effort would greatly decrease the most used form of synthetic fungicides which contain chemicals. Furthermore, chemical modification of these compounds and their use to discover new modes of action greatly expand the scope of future work.

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