

**“ANTIMICROBIAL, ANTIDIABETIC, GLUCOSE UPTAKE ACTIVITY AND  
ANTICANCER (He La CELLS AND DNA DAMAGE) POTENTIALS OF  
*TRITICUM AESTIVUM* AND *ANDROGRAPHIS PANICULATA*”**

**POORNI KIRTHI. V  
(16PFN013)**

**A THESIS SUBMITTED TO THE  
AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER  
EDUCATION FOR WOMEN, COIMBATORE- 641043.**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF  
MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION**

**APRIL 2018**

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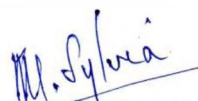
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**Certified as a Bonafide Research Work**



**Signature of the Supervisor**



**Signature of the Head of the  
Department**

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# INTRODUCTION

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# REVIEW OF LITERATURE

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# **METHODOLOGY**

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## **SUMMARY AND CONCLUSION**

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## I.INTRODUCTION

Increased urbanization and unhealthy lifestyle characterized by a shift towards sedentary lifestyle and decreased energy expenditure are considered the main drivers of epidemiological transition. In particular, developing countries are facing a double burden caused by coexisting under and over nutrition, which causes a change in the disease profile from infectious diseases to a chronic degenerative pattern.

Food is the major source of supply to the nutritional needs of the body. But the changing modernized food and life style patterns result in poor nutrition and imbalance between antioxidants and oxidants. This imbalance ultimately leads to production of free radicals by both exogenous and endogenous factors and cause oxidative stress. Oxidative stress is known to be involved in the pathogenesis of lifestyle-related diseases including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies.

India being a land of diverse groups of people with their own religion, beliefs, culture, language and dialects. Thus, diverse medicinal systems have developed in this country. A number of medicinal systems have also been introduced here from outside and have enriched in India. Since ancient times, Indian society depends on traditional medicinal systems. Introduction of allopathic drugs during British era and neglect of Indian traditional medicine by British rulers are responsible for the widespread erosion of Indian traditional medicine (Pandey *et al.*, 2013).

### ***“Nature itself is the best physician” - Hippocrates***

Nature has been a source of medicinal agents for thousands of years and since the beginning of mankind. The application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession. They are considered as an important source for the therapeutic remedies of various ailments.

Cervical cancer lesions are a major threat to the health of the women, representing the second most common cancer worldwide. Human papillomaviruses (HPVs) have been identified as the major etiological factor in cervical carcinogenesis. Cervical cancer is a major cause of cancer mortality in women and more

than a quarter of its global burden is contributed by developing countries. In India, in spite of alarmingly high figures, there is no nationwide government-sponsored screening program. Despite intensive research for the prevention and treatment of cancer, the number of deaths due to cancer is continuously increasing and it remains a leading cause of morbidity and mortality in the world (Dolmenico *et al.*, 2011).

Although the efficacy of chemotherapy for majority of cancer types has increased, these drugs cause several toxic effects, ultimately leading to reduction in the quality of life. Furthermore, a growing worldwide interest in the use of phyto-pharmaceuticals as complementary or alternative medicine, either to prevent or to ameliorate many diseases, has been noted in recent years. It is believed that about 80% of world's population use plants as their primary source of medicinal agents (Sani *et al.*, 2017).

Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. In 2000, India (31.7 million) topped the world with the highest number of people with Diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively (Kaveeshwar *et al.*, 2014).

India is the largest producer of medicinal plants. Around 25,000 effective plant-based formulations are used in traditional and folk medicine. Medicinal plants are always the key source of drug or treatment strategy in different traditional medicinal systems. As powders, extracts, decoctions or infusions, plants are being used in the traditional systems of medicine in many parts of the world, especially in rural communities, for the control, management and treatment of a various degenerative diseases (Sen *et al.*, 2016).

In recent years, many people are choosing plant based medicines or products to improve their health conditions or as curative substance either alone or in combination with others. A long history of natural products in ancient times and in folk medicine around the world is the basis for the use of many therapeutic agents in modern medicine.

Since ages, more than 3000 plant species have been used for the chemotherapy and chemoprevention. According to World Health Organization, 80 percent of population in some Asian and African countries still depends upon tradition herbal medicine for the prevention of many diseases, most of which involve plant extracts. The effectiveness of the plant extracts is mainly due to the presence of bioactive constituents like phenolics, flavonoids and other bioactive components.

Studies have suggested that polyphenols due to the presence of double bond and hydroxyl group, display high antioxidant properties which help in reducing the risk of development of various degenerative diseases, including cancer. Therefore, in recent years many efforts have been made to identify natural, potent anticancer agents with low toxicity and high antioxidant activity.

In 2002, the U.S. National Center for Complementary and Alternative Medicine of National Institutes of Health began funding clinical trials about the effectiveness of herbal medicines. In 2010, a survey of 1000 plants was completed, out of which 356 clinical trials had published evaluating their pharmacological activities and therapeutic applications. One of these plants, wheatgrass, has been an integral part of Indian culture for thousands of years, and has been known to have remarkable healing properties (Rana *et al.*, 2011).

Wheatgrass has a potent antioxidant efficacy and has been used as a health drink to cure many diseases in folk medicine. Wheatgrass packs a nutritional punch of 860 mg protein, 18.5 mg chlorophyll, 15 mg calcium, 38 mg lysine, 7.5 mg vitamin C and an abundance of micronutrients, such as B complex vitamins and amino acids (per 3.5 grams). Wheat grass extract, which contains chlorophyll, an antioxidant, prevents cancer and other metabolic disorders. Additionally, selenium and laetrile present in wheatgrass have anti-cancer properties. Selenium builds a strong immune system, and can decrease the risk of cancer (Sareen *et al.*, 2014).

Dr. Wigmore., (2012) report that the “wheatgrass” used in her program contain abscisic acid and laetrile, both of which may have anti-cancer activity. It was also reported that young grasses and other chlorophyll-rich plants are a safe and effective for treatment of ailments such as high blood pressure, some cancers, obesity, diabetes,

gastritis, ulcers, pancreas and liver problems, fatigue, anemia, asthma, eczema, hemorrhoids, skin problems, halitosis, body odor and constipation.

Abundance of natural fiber in wheatgrass optimizes blood sugar levels. Instrumental characterization of wheatgrass (spray dried powder of juice) confirmed the presence of chlorophyll, which is believed to be the pharmacologically active component in wheatgrass, acting as an anti-diabetic agent. Two ounces of wheatgrass juice has the nutritional equivalent of five pounds of the best raw organic vegetables (Shakya *et al.*, 2012).

*Andrographis paniculata* (kalmegh) belongs to the *Acanthaceae* family and is popularly known as King of bitters in English. It is the main source of the bitter principle 'andrographolide'. It is extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases in Indian traditional system as well as in tribal medicine applications. The therapeutic value of Kalmegh is due to its mechanism of action by enzyme induction (Geetha *et al.*, 2017).

*A. paniculata* extracts have anti-hypertensive effects and decrease plasma angiotensin converting enzyme (ACE) activity, and lipid peroxidation in kidneys. The plant extract also exhibits antityphoid, antifungal, antimalarial, antithrombogenic, anti-snake venom and antipyretic properties. Besides this it is also used as an immunostimulant agent. In vitro anticancer activities of different solvent derived extracts of *A. paniculata* leaves have been reported against different human cancer cell lines are neuroblastoma (IMR-32) and human colon (HT-29) cells.

Synergy is an important concept, as the practice of herbal medicine often entails the combination of several herbs in a thoughtful, rational and systematic way. It can also mean that the combined effect of a number of herbal components is actually greater than the sum of each of the individual components. When herbs are combined, the resulting effect is generally more powerful than when the herbs are taken alone. Currently, combination therapies are employed for the treatment of critical diseases, such as cancer, acquired immunodeficiency syndrome (AIDS) and pulmonary tuberculosis, in order to achieve enhanced therapeutic effects (Glynn *et al.*, 2012).

The “one drug, one target, one disease” approach has for some time remained the conventional pharmaceutical approach to the development of medicines and treatment strategies. However, over the last decade, this mono-substance therapy model has gradually shifted toward the adoption of combination therapies, in which multiple active components are employed. This paradigm shift has been partly driven by its limited effectiveness in chronic diseases, treatment resistance, and side effects of synthetic mono-drugs. Recent evidence has demonstrated that combination therapy could provide greater therapeutic benefits to diseases such as AIDS, cancer, atherosclerosis and diabetes, all of which possess complex etiology and pathophysiology and therefore are difficult to treat using single drug target approach (Zhouet *al.*, 2016)

The quest to find new therapeutic potentials from natural biodiversity, particularly plants, has been the prime interest among researchers. Furthermore, a growing worldwide interest in the use of phyto-pharmaceuticals as complementary or alternative medicine, either to prevent or to ameliorate many metabolic diseases, has been noted in recent years. *Triticum aestivum* and *Andrographis paniculata* are two indigenous plants used widely against a number of diseases according to Indian Traditional Medicine systems. Through these herbs find multifold use, yet there is no scientific evidence on its synergistic potential and Glucose Uptake Activity. Hence the present study titled “**Antimicrobial, Antidiabetic, Glucose Uptake activity and Anticancer (He La Cells and DNA Damage) Potentials of *Triticum aestivum* and *Andrographis paniculata***” was undertaken with the following objectives:

- Grow plants in a suitable environment.
- Harvest *Triticum aestivum* and *Andrographis paniculata* at the right stage of growth for analysis.
- Identify the phytochemicals and antioxidants in exclusive and combination extracts of *Triticum aestivum* and *Andrographis paniculata*.
- Identify the best combination extract of *Triticum aestivum* and *Andrographis paniculata*.

- Study the efficacy of synergistic potential of best combination extract on antimicrobial, DNA Damage, antidiabetic, anticancer and Glucose Uptake Activity.

## II. REVIEW OF LITERATURE

The review of literature pertaining to the study entitled “**Antimicrobial, Antidiabetic, Glucose Uptake activity and Anticancer (He La Cells and DNA Damage) Potentials of *Triticum aestivum* and *Andrographis paniculata***” is reviewed under the following headings:

- A. *Triticum aestivum* – The Green blood
- B. *Andrographis paniculata* – The King of Bitters
- C. Cervical Cancer and Diabetes mellitus –An overview
- D. Synergistic Activity of Plants

### **A. TRITICUM AESTIVUM – THE GREEN BLOOD**

#### **1. *Triticum aestivum***

Wheat grass (*Triticum aestivum*), belong to *Poaceae* family. At the germinated stage, the leaves of wheat over 6-10 days are commonly called wheatgrass (Sharma *et al.*, 2013). The culms are simple, hollow or pithy, glabrous, and the leaves are approximately 20-38 cm long, flat, narrow and 1.3 cm broad. The spikes are long, slender, dorsally compressed and somewhat flattened (Rana *et al.*, 2011). Wheat grass is an inexpensive and efficient source of food to provide all the required nutrients and therapeutic potentials for a healthy and rejuvenating body (Ashish *et al.*, 2012). It is a nutrient-rich type of young grass in the wheat family, is many times richer in levels of vitamins, minerals and proteins as compared to seed kernel, or grain products of the mature cereal plant (Tirgarl *et al.*, 2011).

#### **2. Nutrient content**

Scientific reports reveal that wheat grass is rich in chlorophyll, minerals like magnesium, selenium, zinc, chromium, antioxidants like beta-carotene (provitamin A), vitamin E, vitamin C, antianemic factors like vitamin B12, iron, folic acid, pyridoxine and many other minerals, amino acids and enzymes, which have significant nutritious and medicinal value. (shodhganga .infibinet .ac.in /2012)

Wheat grass can be followed back in history more than 5000 years, to antiquated Egypt and maybe even early Mesopotamian civilizations. It is indicated that old Egyptians discovered the youthful verdant razor sharp edges of wheat and prized them for their constructive outcome on their wellbeing and imperativeness. The consumption of wheatgrass in the Western world started in the 1930s as a consequence of tests led by Charles F. Schnabel in his endeavours to promote the plant. By 1940, jars of Schnabel's powdered grass were at a bargain in significant medication stores all through the United States and Canada. The wheatgrass that is ensured natural delivers high contents of chlorophyll, catalysts and vitamins. The wheatgrass seed started engrossing regular supplements from dirt arranged utilizing natural systems containing no hereditarily built items, in readiness to turn into a tall, productive plant. At this stage, the plant has the supplement profile like that of other verdant green vegetables and will keep on growing until these supplements achieve their crest levels, at around three inches long (Panwar ., 2015).

Wheat grass juice ( Green Blood) is the richest source of vitamins A, B, C, E and K, calcium, potassium, iron, magnesium, sodium, sulphur and 17 forms of amino acids (Mujoriya *et al.*, 2011). It provides excellent nutrition for humans and animals. It is rich in vitamin A, C, E, small amounts of niacin, thiamine, riboflavin, pantothenic acid and vitamins B12 and B6 (<https://www.thesuperfoods.net/wheatgrass/wheatgrass-nutritional-value>).

Wheatgrass has plenty of phosphorous and potassium. It also has good quantities of sodium, calcium, magnesium and small quantities of selenium, zinc, iron, choline and folic acid (<https://www.thesuperfoods.net/wheatgrass/wheatgrass-nutritional-value>). Suryavathana *et al.*, (2016) found that the amount of carbohydrate and protein present in *Triticum aestivum* was 160 mg/g and 120 mg/g.

Wheatgrass packs a nutritional punch of 860 mg protein, 18.5 mg chlorophyll, 15 mg calcium, 38 mg lysine, 7.5 mg vitamin C(per 3.5 grams) and an abundance of micronutrients, such as B complex vitamins and amino acids (Sareen *et al.*, 2014).

Wheat grass contains vitamins A, B1, 2, 3, 5, 6, 8, and 12; C, E and K, ascorbic acid, dehydrated ascorbic acid, carotene, sulfur, sodium, aluminium, copper, calcium, iodine, phosphorus, magnesium, alkaline earth metal, potassium, selenium, iron, zinc, boron and molybdenum (Singh *et al.*, 2012). Sareen *et al.*, 2014 suggested the dosage of thirty ml of fresh wheat grass juice twice a day or three gram wheat grass powder in a glass of water for physical well being. Little honey may be added to juice to enhance taste.

### **3. Therapeutic potentials**

The molecular structure of chlorophyll in wheat grass and haemoglobin in the human body is similar and because of this wheat grass is called "GREEN BLOOD". The pH factor of human blood is 7.4. The pH factor of the wheat grass juice is also 7.4 which may be the reason of quick absorption of wheat grass juice in the blood. The various enzymes responsible for its pharmacological actions are protease, amylase, lipase, cytochrome oxidase, transhydrogenase and super oxide dismutase (SOD) (Kumaret *al.*, 2016). Instrumental characterization of wheatgrass (spray dried powder of juice) confirmed the presence of chlorophyll, which is believed to be the pharmacologically active component in wheatgrass, acting as an anti-diabetic agent (Shirudeet *al.*, 2011).

*Triticum aestivum* leaf extract plays a vital role in liver enzyme activities as well as lipid peroxidation (Arya *et al.*, 2011). Jain *et al.*, (2007) reported the hepatoprotective role of fresh wheatgrass juice has in CCl<sub>4</sub> treated rats. It showed a significant hepatoprotective effect with a dose of 100mg/kg/day in terms of SGOT, SGPT, ALP and Bilirubin in serum.

Wheatgrass juice may also inhibit hematological toxicity related to chemotherapy in breast cancer patients (Barsela *et al.*, 2007). It is also observed that people undergoing chemotherapy, who are on wheatgrass, have sailed through this treatment, and have not required hospital admission, while side effects such as nausea, vomiting and mouth ulcers have been virtually non-existent (Cassileth *et al.*, 2017). The use of orally consumed wheatgrass juice improved hemoglobin values in patients with intermediate thalassemia. It also possibly reduced the need for blood transfusions.

Wheatgrass juice dose-dependently chelates iron, which could be helpful in patients who require frequent transfusions.

Rajagopalan *et al.*, (2012) report that GC-MS analysis of methanol extract of wheatgrass powder shows the presence of nine bioactive components. Squalene is one of the active compounds with peak area of eight percent, found in GC-MS reports. Squalene is found to have good antioxidant and antitumor activity. In vitro experiments have suggested that squalene is a highly effective singlet oxygen scavenging agent. Moreover, squalene is also found to inhibit aberrant hyperproliferation, an event that precedes mammary tumorigenesis.

## **B. ANDROGRAPHIS PANICULATA – THE KING OF BITTERS**

### **1. *Andrographis paniculata***

*Andrographis paniculata* belongs to the family *Acanthaceae* grows erect to a height of 30– 110 cm in moist, shady places. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. The lance shaped leaves have hairless blades measuring up to 8×2.5 cm. Small flowers are borne in spreading racemes. The fruit is a capsule around 2 centimeters long and a few millimeters wide. It contains many yellow brown seeds (Rajalakshmi *et al.*, 2016).

### **2. Nutrient content**

*A. paniculata* contains 73.02% of moisture, 0.98% of fat, 2.85% of protein, 12.16% of carbohydrate, 250.13mg/100g of P, 152.50mg/100g of Na, 318.62mg/100g of Ca, 7.68 mg/100g of Mg and 45.01mg/100mg of vitamin C which may control physiological oxidative stress (Puranik *et al.*, 2012).

### **3. Therapeutic potentials**

Preliminary phytochemical screening of crude extracts of *A. paniculata* shown the presence of Alkaloids, Tannins, Flavonoids, Steroids, Terpenoids, Saponins, Phenols, Antraquinones, Phlobatannins and Organic Acids (Deepak *et al.*, 2014).

*Andrographis paniculata* contains diterpenes, lactones and flavonoids. Flavonoids mainly exist in the root, but have also been isolated from the leaves. Aerial

parts contain alkanes, ketones, and aldehydes and the bitter principles in the leaves were due to presence of the lactone andrographolide named kalmegin. Four lactones Chuaxinlian A (deoxyandrographolide), B (andrographolide), C (neoandrographolide) and D (14-deoxy-11, 12-didehydroandrographolide) were isolated from the aerial parts (Das *et al.*, 2014).

*Andrograhis paniculata* was recommended in Charaka Samhita dating to 175 BC for treatment of Jaundice along with other plants in multi plant preparations. It has also been used traditionally for sluggish liver as antidote in case of colic dysentery and dyspepsia. It is used as bitter tonic, antispasmodic, antiperistaltic and also an antihelmintic(Vijayakumar *et al.*, 2007).

*Andrograhis paniculata* extract exhibits anti-typhoid, antifungal activity, antioxidants, anti-inflammatory, anti-snake venom and antipyretic properties. It is also used as an immune stimulant agent (Shama *et al.*, 2011).

Methanol extract of *Andrographis paniculata* (at dose 100mg/kg) produced an anti- diabetic action by decreasing the blood glucose level in the model of alloxan-induced diabetes in mice (Das *et al.*, 2014).

Yusuf *et al.*, (2017) recorded that dietary supplementation of leaves and whole plant of *Andrographis paniculata* can be used to manipulate rumen metabolism for improved nutrient digestibility in goats.

Andrographolide exhibited both direct and indirect effects on cancer cells by inhibiting proliferation of cancer cells, cell-cycle arrests, or cell differentiation, enhancing body's own immune system against cancer cells; and inducing apoptosis and necrosis of cancer cells (Vojdani *et al.*, 2006).

According to Rajkumar *et al.*, (2015) ethanol extract of *Andrographis paniculata* has alkaloids and flavonoids act against cancer cell lines and induce apoptosis by DNA fragmentation, nuclear condensation and cell shrinkage.

According to Roy *et al.*, (2010) chloroform extract of *Andrographis paniculata*, antimicrobial results showed the diameter of inhibition zones ranging from 15 to 35 mm,

with the highest inhibition zone observed against *E. faecalis* (35 mm), followed by *E. cloacae* (30 mm) *P. aeruginosa* (28 mm) and *E. coli* (25 mm). Least inhibition zone was observed against *S. aureus* (15 mm).

In the preliminary screening of antibacterial activity of methanol leaf extract of *Andrographis paniculata* exhibited maximum activity when compared with other plant parts and also from different solvent extracts (Monoharan and Monoharan, 2013). The maximum activity was observed for the pathogens *E. coli* followed by *Salmonella typhi*, *Staphylococcus* species and *Pseudomonas* species.

Increased concentration (50 to 300 µg / ml) of Andrographolide extract of *Andrographis paniculata*, has shown reduction of viability and induction of cell apoptosis in HEP2 cell lines (Padmalochana *et al.*, 2017).

Oral administration of andrographolide at a dose of 300mg/kg daily had shown significant analgesic activity on acetic-induced writhing in mice and on the Randall-Selitto test in rats, but there was no effect on the hot plate test in mice. Oral administration of andrographolide at 30, 100, and 300mg/kg also showed anti-inflammatory activity in different models in rats (Madavet *et al.*, 2007).

Ethanol extract of *Apaniculata* possesses antidiabetic property and caused 49.8% reduction of fasting serum triglyceride levels, compared to 27.7% with metformin (Zhang *et al.*, 2000). Similarly Nugthroho *et al.*, (2012) depicted that purified extract and andrographolide of *A paniculata* significantly ( $P < 0.05$ ) decreased the levels of blood glucose, triglyceride, and LDL compared to controls. However, no changes were observed in serum cholesterol and rat body weight. Metformin also showed similar effects on these parameters.

Administration of *A. paniculata* ethanol leaf extract to Streptozotocin induced diabetic rats for four weeks produced a significant blood glucose reduction (Premnath *et al.*, 2015).

## **C. CERVICAL CANCER AND DIABETES MELLITUS – AN OVERVIEW**

### **1. Cervical cancer**

About three and a half decade ago the link between Human Papilloma Virus (HPV) and cervical cancer was proposed by Harald zur Hausen. Today HPV infection is known to cause about five percent of global burden of human cancers and in females it accounts for over 60 per cent of all infection-related cancers (IMJ, 2009).

Cervical cancer is the fifth most common cancer in humans, the second most common cancer in women, worldwide and the most common cancer cause of death in the developing countries. Sexually transmitted human papilloma virus (HPV) infection is the most important risk factor for cervical intraepithelial neoplasia and invasive cervical cancer (Karthigeyan *et al.*, 2012).

Cervical cancer starts in the cells on the surface of the cervix, the lower part of the uterus (womb) that opens at the top of the vagina. Most cervical cancers are squamous cell carcinomas with adenocarcinomas that involve the glandular epithelial cells, being the second most common type. Cervical cancer usually develops very slowly and starts as a precancerous condition called dysplasia. This condition can be detected by a Pap smear and is 100% treatable. The early stages of cervical cancer are completely asymptomatic and it can take years for precancerous changes to turn into cervical cancer (NCBI, 2012). Many studies reveal that the disease is more common in the low economic status, possibly due to poor personal and sexual hygiene, nutritional deficiency and lack of health care (Molshey *et al.*, 2016).

Majority of cancers in developing countries like India are detected in late stages, predominantly due to lack of awareness about the condition, lack of availability of screening programs and prevention services (Diaz *et al.*, 2008).

In India, cervical cancer is the most common cancers among women between 15 and 44 years of age, followed by breast cancer. Current estimates report the age adjusted incidence rate of cervical cancer per year is 27.0 per 100,000 women with an age adjusted mortality rate of 15.2 per 100,000 women (WHO/ICO, 2010).

Several risk factors increase the chance of developing cervical cancer. Most common risk factor for developing cervical cancer is infection of HPV. There are some risk factors that may influence the progression of cervical cancer such as leaving the HPV infection untreated and allowing it to persist for a long period of time, smoking cigarettes, weakened immune system, multiple partners etc (Dutta *et al.*, 2012).

Prevention and control of cervical cancer requires primary prevention with vaccination of high-risk patients, secondary prevention with regular screening and treatment of precancers, and availability of cancer treatment and palliative care (WHO/UNFPA, 2006).

Recently, two HPV prophylactic vaccines, Cervarix and Gardasil based on papillomavirus like particles (HPV-VLPs) are produced by self-assembly of the major viral L-1 capsid protein using recombinant DNA technology. Both have been shown in several large clinical trials to be safe, well tolerated and can induce strong immunity against the HPV genotypes incorporated in the vaccine. This is the first antiviral vaccine developed specially to prevent human cancer (IMJ 2009).

Despite the fact that more than 80% of cervical cancer cases are in developing countries, only 5% of women in these countries have been ever tested for abnormalities of the cervix (WHO 2006). Cytology screening programs have resulted in a marked decline of this disease in developed countries.

Results from an Indian cluster randomized control trial based in Tamil Nadu suggests that screening using the VIA method substantially reduces the incidence of, and mortality from cervical cancer (Sankaranarayanan *et al.*, 2007).

## **2. Diabetes mellitus**

Diabetes mellitus is an endocrinological and metabolic disorder with an increasing global prevalence and incidence. High blood glucose levels are symptomatic of diabetes mellitus as a consequence of inadequate pancreatic insulin secretion or poor insulin-directed mobilization of glucose by target cells. Diabetes mellitus is aggravated by and associated with metabolic complications that can subsequently lead to premature death (Prieto *et al.*, 2015).

According to the official WHO data, India tops the list of countries with the highest number of diabetes; China, America, Indonesia, Japan, Pakistan, Russia, Brazil, Italy and Bangladesh follow. In the year 2000, the total number of diabetics in India stood at 31.7 million and is expected to rise by more than 100% in the year 2030.

According to Ramachandran (2014), In 2013, of the estimated 382 million people with diabetes globally, more than 80 per cent lived in LMIC. It was estimated that India had 65.1 million adults with diabetes in 2013, and had the 2<sup>nd</sup> position among the top 10 countries with the largest number of diabetes. This number is predicted to increase to 109 million by 2035 unless steps are taken to prevent new cases of diabetes.

Although persistent hyperglycemia is acknowledged as a primary driver of diabetic complications, it is not the only factor. Many of these complications are exacerbated by other co-pathologies commonly occurring with diabetes, particularly high blood pressure and lipid abnormalities. Hence, the current clinical approaches that aim to prevent diabetic complications are focused on achieving targets for glycemic, blood pressure, and lipid control (ADA 2012)

The classic symptoms of diabetes such as polyuria, polydipsia and polyphagia occur commonly in type 1 diabetes, which has a rapid development of severe hyperglycaemia and also in type 2 diabetes with very high levels of hyperglycaemia. Severe weight loss is common only in type 1 diabetes or if type 2 diabetes remains undetected for a long period. Unexplained weight loss, fatigue and restlessness and body pain are also common signs of undetected diabetes. Symptoms that are mild or have gradual development could also remain unnoticed (Ramachandran., 2014)

Diabetic nephropathy, neuropathy, and retinopathy are the main microvascular complications induced by chronic hyperglycemia via several mechanisms such as the production of advanced glycation end products (AGEs), the creation of a proinflammatory microenvironment, and the induction of oxidative stress(Chilleli *et al.*, 2013).

Atherosclerosis is more common in people with Diabetes mellitus than in those without. Coronary Computed Tomography Angiography to evaluate coronary plaque

subtypes and luminal narrowing in patients with and without type 2 DM report that patients with DM are more prone to have significant stenosis with calcified plaques and such findings are accompanied by higher hs-CRP levels. Diabetic cardiomyopathy is a specific complication that develops independently of coronary artery disease or hypertension and it is possible to lead to increased morbidity and mortality (Jia *et al.*, 2016).

Better understanding of genetics could also lead to individualized therapy, and a potential paradigm for Diabetes is outlined in the review by Costacou and Levy., (2012) where a haptoglobin genotype may not only impact risk of diabetic complications, but also potentially the response to certain kinds of treatment.

There are seven essential self-care behaviors in people with diabetes which predict good outcomes. These are healthy eating, being physically active, monitoring of blood sugar, compliant with medications, good problem-solving skills, healthy coping skills and risk-reduction behaviors(ADA 2008).

The American Diabetes Association had reviewed the standards of diabetes self management education and found that there was a four-fold increase in diabetic complications for those individuals with diabetes who had not received formal education concerning self-care practices.

It is known that early insulin initiation is needed for tight glycemic control and delay in the onset of complications. Patients feel using insulin therapy is inconvenient due to its interference with eating, exercise and daily routines and dissatisfaction associated with hypoglycemia, injection pain, time required to administer, and embarrassment(Pyerotet *al.*, 2010).

## **D. SYNERGISTIC ACTIVITY OF PLANTS**

### **1. *Triticum aestivum***

Study on synergistic antimicrobial potential of the three combinations of aloe – wheat grass extract were found to be encouraging and results of phytochemical

screening contained a number of phytochemicals like alkaloids, glycosides, tannins, phenolics and flavonoids (Kakkar *et al.*, 2012).

Cell viability assay of combination of wheatgrass and cisplatin minimized the side effects and potentiate the chemotherapeutic activity of cisplatin. Cells (MCF-7/ HeLa) were treated with different concentrations of cisplatin and wheatgrass alone, and in combination for 24 h and their viability was evaluated. It was observed that 1  $\mu$ M of cisplatin (C1) used in combination with 1% (W1) and 5% (W2) wheatgrass resulted in a significant decrease in cell viability (85 and 80%, respectively) of MCF-7 cells as compared to either of the compounds alone (98% for C1, 97% and 90% for W1 and W2). When these combinations were used on HeLa cells, the combinations resulted in 60 and 53% (for C1W1 and C1W2) decrease in cell viability while individual drugs decreased the cell viability by 84% for C1 and 81 and 75% with W1 and W2, respectively. Further combinational index was calculated and it was found to be less than 1 for all the combinations indicative of synergistic action of these combinations on both MCF-7 and HeLa cells (Hussain *et al.*, 2014).

Laboratory in vitro studies, mostly using the fermented wheat germ extract, have demonstrated anti-cancer potential and have identified apoptosis as a possible mechanism. In animal experiments, wheatgrass demonstrated benefits in cancer prevention and as an adjunct to cancer treatment, as well as benefits to immunological activity and oxidative stress. Clinical trials show that wheatgrass may induce synergistic benefits to chemotherapy and may attenuate chemotherapy-related side effects, as well as benefit rheumatoid arthritis, ulcerative colitis, hematological diseases, diabetes, obesity, and oxidative stress (Sela *et al.*, 2015).

Growth of rats increases on a diet of milk produced on summer pasture compared with milk produced from winter feeding conditions. i.e. fodder. When wheat grass juice was added to the winter milk diet, growth doubled from 2 to 4 grams a day. It was evident there are important water-soluble substances in the juice that directly stimulate growth when added to winter milk (Kohler *et al.*, 1985).

## **2. *Andrographis paniculata***

The combination of paclitaxel and Andrographolide exert significant synergistic anticancer effect on A549 cells *in vitro* and *in vivo*. The synergy may be the result of the accumulation of ROS. The combination of Andrographolide and paclitaxel represents a potential strategy for the treatment of A549 cells (Yuan *et al.*, 2016).

The antitussive activity of arabinogalactan was increased by synergistic action with andrographolide and traditional aqueous extraction method provides an arabinogalactan from *A. paniculata*, which stimulate biological response but without addiction (Nosalova *et al.*, 2016).

Combination of erythromycin with chloroform extract of *A. paniculata* and erythromycin with methanol extract of *A. paniculata* lead to synergistic effects with the zone of inhibition were 31.33 mm and 32.67 mm against *Streptococcus agalactiae* respectively. Comparing two sets of data of synergism, combination of erythromycin and methanol produced better synergism than combination of erythromycin and chloroform extract. Methanol solvent is better to be used for extracting the compounds from *Andrographis paniculata*. The reason may be the different polarity of the solvent were used and the degree of solubility of the bioactive compounds in the respective solvents (Sivananthan *et al.*, 2016).

### III. METHODOLOGY

The methodology involved in the conduct of the present study titled “**Antimicrobial, Antidiabetic, Glucose Uptake activity and Anticancer (He La Cells and DNA Damage) Potentials of *Triticum aestivum* and *Andrographis paniculata***” is discussed under the following headings:

- A) Selection of Plants and Preparation of Aqueous Extracts
- B) Phytochemical and In vitro Antioxidant Analysis
- C) Antimicrobial Activity and DNA Damage Study
- D) Antidiabetic Activity and Glucose uptake Activity
- E) Anticancer Potentials

#### **A) SELECTION OF PLANTS AND PREPARATION OF AQUEOUS EXTRACTS**

Wheatgrass (*Triticum aestivum*) extract is a rich source of vitamins, minerals, chlorophyll and antioxidant enzymes like superoxide dismutase and cytochrome oxidase which are responsible for inhibiting the metabolic activation of carcinogens (Rajagopalan *et al.*, 2012). Morphological and pharmacological applications of Kalmegh (*Andrographis paniculata*) as a multipurpose drug show efficient activity in curing various diseases (Elumalai *et al.*, 2016).

*Triticum aestivum* and *Andrographis paniculata* are two important plants with promising nutraceutical and therapeutic potentials according to Folk Medicine (Rajagopalan *et al.*, 2012; Kumar *et al.*, 2004) and hence these plants were selected for the present study.

The grass of *T. aestivum* was used in this study was grown under suitable environmental conditions. Wheat seeds were bought from Tamilnadu Agricultural University, Coimbatore. They were rinsed in tap water prior to soaking. After rinsing, the seeds were soaked in water for 15 hours. Water was drained and the seeds were left to germinate for 12 hours until sprouts were formed. A rectangular growing tray was taken and filled with ½-1 inch of soil (with vegetable waste manure) mixed with cow dung in the ratio of 2:1 (Ashish *et al.*, 2012). The sprouted seeds were spread in an even layer across the top of the soil. Small quantities of water were sprinkled evenly over the soil in

the tray and covered with moistened newspaper for two days and then moved into indirect sunlight. Placing the tray in direct sunlight can cause drying out of the soil and inhibits growth, hence it was avoided. On the seventh day, grass was harvested and used for further investigations (Mohan *et al.*, 2013).

*Andrographis paniculata* was collected from its native habitat in Coimbatore and grown in a mud pot. The leaves of the plant were used for further investigations. The seeds of *Triticum aestivum* and *Andrographis paniculata* were authenticated by the Botanical Survey of India, TNAU, Coimbatore (Appendix - 2).

In order to know the synergistic activity of *Triticum aestivum* juice with *Andrographis paniculata*, exclusive aqueous extracts and combination aqueous extracts were prepared in the present study according to the methodology outlined by Soundarajan *et al.*, (2017).

## **1. Exclusive Extracts**

### **a) Extract - 1**

10g of fresh grass of *Triticum aestivum* was weighed, washed and cut into small pieces. Then it was ground in a mortar and pestle into a fine paste with the addition of 2ml of distilled water. The mixture was transferred into a 100 ml clean and dry conical flask and kept in Shaker Incubator at 40° C and 50rpm for 24 hours. After incubation the mixture was filtered with No.1 Whatman filter paper and the clear aqueous extract was used for the analysis.

### **b) Extract -2**

10g of fresh leaves of *Andrographis paniculata* was weighed, washed and cut into small pieces. Then it was ground in a mortar and pestle into a fine paste with the addition of 2ml of distilled water. The mixture was transferred into a 100 ml clean and dry conical flask and kept in Shaker Incubator at 40° C and 50rpm for 24 hours. After incubation the mixture was filtered with No.1 Whatman filter paper and the clear aqueous extract was used for the analysis.

## **2. Combination Extracts**

Kakkaret *al.*, (2012) report that evaluation of antimicrobial potential of aloe-wheatgrass combination taken in the ratios of C<sub>1</sub> - 3:1, C<sub>2</sub>- 2:2 and C<sub>3</sub> - 1:3 were found to be encouraging. Taking this to be the guideline, combination extracts were prepared based upon these ratios in the present study.

### **c) Extract -3**

5g each of fresh grass of *Triticum aestivum* and fresh leaves of *Andrographis paniculata* were weighed, washed and cut into small pieces. Then it was ground in a mortar and pestle into a fine paste with the addition of 2ml of distilled water. The mixture was transferred into a 100 ml clean and dry conical flask and kept in Shaker Incubator at 40° C and 50rpm for 24 hours. After incubation the mixture was filtered with No.1 Whatman filter paper and the clear aqueous extract was used for the analysis.

### **d) Extract -4**

2.5g of fresh grass of *Triticum aestivum* and 7.5g of fresh leaves of *Andrographis paniculata* were weighed, washed and cut into small pieces. Then it was ground in a mortar and pestle into a fine paste with the addition of 2ml of distilled water. The mixture was transferred into a 100 ml clean and dry conical flask and kept in Shaker Incubator at 40° C and 50rpm for 24 hours. After incubation the mixture was filtered with No.1 Whatman filter paper and the clear aqueous extract was used for the analysis.

### **e) Extract -5**

7.5g of fresh grass of *Triticum aestivum* and 2.5g of fresh leaves of *Andrographis paniculata* was weighed, washed and cut into small pieces. Then it was ground in a mortar and pestle into a fine paste with the addition of 2ml of distilled water. The mixture was transferred into a 100 ml clean and dry conical flask and kept in Shaker Incubator at 40° C and 50rpm for 24 hours. After incubation the mixture was filtered with No.1 Whatman filter paper and the clear aqueous extract was used for the analysis.

## **B) PHYTOCHEMICAL AND IN - VITRO ANTIOXIDANT ANALYSIS**

### **1. Phytochemical Analysis**

Phytochemicals are the chemicals that present naturally in plants. Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as “manfriendly medicines”(Banu *et al.*, 2015).Hence it was thought of interest to analyse the phytochemical analysis of the aqueous extracts. Preliminary phytochemical analysis was carried out for the extracts as per standard method outlined by Monisha *et al.*,(2017).

#### **Test for alkaloids**

To 1 ml of extract, a drop of iodine and 1ml of Mayer’s reagent are added along the sides of test tube. Appearance of yellow colour indicates the presence of alkaloids

#### **Test for terpenoids**

To 2 ml of extract, equal amount of chloroform followed by few drops of concentrated Sulphuric acid are added along the sides of test tube and heated for 4-5 minutes. Appearance of grey colour indicates the presence of terpenoids.

#### **Test for phenol**

To 1 ml of extract, 2ml of 2 percent ferric chloride was added. Appearance of blue/green/black colour indicates the presence of phenol.

#### **Test for saponins**

2-3 ml of distilled water was mixed with 1ml of extract in a test tube and it was shaken vigorously. The 1cm length of foam appearance showed the presence of saponins.

#### **Test for flavonoids**

To 2 ml of extract, a piece of Mg ribbon and few drops of concentrated Hydrochloric acid were added. Appearance of scarlet pink colour indicates the presence of terpenoids.

### **Test for quinine**

To 2 ml of extract, 1ml of 2 percent NaOH was added. Appearance of blue/green colour indicates the presence of quinine.

### **Test for protein**

To 2 ml of extract, a drop of concentrated Nitric acid was added. Appearance of yellow colour indicates the presence of protein.

### **Test for sterols**

To 2 ml of extract, equal amount of chloroform followed by few drops of concentrated Sulphuric acid are added along the sides of test tube. A red coloured ring indicates the presence of sterols.

### **Test for tannins**

To 1 ml of extract, 2 ml of 2 percent gelatin solution was added. Appearance of curdy white precipitate indicates the presence of tannins.

## **2. In vitro Antioxidant Analysis**

Antioxidants are substances that inhibit the oxidation of oxidisable substrate in the chain reaction and play a significant role in the prevention of many degenerative diseases. It may function as a free radical scavenger, metal chelator, reducing agent and quencher of singlet oxygen (Barreira *et al.*, 2008).

### **a) Determination of Total alkaloids**

To 1g of sample, 20 ml of 10percent acetic acid in ethanol was added and kept in incubator at 40° C for 4 hours after mixing the mixture well. After incubation 5 ml of distilled water and concentrated ammonium hydroxide was added drop by drop and mixed well. The precipitate was collected and washed with ammonium hydroxide.

Then the sample was filtered with No.1 Whatman filter paper and the residue was kept for drying. After drying the residue was weighed and noted. The calculation was done based on the formula given (Monisha *et al.*, 2017):

**Total alkaloids = Weight of the beaker with sample – Final weight of the beaker  
with sample (after drying)**

### **b) Determination of Total flavonoids**

Aluminum chloride method was used for the determination of the total flavonoid content of the extracts. To 1 ml of extract, 0.1 ml of 10percentAluminium chloride solution was added and mixed well. To this mixture 0.1 ml of 0.1M sodium potassium tartarate was added and mixed well. 2.8 ml of distilled water was added and mixed well and then the mixture was kept in incubator at room temperature for 30 minutes. After incubation absorbance was measured at 415nm using UV-VIS spectrophotometer. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample (Upadhyaya *et al.*, 2011).

### **c) DPPH-Free Radical Scavenging Assay**

The free radical scavenging activity of the extract was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH).To 1 ml of extract, 0.1 ml of DPPH solution was added and mixed well. To this mixture 0.4 ml of 50mmoltrisHydrochloric acid was added and kept in incubator at room temperature for 30 minutes. After incubation absorbance was measured at 517nm using UV-VIS spectrophotometer (Monisha *et al.*, 2017). Ascorbic acid was used as the standard. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula:

$$\text{Percentage of inhibition (DPPH Activity)} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

#### **d) Determination of Total Antioxidant Activity**

To 1 ml of extract, 1 ml of reaction mixture was added and mixed well. 1mmol reaction mixture contains 0.6 mol Sulphuric acid, 28mmol sodium sulphate and 4 mmol ammonium molybdate. The mixture was kept in incubator at 50°C for 90 minutes. After incubation absorbance was measured at 695nm using UV-VIS spectrophotometer (Prieto *et al.*, 1999).

#### **e) Superoxide dismutase Activity**

Superoxide Dismutase (SOD) catalyzes the dismutation of the superoxide radical ( $O_2^-$ ) into hydrogen peroxide ( $H_2O_2$ ) and elemental oxygen ( $O_2$ ) and as such provides an important defense against the toxicity of the superoxide radical. In fact, overexpression of SOD protects murine fibrosarcoma cells from apoptosis and promotes cell differentiation. SOD also inhibits adriamycin-induced apoptosis in murine peritoneal macrophages. To 1 ml of extract, 1 ml of reaction mixture was added and mixed well. The mixture was incubated for 10 minutes at 30°C. Reaction mixture I contains 1 ml of 50 mmol phosphate buffer, 0.075 ml of 20 mmol alpha methionine, 0.04 ml of 10 mmol hydroxylamine hydrochloride and 0.1 ml of 50 mmol EDTA. After incubation 80µl of 50 mmol riboflavin was added and mixed well. Then the mixture was exposed under 200 volt florescent light for 5 minutes. 1 ml of reaction mixture II was added to the mixture and mixed well. Reaction mixture II contains 1 percent Sulphanilamide in 5percent Phosphoric acid. The absorbance was measured at 543nm using UV-VIS spectrophotometer (Das *et al.*, 2000).

### **3. UV Analysis**

UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of compounds in both pure and biological mixtures. Natural compounds can be determined by using UV- VIS spectroscopy. Phenolic compounds including anthocyanins, tannins, polymer dyes, and phenols form complexes with iron that have been detected by the ultraviolet/visible (UV- VIS) spectroscopy. This technique is not time-consuming, and presents reduced cost compared to other techniques (Altemimi *et al.*, 2017). UV-VIS confirms the identity of a compound by matching the absorbance spectrum and also a very popular detector for other analytical techniques, such as chromatography, because it can detect many compounds. The selected concentration of plant extract was scanned in the wavelength ranging from 200- 500 nm by using UV-VIS Spectrophotometer.

### **C) ANTIMICROBIAL ACTIVITY AND DNA DAMAGE STUDY**

#### **1. Antimicrobial Activity**

##### **a) Antibacterial Activity**

The effect of selected concentration of combination aqueous extract on three bacterial strains was assayed by Agar well diffusion method (Nithya *et al.*, 2012).

##### **Test Microorganisms**

The three bacterial strains used in the present study were the clinical isolates obtained from Centre for Bioscience and Nanoscience Research, Coimbatore. The bacteria used were *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa*.

##### **Principle**

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly swabbed with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

## Reagents

### 1. Mueller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.9 g of the commercially available Mueller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

### 2. Amikacin disc (AK 30) as standard antibacterial agent.

## Procedure

Sterile petriplates containing 20ml Mueller Hinton medium were swabbed with 24hr culture of bacterial strains. Well with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer and 20 µl of the selected concentration of plant extract was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Amikacin disc (AK 30) was used as a positive control.

## **b) Antifungal Activity**

The effect of selected concentration of combination aqueous extract on two fungal strains was assayed by Agar well diffusion method (Nithya *et al.*, 2012).

## Test Microorganisms

The two fungal strains used in the present study were the clinical isolates obtained from Centre for Bioscience and Nanoscience Research, Coimbatore. The fungi used were *Aspergillusniger* and *Trichodermaviride*.

## Principle

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly swabbed with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

## Reagents

### 1. Malt Agar Medium (1 L)

The medium was prepared by dissolving 45 g of the commercially available Malt Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

## Procedure

Sterile petriplates containing 20ml Malt Agar Medium were swabbed with spore of fungal strains. Well with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer and 20 µl of the selected concentration of plant extract was added. The plates were then incubated at 37°C for 3-5 days. The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well.

## 2. DNA Damage Study

The study was done using Agarose gel Electrophoresis (AGE) apparatus. Genomic DNA was isolated from *Escherichia coli*. 3µL of selected concentration of plant extract was mixed with 0.5 µg of DNA and kept for incubation for 2 hours at 37°C. 0.9percent Agarose gel was prepared in Tris Acetate EDTA Buffer. The mixture was then heated in an oven for 50°C for 50 seconds. 20 µL of Ethidium bromide was added to the mixture. The mixture was then poured into Gel casting tray in AGE Apparatus and left aside for solidification. AGE apparatus filled with TAE Buffer was connected with 50V power supply. DNA and treated DNA sample was loaded and run until the dye has

migrated 40 to 60 percent of the length of the gel (Soundarajan *et al.*, 2015). After running the DNA damage was visualized under UV-transilluminator.

## **D) ANTIDIABETIC ACTIVITY AND GLUCOSE UPTAKE ACTIVITY**

### **1. Antidiabetic Activity**

One therapeutic approach for treating diabetes is to decrease post-prandial hyperglycemia. This is done by hindering the absorption of glucose through inhibition of the carbohydrate hydrolyzing enzymes,  $\alpha$ -amylase and  $\alpha$ -glucosidase, in the digestive tract. In the investigation, the inhibitory activity of selected plant extract against both mentioned enzymes was carried out according to the method outlined by Russo *et al.*, (2015). The effect of the selected concentration of plant extract from phytochemical and in vitro antioxidant analysis was done in the following enzyme inhibition assay.

- a)  $\alpha$ -amylase inhibition assay
- b)  $\alpha$ -glucosidase inhibition assay

#### **a) $\alpha$ -amylase inhibition assay**

1 ml of selected concentration of plant extract was taken in a clean dry test tube and 0.5 ml of 0.1 percent starch solution in 16 mM sodium phosphate buffer was added. The mixture was then added with 0.2 ml of  $\alpha$ -amylase and 0.5 ml of 96 mM of sodium potassium tartarate and 3, 5 dinitro salicylic acid and mixed well. The mixture incubated in alkaline condition at 25°C for 5- 10 minutes. The absorbance was measured at 540 nm using UV-VIS spectrophotometer.

#### **b) $\alpha$ -glucosidase inhibition assay**

1 ml of selected concentration of plant extract was taken in a clean dry test tube and 1 ml of 2 percent starch solution was added. 1 ml of 0.2 M tris buffer was added and mixed well. The mixture was then incubated for 30 minutes at 37°C. After preincubation 0.2 ml of  $\alpha$ -glucosidase was added to the mixture and again incubated at 35°C for 45 minutes. The reaction was stopped with the addition of 2 ml of 6N Hydrochloric acid and mixed well.

The absorbance was measured at 540 nm using UV-VIS spectrophotometer. The inhibition activity of both the enzymes was calculated using the formula given below:

$$\text{Percentage of inhibition (percent)} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

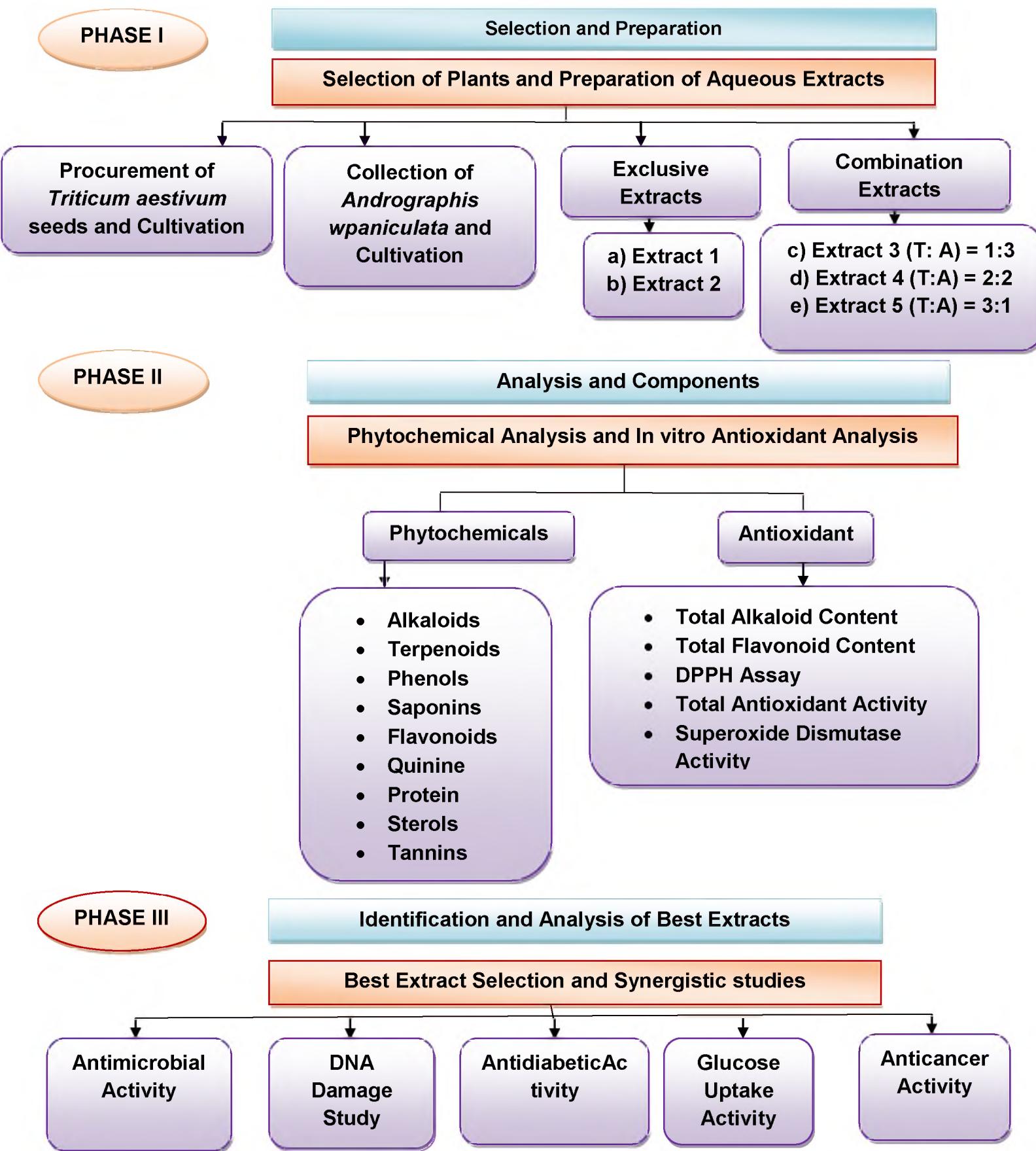
## 2. Glucose Uptake Activity

Glucose uptake activity of selected concentration of plant extract was determined in He La cell lines. In brief, the 24 hr cell cultures with 70-80 percent confluency in 40mm petriplates were allowed to differentiate by maintaining in DMEM with 2 percent FBS for 4-6 days. The extent of differentiation was established by observing multinucleation of cells. The differentiated cells were serum starved over night and at the time of experiment cells were washed with HEPES buffered Krebs Ringer Phosphate solution (KRP buffer) once and incubated with KRP buffer with 0.1 percent BSA for 30 min at 37°C. Cells were treated with different non-toxic concentrations of selected concentration of plant extract and standard drug (insulin) for 30 min along with negative controls at 37°C. 20µl of D-glucose solution was added simultaneously to each well and incubated at 37°C for 30 min. After incubation, the uptake of the glucose was terminated by aspiration of solutions from wells and washing thrice with ice-cold KRP buffer solution. Cells were lysed with 0.1M NaOH solution and an aliquot of cell lysates were used to measure the cell-associated glucose. The glucose levels in cell lysates were measured using autoanalyser. Three independent experimental values in duplicates were taken to determine the percentage enhancement of glucose uptake over controls (Mathews *et al.*, 2013).

## E) ANTICANCER ACTIVITY

He La cells were procured from the National Centre for Cell Science, Pune, India. The cells were subcultured in Dulbecco modified eagle's medium (DMEM Medium) supplemented with Bovine serum albumin, sodium carbonate and glucose in tissue culture flasks, and incubated at 37°C in a CO<sub>2</sub> incubator in a 5percent CO<sub>2</sub>, pH – 7 and 70-80percent humidity atmosphere for 24-72 hours. A known number of cells (2 × 10<sup>3</sup> cell/well in 100 µl of medium) were seeded into 96-well plates respectively for carrying out a MTT assay. The cells were then treated with different concentration range of selected concentration of plant extract (5-10 µL) and incubated at 37°C in a CO<sub>2</sub> incubator in a 5percent CO<sub>2</sub>, pH – 7 and 70-80percent humidity atmosphere for 24 hours. After the pre-incubation the cells were washed with DMSO and trypsin and 20 µL of MTT Dye was added and kept for incubation at 37°C in a CO<sub>2</sub> incubator in a 5percent CO<sub>2</sub>, pH – 7 and 70-80percent humidity atmosphere for 24 hours. The absorbance was recorded at 540 nm by an enzyme-linked immunosorbent assay reader and the results were analyzed in triplicate and the percentage was calculated. The viability of the cells was calculated using the formula given (Soundarajan *et al.*, 2015).

$$\text{Viable cells} = \frac{\text{Test}}{\text{Control}} \times 100$$



RESEARCH DESIGN

FIGURE 2

## IV. RESULTS AND DISCUSSION

The findings of the present study titled “Antimicrobial, Antidiabetic, Glucose Uptake activity and Anticancer (He La Cells and DNA Damage) Potentials of *Triticum aestivum* and *Andrographis paniculata*” is discussed under the following headings:

- A) Phytochemicals and Antioxidants
- B) Antimicrobial Activity and DNA Damage Study
- C) Antidiabetic Potential and Glucose Uptake Activity
- D) Anticancer Activity

### A) PHYTOCHEMICALS AND ANTIOXIDANTS

#### 1. Phytochemicals

The results of preliminary phytochemical analysis of aqueous extracts namely exclusive (1 and 2) and combination (3, 4 and 5) extracts are given in Table I.

TABLE I  
PHYTOCHEMICALS

Phytochemicals	Extract - 1	Extract - 2	Extract - 3	Extract - 4	Extract - 5
Alkaloids	+	+	+	+	+
Terpenoids	+	+	+	+	+
Phenol	-	+	-	-	+
Saponins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Quinine	+	-	-	-	+
Protein	+	+	+	+	+
Sterols	+	+	+	-	+
Tannins	+	-	-	-	-

Extract - 1 contained alkaloids, terpenoids, saponins, flavonoids, quinine, protein, sterols and tannins. Phenols were absent in this extract. Extract – 2 contained alkaloids, terpenoids, phenols, saponins, flavonoids, protein and sterols. Quinine and tannins were absent in Extract - 2. Extract – 3 contained alkaloids, terpenoids, saponins, flavonoids, protein and sterols. Phenol, quinine and tannins were absent in Extract - 3. Extract - 4 contained alkaloids, terpenoids, saponins, flavonoids and protein. Phenol, quinine, sterols and tannins were absent in Extract - 4. Extract - 5 contained alkaloids, terpenoids, phenol, saponins, flavonoids, quinine, protein and sterols. Tannins were absent in Extract - 5.

Exclusive extracts namely Extract – 1 contained eight phytochemicals and Extract – 2 contained seven phytochemicals. Combination extracts namely Extract – 3, 4 and 5 contained six, five and seven phytochemicals respectively. Hence based on this result Extract – 1, 2, 3 and 5 were selected for further investigations.

According to Suryavathana *et al.*, (2016), the phytochemical screening analysis of *Triticum aestivum* using various solvents revealed the presence of carbohydrate, protein, alkaloids, tannins and phenols in the methanolic and aqueous extracts, while the presence of saponins was noted in chloroform extract. Rajagopalan *et al.*, (2012) also reported that phytochemical analysis of *Triticum aestivum* with methanolic and aqueous extracts showed the presence of different types of active compounds such as alkaloids, saponins, amino acids and proteins, carbohydrates, cardioglycosides, coumarin, terpenoids, tannins, flavonoids and phenolics. Tannin was absent in chloroform extract. Rajalakshmi *et al.*, (2016) reports that phytochemical screening of the ethanolic extracts of *Andrographis paniculata* revealed the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoid and glycoside.

## 2. Antioxidants

Based on the results of phytochemical analysis of aqueous extracts, exclusive extracts (1 and 2) and combination extracts (3 and 5) were selected for in - vitro antioxidant analysis. Extract – 4 was excluded as it possessed the lowest phytochemical content among the aqueous extracts. The result of quantitative antioxidant analysis of the selected aqueous extracts is given as follows.

### a) Total Alkaloid Content

The total alkaloid content of the selected aqueous extracts is presented in Table II.

**TABLE II**  
**TOTAL ALKALOID CONTENT (TAC= mg/g)**

<b>Aqueous Extracts</b>	<b>TAC mg/g (Mean ± S.D)</b>
<b>Extract – 1</b>	<b>131.8 ± 0.76</b>
<b>Extract –2</b>	<b>65.56 ± 0.51</b>
<b>Extract –3</b>	<b>170.6 ± 0.57</b>
<b>Extract - 5</b>	<b>190.26 ± 0.64</b>

Extract – 1 contained  $131.83 \pm 0.76$  mg/g of total alkaloid whereas Extract – 2 contained  $65.56 \pm 0.51$  mg/g of total alkaloids. Extract – 3 and 5 contained  $170.66 \pm 0.57$  mg/g and  $190.26 \pm 0.64$  mg/g of total alkaloids. Extract – 5 showed the highest total alkaloids while Extract – 2 revealed the lowest content among other selected aqueous extracts.

Suryavathanaet *et al.*, (2016) report a total alkaloid content in *Triticum aestivum* of 150 mg/g. Kurzawa *et al.*, (2015) report the total alkaloid content in roots and leaves of *Andrograhis paniculata* varies from  $50.71 \pm 0.36$  mg/g to  $78.71 \pm 0.48$  mg/g. According to Uddin *et al.*, (2014) maximum crude alkaloids were determined in leaf (21.78 mg to 3.64 mg) of early monsoon (March-May) and minimum in stem (17.60 mg to 2.71 mg) of winter, grown *Andrograhis paniculata*.

## b) Total Flavonoid Content

The total flavonoid content of the selected aqueous extracts is given in Table III.

**TABLE III**  
**TOTAL FLAVONOID CONTENT (TFC= mg/ml)**

<b>Aqueous Extracts</b>	<b>TFC mg/ml (Mean ± S.D)</b>
<b>Extract – 1</b>	<b>0.41 ± 0.01</b>
<b>Extract –2</b>	<b>0.48 ± 0.00</b>
<b>Extract –3</b>	<b>0.42 ± 0.00</b>
<b>Extract - 5</b>	<b>0.52 ± 0.02</b>

Extract 1 contained  $0.41 \pm 0.01$  mg/ml of total flavonoids whereas Extract – 2 contained  $0.48 \pm 0.00$  mg/ml of total flavonoids. Extract – 3 and 5 contained  $0.42 \pm 0.00$  mg/ml and  $0.52 \pm 0.02$  mg/ml of total flavonoids. Extract – 5 shows the highest total flavonoids and Extract – 1 shows the lowest content among other selected aqueous extracts.

Zendehbad *et al.*, (2014) report the total flavonoids of *Triticum aestivum* were more in the chloroform fraction ( $40.420 \pm 0.13$  µg/mL) when compared with the methanol fraction ( $22.873 \pm 0.05$ ). The total flavonoid content of *Andrograhis paniculata* was found to be 0.43 mg/ml (Sani *et al.*, 2017). Premnath *et al.*, (2011) reported that ethanol extracts of *A. paniculata* has total flavonoid of 0.86 mg/ml.

## c) DPPH-Free radical Scavenging

The DPPH radical scavenging activity of the selected aqueous extracts is given in Table IV.

**TABLE IV**  
**DIPHENYL 2-PICRYL HYDRAZYLSCAVENGING (%)**

Aqueous Extracts	Percentage of Inhibition(%) (Mean ± S.D)
<b>Extract – 1</b>	<b>48.03 ± 0.05</b>
<b>Extract –2</b>	<b>48.53 ± 0.50</b>
<b>Extract –3</b>	<b>49.06 ± 0.10</b>
<b>Extract - 5</b>	<b>51.13± 0.23</b>

The stable radical DPPH has been used widely for the determination of primary anti-oxidant activity. The DPPH anti-oxidant assay is based on the ability of DPPH, a stable free radical to decolorize in the presence of anti-oxidants. Extract – 5 recorded the highest scavenging activity of  $51.13 \pm 0.23$  % and Extract – 1 recorded the lowest scavenging activity of  $48.03 \pm 0.05$ %. Extract – 2 and 3 showed  $48.53 \pm 0.50$  % and  $49.06 \pm 0.10$  % of scavenging activity.

According to Dhaliwal *et al.*, (2015) the aqueous extract of *Triticum aestivum* was found to be 35.1% of free radical scavenging activity. Sharma *et al.*, (2011) reported the methanol extracts of leaves of *A. paniculata* recorded the highest scavenging activity of 54% at 0.7 mg/ml and 52% at 0.9 mg/ml.

Based on the results of TAC, TFC and DPPH analysis of selected aqueous extracts, combination extracts (3 and 5) were selected for further in - vitro antioxidant analysis. Exclusive extracts – 1 and 2 were excluded as they possessed lowest TAC, TFC content and DPPH activity than combination extracts. The result of further in - vitro antioxidant analysis of combination extracts (3 and 5) is given as follows.

#### d) Total Antioxidant Activity

The Total antioxidant activity of Extract – 3 and Extract – 5 were carried out and the result is given in Table V.

**Table V**  
**TOTAL ANTIOXIDANT ACTIVITY (TAA = mg/ml)**

<b>Combination Extracts</b>	<b>TAA mg/ml (Mean <math>\pm</math> S.D)</b>
<b>Extract –3</b>	<b>72 <math>\pm</math> 0.00</b>
<b>Extract - 5</b>	<b>94.03 <math>\pm</math> 0.05</b>

Total Antioxidant Activity (TAA) is an analyte frequently used to assess the antioxidant status of biological samples and can evaluate the antioxidant response against the free radicals produced in a disease. Extract – 3 contained  $72 \pm 0.00$  mg/ml whereas Extract – 5 contained  $94.03 \pm 0.05$  mg/ml of total antioxidant activity concentration. Sangeetha *et al.*, (2014) reported the total antioxidant concentration of the aqueous extract of *Andrographis paniculata* was found to be 75 mg/ml whereas Shukla *et al.*, (2009) reported the TAA of *Triticum aestivum* was  $0.278 \pm 0.024$  mg/ml.

#### e) Superoxide dismutase

The SOD catalyzes the dismutation of superoxide anion to H<sub>2</sub>O<sub>2</sub>. The amount of SOD present in the Extract – 3 and Extract – 5 is given in Table VI.

**TABLE VI**  
**SUPEROXIDE DISMUTASE (SOD = mg/ml)**

<b>Combination Extracts</b>	<b>SOD mg/ml (Mean <math>\pm</math> S.D)</b>
<b>Extract –3</b>	<b>86 <math>\pm</math> 0.00</b>
<b>Extract –5</b>	<b>232 <math>\pm</math> 0.00</b>

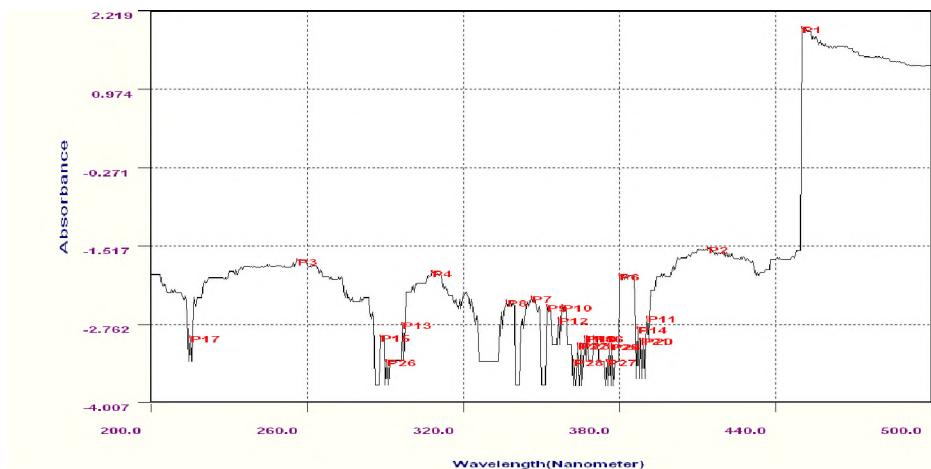
Extract – 3 contained  $86 \pm 0.00$  mg/ml whereas Extract – 5 contained  $232 \pm 0.00$  mg/ml of superoxide dismutase activity concentration. According to Vidya *et al.*, (2014), the superoxide dismutase activity of *Triticum aestivum* was  $18.32 \pm 0.28$  units/g (1 Unit of enzyme is the amount that causes 50% inhibition in NBT reduction). The SOD activity of *Andrograhis paniculata* leaf extract showed 80.41% of highest inhibition rate (Rafat *et al.*, 2010).

### Best Combination Extract

From the findings of phytochemical analysis and in - vitro antioxidant analysis, Extract – 5 containing 0.75 g of *Triticum aestivum* and 0.25 g of *Andrograhis paniculata* recorded high free radical scavenging potential since the presence of eight phytochemicals and antioxidant contents (TAC:  $190.26 \pm 0.64$  mg/g, TFC:  $0.52 \pm 0.02$  mg/ml, DPPH:  $51.13 \pm 0.23\%$ , TAA:  $94.03 \pm 0.05$  mg/ml and SOD:  $232 \pm 0.00$  mg/ml) were more than the other extract concentration of *Triticum aestivum* and *Andrograhis paniculata* taken in different combinations. Hence Extract – 5 was selected for further assays in order to identify the synergistic potential of selected extract concentration against He La cell lines.

### 3. UV Analysis

UV analysis of Extract – 5 is depicted in Figure 2.



**UV ANALYSIS**

**FIGURE 2**

From the figure, it is evident that Extract – 5 contained numerous phytochemicals and antioxidants responsible for the inhibition of free radical scavenging activity thereby increasing the therapeutic potentials ranging from the peak of 200 nm to 500nm. UV Study on *Triticum aestivum* by Khan *et al.*, (2015) showed anti-cancer, anti-ulcer, antioxidant, and anti-arthritis activity due to the presence of biologically active compounds, and minerals.

## B) ANTIMICROBIAL ACTIVITY AND DNA DAMAGE STUDY

### 1. Antimicrobial Activity

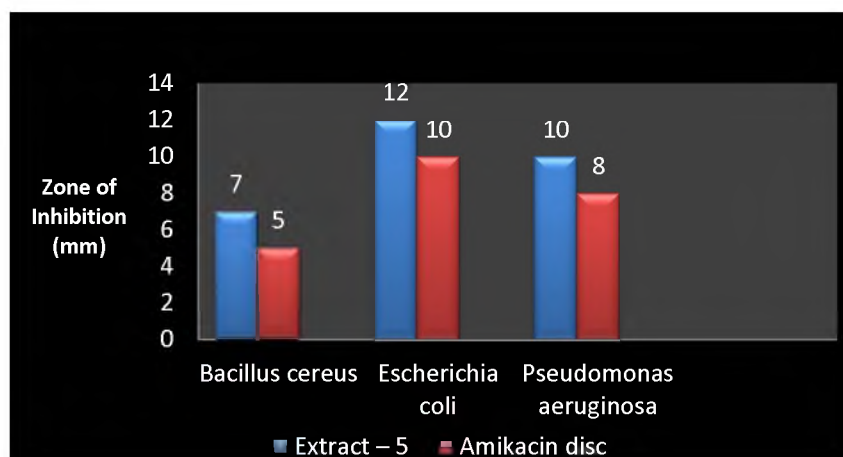
#### a) Antibacterial Activity

The effect of selected combination extract (Extract – 5) on three bacterial strains is shown in Table VII and Figure 3.

TABLE VII

#### ANTIBACTERIAL ACTIVITY

Microbes- Bacteria	Zone of inhibition(mm)		
	Extract – 5	Distilled Water	Amikacin disc
<i>Bacillus cereus</i>	7 mm	0	5 mm
<i>Escherichia coli</i>	12 mm	0	10 mm
<i>Pseudomonas aeruginosa</i>	10 mm	0	8 mm



### ANTIBACTERIAL ACTIVITY

**FIGURE 3**

Extract – 5 showed good antibacterial activities against selected bacterial strains. Among three bacterial strains the highest antibacterial activity was observed against *Escherichia coli* with a zone of inhibition of 12mm, *Pseudomonas aeruginosa* with a zone of inhibition of 10mm and the lowest antibacterial activity was observed against *Bacillus cereus* with a zone of inhibition of 7mm.

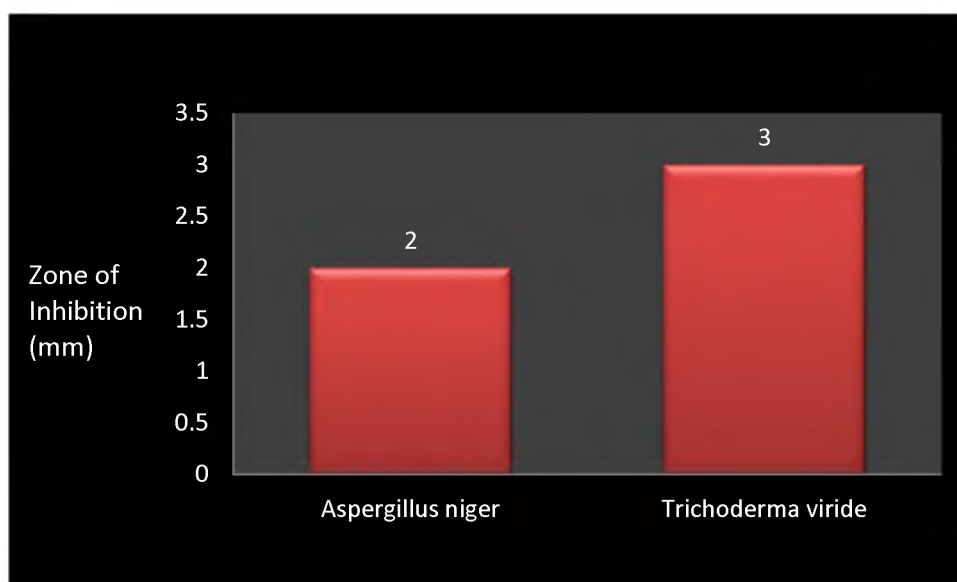
Khan *et al.*, (2015), record that 200 mg of *Triticum aestivum* showed a zone of inhibition of 5mm against *Escherichia coli* and a zone of inhibition of 3mm against *Pseudomonas aeruginosa*. According to Mishra *et al.*, (2013), Methanol extract of *A. paniculata* leaves exhibited strong *in vitro* antibacterial activity against Gram positive bacteria including clinical isolates of *Mycobacterium tuberculosis*, *Enterococcus faecalis* and methicillin resistant *Staphylococcus aureus*. The growth of *E. coli* was inhibited by the mid (150 mg) and higher dose (200 mg) of chloroform and methanol extract of *A. paniculata* with the zone of inhibition ranging from 10 - 13 mm. None of the extracts inhibited the growth of *Pseudomonas aeruginosa* according to Geetha *et al.*, (2017).

#### **b) Antifungal Activity**

The effect of selected combination extract (Extract – 5) on two fungal strains is shown in Table VIII and Figure 4.

**TABLE VIII**  
**ANTIFUNGAL ACTIVITY**

Microbes – Fungi	Zone of inhibition(mm)	
	Extract – 5	Distilled Water
<i>Aspergillusniger</i>	2 mm	0
<i>Trichodermaviride</i>	3 mm	0



**ANTIFUNGAL ACTIVITY**

**FIGURE 4**

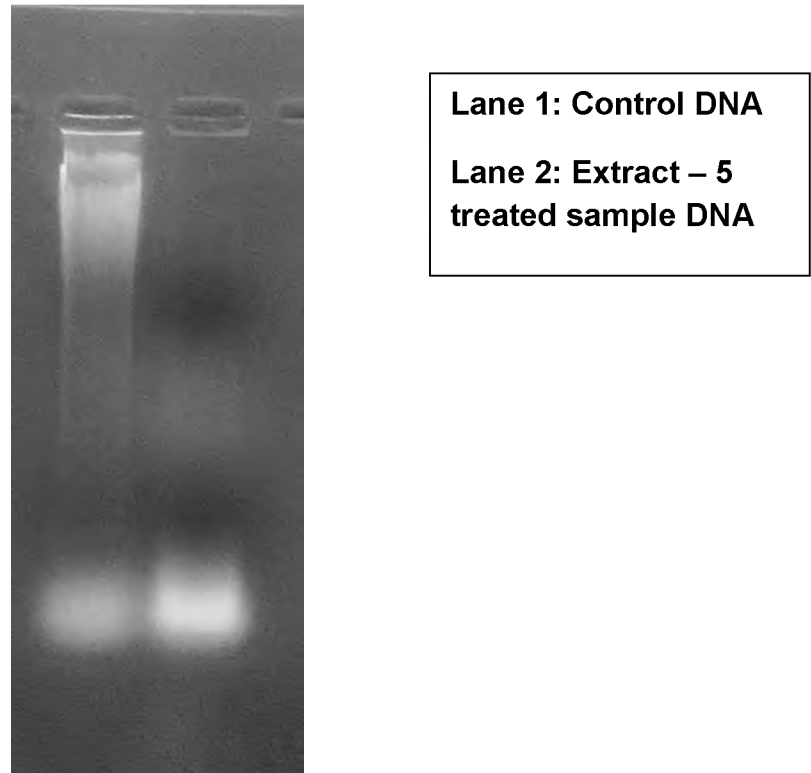
Extract – 5 showed antifungal activities against selected fungal strains. Among two fungal strains the highest antifungal activity was observed against *Trichoderma viride* with a zone of inhibition of 3mm whereas the lowest antifungal activity was observed against *Aspergillus niger* with a zone of inhibition of 2mm.

In the study done by Prasanna *et al.*, (2016), 60 µg of wheatgrass extract showed antifungal action and zone of inhibition (22, 20,17) against *Asperigillus niger*, *Asperigillus flavus*, and *Trichoderma viride*. According to Premnath *et al.*, (2016), *A. paniculata* leaf ethanol extract against two dermatophytes, *T. rubrum* and *E. floccosum*

revealed that *E. floccosum* was found to be more susceptible to the ethanol leaf extract with an MIC value of  $1.75 \pm 0.05$ mg/ml (74.6% growth inhibition) than *T. rubrum* which had an MIC value of  $3.0 \pm 0.04$  mg/ml (70.9% growth inhibition).

## 2. DNA Damage Study

The DNA damage was visualized under UV-transilluminator and the image is shown in Figure 5. The result revealed that the Extract - 5 cleaved the sample DNA and showed the DNA damage.



**EFFECT OF EXTRACT – 5 ON SAMPLE DNA**

**FIGURE 5**

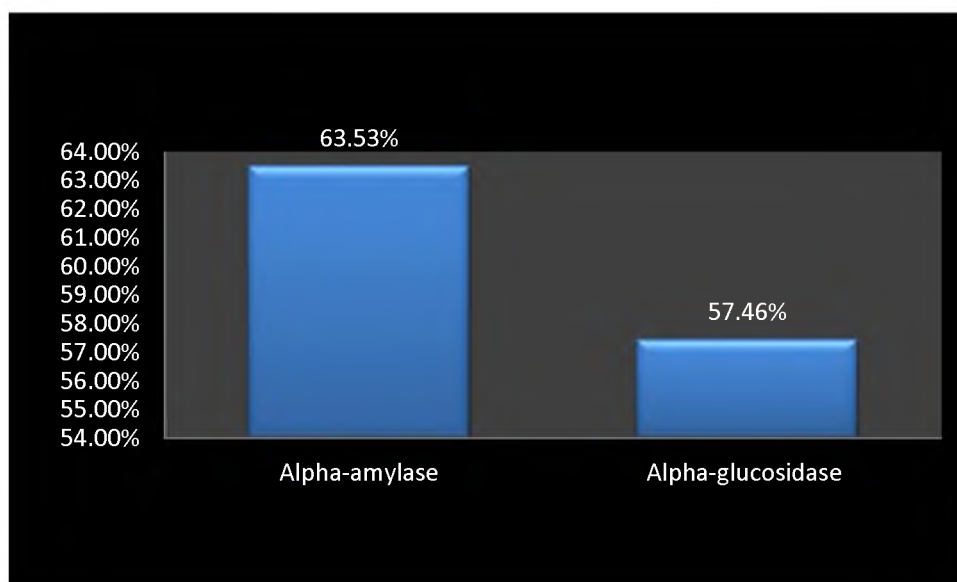
## C) ANTIDIABETIC POTENTIALS AND GLUCOSE UPTAKE ACTIVITY

### 1. Antidiabetic Activity

The inhibitory activity of Extract – 5on alpha amylase and alpha glucosidase was investigated in this study and the results are shown in Table IX and Figure6.

**TABLE IX**  
**ANTIDIABETIC ACTIVITY**

<b>Enzymes</b>	<b>Percentage of Inhibition (%) (Mean ± S.D)</b>
<b>Alpha-amylase</b>	<b>63.53 ± 0.00</b>
<b>Alpha-glucosidase</b>	<b>57.46 ± 0.02</b>



**ANTIDIABETIC ACTIVITY**  
**FIGURE 6**

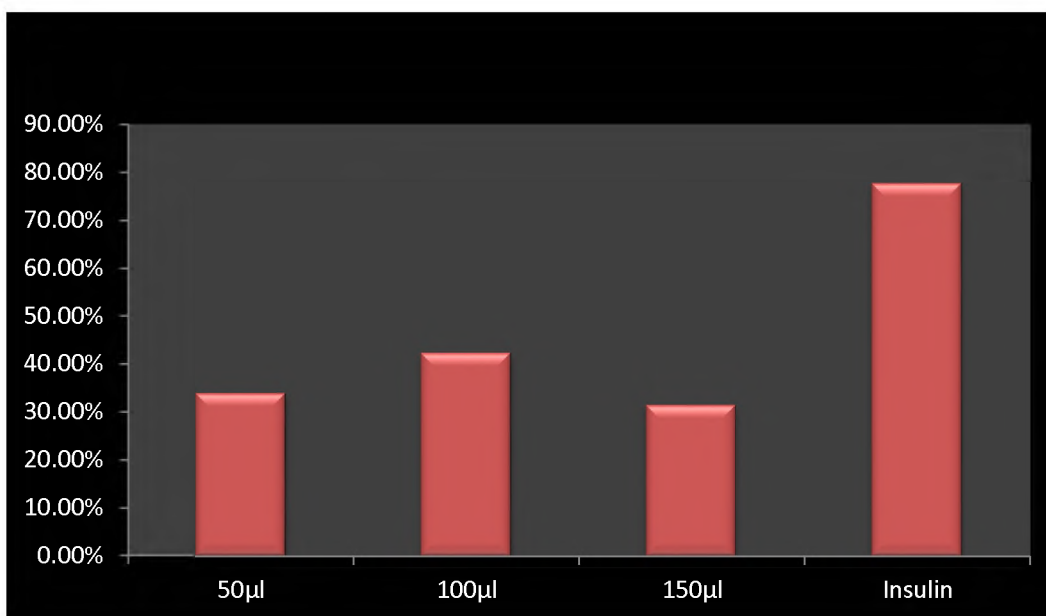
Extract – 5 showed highest inhibitory activity on Alpha-amylase of  $63.53 \pm 0.00\%$  and lowest inhibitory activity on Alpha-glucosidase of  $57.46 \pm 0.02\%$ . The similar findings were reported by Sathiavelu *et al.*, (2013) in- vitro antidiabetic study of *Andrographis paniculata* showed the highest value of 65.78% seen at 100mg/ml concentration of the plant extract. Ashoket *et al.*, (2011) performed Anti-hyperglycemic activity of *Triticum aestivum* by in vivo method involving Wistar rat 21 day model. The result obtained was expressed in terms of blood glucose level (mg/dl) and compared with standard drug Gliclazide. Among the two doses, higher dose (100 mg, p.o.) of *Triticum aestivum* showed significant anti-hyperglycemic activity.

## 2. Glucose Uptake Activity

Medicinal plants enhance the glucose uptake by GLUT4 translocation and were proven by in vitro glucose model of Daset *et al.*, (2015). Hence, in this study He La cells are used to determine the glucose uptake activity and GLUT4 translocation of Extract – 5 and the results are presented in Table X and Figure 7

**TABLE X**  
**GLUCOSE UPTAKE ACTIVITY**

<b>Concentrations of Extract – 5/ Standard</b>	<b>Glucose uptake activity (%) (Mean <math>\pm</math> S.D)</b>
<b>50 <math>\mu</math>l</b>	<b>33.99 <math>\pm</math> 0.00</b>
<b>100 <math>\mu</math>l</b>	<b>42.32 <math>\pm</math> 0.02</b>
<b>150 <math>\mu</math>l</b>	<b>31.53 <math>\pm</math> 0.05</b>
<b>Insulin</b>	<b>77.63 <math>\pm</math> 0.03</b>



### GLUCOSE UPTAKE ACTIVITY

FIGURE 7

Extract - 5 enhance the glucose uptake by  $33.99 \pm 0.00$  %,  $42.32 \pm 0.02$  % and  $31.35 \pm 0.05$  % at 50 µl, 100 µl and 150 µl concentrations over control. 100 µl showed maximum glucose uptake activity while 150 µl showed minimum glucose uptake activity. Insulin used as the standard anti diabetic drug (1IU/ml) enhances the glucose uptake by  $77.63 \pm 0.03$  % over control. The results showed Extract - 5 did not confer any cytotoxicity and showed better glucose uptake potential.

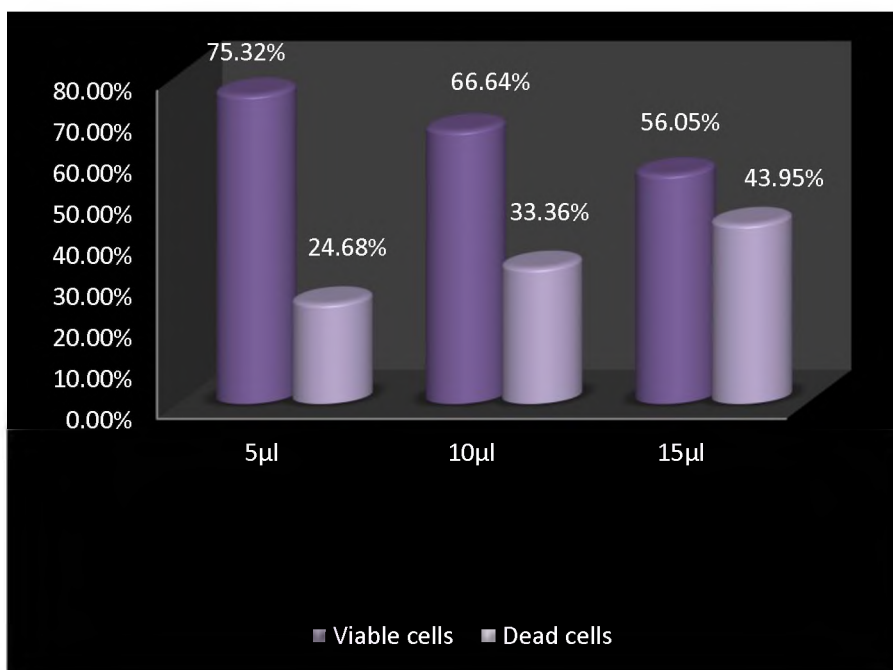
Daset *al.*, (2015) record that Vero, L6 and 3T3 cell lines enhance the glucose uptake by  $27.15 \pm 1.19$ ,  $18.73 \pm 1.29$  and  $24.43 \pm 0.88$  at 500 µg/ml concentration of ethanolic extract of fruits of Terminalia bellirica.

#### D) ANTICANCER ACTIVITY

An in vitro anticancer activity of Extract – 5 was analyzed in He La cells using MTT Assay and the findings are represented in Table XI and Figure 8.

**TABLE XI**  
**ANTICANCER ACTIVITY**

Concentrations	Viable cells(%) (Mean $\pm$ S.D)	Dead cells (%) (Mean $\pm$ S.D)
5 $\mu$ l	75.32 $\pm$ 0.00	24.68 $\pm$ 0.00
10 $\mu$ l	66.64 $\pm$ 0.03	33.36 $\pm$ 0.03
15 $\mu$ l	56.05 $\pm$ 0.07	43.95 $\pm$ 0.07



**ANTICANCER ACTIVITY**  
**FIGURE 8**

MTT assay is based on the metabolic reduction of MTT into formazan crystals on treatment with cancer cell lines. The inhibitory activity of Extract - 5 was observed in HeLa cancer cell lines. The cancer cell viability percentage was found to be at different concentration of extracts. Anticancer activity at the different concentrations of 5  $\mu$ l, 10  $\mu$ l and 15  $\mu$ l showed effective inhibition against cancer cell lines such as  $24.68 \pm 0.00\%$ ,  $33.36 \pm 0.03\%$  and  $43.95 \pm 0.07\%$ . Increased percentage of Cell line inhibition by suppressing viability was observed in 15  $\mu$ l. Minimum inhibitory concentration was observed based on the percentage of cell viability was  $24.68 \pm 0.00\%$  at 5  $\mu$ l.

According to Kumaret al., (2015), Ethanol extracts of *Andrographis paniculata* was subjected to different concentrations on IMR-32 and HT-29 cancer cell lines resulted in  $51.25 \pm 0.85$  and  $50.25 \pm 1.6\%$  inhibition at 200  $\mu$ g/ml, respectively with some significant differences ( $p < 0.01$ ). Dhamodaran et al., (2015) report the anticancer activity of silver nanoparticles in terpenoid for *A. Paniculata* (Ag-NPsTAP) has been examined over human cell line such as HeLa, Hep-2 at different concentrations by MTT assay method showed a maximum activity against human cervical cancer cells (HeLa) and human liver cancer cells (Hep-2) and it was detected to be 59.01% and 48.79% at 250  $\mu$ g/ml respectively. A 41.4% of OSCC cell inhibition was observed at 1000  $\mu$ g/ml dilution of aqueous wheatgrass extract in 24 hours (Goreet al., 2017). Hussain et al., (2014) also record that AWE showed dose and time dependent selective cytotoxicity towards the cancer highlighting its safe profile. Lower dose combinations of AWE and cisplatin induced increased growth inhibition compared with the individual drugs on both cell lines (combination index  $< 1$ ) indicating strong synergistic interactions in MCF-7 and HeLa cells.

On phytochemical analysis, exclusive extracts namely Extract – 1 and Extract – 2 contained eight and seven phytochemicals while combination extracts namely Extract – 3, 4 and 5 contained six, five and seven phytochemicals respectively. Hence based on this result Extract – 4 was excluded and Extract – 1, 2, 3 and 5 were selected for further in-vitro antioxidant analysis. Based on the result of in-vitro antioxidant analysis Extract – 5 (TAC:  $190.26 \pm 0.64$  mg/g, TFC:  $0.52 \pm 0.02$  mg/ml, DPPH:  $51.13 \pm 0.23\%$ , TAA:  $94.03 \pm 0.05$  mg/ml and SOD:  $232 \pm 0.00$  mg/ml) was selected for further assays

in order to identify the synergistic potential of selected extract concentration against He La cell lines. Extract – 5 on synergistic studies revealed the increased efficacy on therapeutic potential and enzymatic antioxidants than the antioxidant contents and therapeutic potential of exclusive extracts of *Triticum aestivum* and *Andrographis paniculata*. Glucose Uptake Activity showed maximum activity at 100 µl concentration of Extract – 5. Hence it can be concluded that the synergistic potential of Extract – 5 is found to be effective in antioxidant contents, antimicrobial, DNA Damage, antidiabetic, anticancer activity and in glucose uptake.

## V. SUMMARY AND CONCLUSION

More than 80 percent of people in developing countries cannot afford the most basic medical procedures, drugs, and vaccines. Among wealthier populations in both developed and developing countries, complementary and alternative practices are popular although proof of their safety and effectiveness is modest. Combining herbs to achieve the best therapeutic effect is a skilled process. Fortunately, many of the herbal medicines fixed combination have been designed according to experience gained over many generations. At present it has been estimated that about 80 percent of the world population rely on botanical preparations as medicine as they are considered safe and have been proved to be effective against certain ailments.

Studies on *Triticum aestivum* and *Andrographis paniculata* shows excellent therapeutic potentials and considered as multipurpose drugs. Synergistic studies of both the plants also revealed the highest activity in free radical scavenging and other pharmacological activities. With this background the present study on **“Antimicrobial, Antidiabetic, Glucose Uptake activity and Anticancer (He La Cells and DNA Damage) Potentials of *Triticum aestivum* and *Andrographis paniculata*”** was undertaken with the following objectives to grow plants in a suitable environment, harvest *Triticum aestivum* and *Andrographis paniculata* at the right stage of growth for analysis, identify the phytochemicals and antioxidants in exclusive and combination extracts of *Triticum aestivum* and *Andrographis paniculata*, select the best combination extract and study the efficacy of the synergistic potential of best combination extract on antimicrobial, DNA Damage, antidiabetic, anticancer and Glucose Uptake Activity.

The methodology followed for the present study is as follows:

The grass of *T. aestivum* was used in this study was grown under suitable environmental conditions. Wheat seeds were bought from Tamilnadu Agricultural University, Coimbatore. They were rinsed in tap water prior to soaking. After rinsing, the seeds were soaked in water for 15 hours. Water was drained and the seeds were left to germinate for 12 hours until sprouts were formed. A rectangular growing tray was taken and filled with ½-1 inch of soil (with vegetable waste manure) mixed with cow dung in

the ratio of 2:1. The sprouted seeds were spread in an even layer across the top of the soil. Small quantities of water were sprinkled evenly over the soil in the tray and covered with moistened newspaper for two days and then moved into indirect sunlight. Placing the tray in direct sunlight can cause drying out of the soil and inhibits growth, hence it should be avoided.

On the seventh day, grass was harvested and used for further investigations. *Andrographis paniculata* was collected from its native habitat in Coimbatore and grown in a mud pot. The leaves of the plant were used for further investigations. In order to know the synergistic activity of *Triticum aestivum* juice with *Andrographis paniculata*, exclusive aqueous extracts of the two wheat grass and kalmegh leaves and combination aqueous extracts (of the two ingredients together) were prepared. Combination extracts were prepared based upon the combination index followed in synergistic studies.

Based upon the ratio, the plants were weighed, washed and cut into small pieces and ground in a mortar and pestle into a fine paste with the addition of 2ml of distilled water. The mixture was transferred into a 100 ml clean and dry conical flask and kept in Shaker Incubator at 40° C and 50rpm for 24 hours. After incubation the mixture was filtered with No.1 Whatman filter paper and the clear aqueous extracts (namely Extract – 1 and 2 – Exclusive extracts and Extract – 3,4 and 5 – Combination extracts) were used for analysis.

The phytochemicals tested include alkaloids, terpenoids, phenol, saponins, flavonoids, quinine, protein, sterols and tannins. In vitro antioxidant analysis include Total alkaloid content, Total flavonoid content, DPPH free radical scavenging assay, Total antioxidant activity and SOD activity were carried out. Based upon the results evaluated from phytochemical and in vitro antioxidant analysis, best combination extract was selected. Best combination extract (Extract – 5) containing 0.75 g of *Triticum aestivum* and 0.25 g of *Andrographis paniculata* was tested for antimicrobial, DNA Damage, antidiabetic, glucose uptake and anticancer activity against He La cells. The experimental results are expressed as Mean  $\pm$  Standard deviation (SD) of triplicate measurements.

The results of the present study are summarized as follows:

Extract - 1 contained alkaloids, terpenoids, saponins, flavonoids, quinine, protein, sterols and tannins. Phenols were absent in this extract. Extract - 2 contained alkaloids, terpenoids, phenols, saponins, flavonoids, protein and sterols. Quinine and tannins were absent in Extract - 2. Extract - 3 contained alkaloids, terpenoids, saponins, flavonoids, protein and sterols. Phenol, quinine and tannins were absent in Extract - 3. Extract - 4 contained alkaloids, terpenoids, saponins, flavonoids and protein. Phenol, quinine, sterols and tannins were absent in Extract - 4. Extract - 5 contained alkaloids, terpenoids, phenol, saponins, flavonoids, quinine, protein and sterols. Tannins were absent in Extract - 5.

Exclusive extracts namely Extract – 1 contained eight phytochemicals and Extract – 2 contained seven phytochemicals. Combination extracts namely Extract – 3, 4 and 5 contained six, five and seven phytochemicals respectively. Hence based on this result Extract – 1, 2, 3 and 5 were selected for further investigations.

Based on the results of phytochemical analysis of aqueous extracts, exclusive extracts (1 and 2) and combination extracts (3 and 5) were selected for in - vitro antioxidant analysis. Extract – 4 was excluded as it possessed the lowest phytochemical content among the aqueous extracts.

Extract – 1 contained  $131.83 \pm 0.76$  mg/g of total alkaloid whereas Extract – 2 contained  $65.56 \pm 0.51$  mg/g of total alkaloids. Extract – 3 and 5 contained  $170.66 \pm 0.57$  mg/g and  $190.26 \pm 0.64$  mg/g of total alkaloids. Extract – 5 showed the highest total alkaloids while Extract – 2 revealed the lowest content among other selected aqueous extracts.

Extract 1 contained  $0.41 \pm 0.01$  mg/ml of total flavonoids whereas Extract – 2 contained  $0.48 \pm 0.00$  mg/ml of total flavonoids. Extract – 3 and 5 contained  $0.42 \pm 0.00$  mg/ml and  $0.52 \pm 0.02$  mg/ml of total flavonoids. Extract – 5 shows the highest total flavonoids and Extract – 1 shows the lowest content among other selected aqueous extracts.

The stable radical DPPH has been used widely for the determination of primary anti-oxidant activity. The DPPH anti-oxidant assay is based on the ability of DPPH, a stable free radical to decolorize in the presence of anti-oxidants. Extract – 5 recorded the highest scavenging activity of  $51.13 \pm 0.23$  % and Extract – 1 recorded the lowest scavenging activity of  $48.03 \pm 0.05$ %. Extract – 2 and 3 showed  $48.53 \pm 0.50$  % and  $49.06 \pm 0.10$  % of scavenging activity.

Based on the results of TAC, TFC and DPPH analysis of selected aqueous extracts, combination extracts (3 and 5) were selected for further in - vitro antioxidant analysis. Exclusive extracts – 1 and 2 were excluded as they possessed lowest TAC, TFC content and DPPH activity than combination extracts.

Total Antioxidant Activity (TAA) is an analyte frequently used to assess the antioxidant status of biological samples and can evaluate the antioxidant response against the free radicals produced in a disease. Extract – 3 contained  $72 \pm 0.00$  mg/ml whereas Extract – 5 contained  $94.03 \pm 0.05$  mg/ml of total antioxidant activity concentration. The SOD catalyzes the dismutation of superoxide anion to  $H_2O_2$ . Extract – 3 contained  $86 \pm 0.00$  mg/ml whereas Extract – 5 contained  $232 \pm 0.00$  mg/ml of superoxide dismutase activity concentration.

From the findings of phytochemical analysis and in - vitro antioxidant analysis, Extract – 5 containing 0.75 g of *Triticum aestivum* and 0.25 g of *Andrograhis paniculata* recorded high free radical scavenging potential since the presence of eight phytochemicals and antioxidant contents (TAC:  $190.26 \pm 0.64$  mg/g, TFC:  $0.52 \pm 0.02$  mg/ml, DPPH:  $51.13 \pm 0.23$ %, TAA:  $94.03 \pm 0.05$  mg/ml and SOD:  $232 \pm 0.00$  mg/ml) were more than the other extract concentration of *Triticum aestivum* and *Andrograhis paniculata* taken in different combinations. Hence Extract – 5 was selected for further assays in order to identify the synergistic potential of selected extract concentration against He La cell lines.

UV Study on Extract – 5 contained numerous phytochemicals and antioxidants responsible for the inhibition of free radical scavenging activity thereby increasing the therapeutic potentials ranging from the peak of 200 nm to 500nm.

Extract – 5 showed good antibacterial activities against selected bacterial strains. Among three bacterial strains the highest antibacterial activity was observed against *Escherichia coli* with a zone of inhibition of 12mm, *Pseudomonas aeruginosa* with a zone of inhibition of 10mm and the lowest antibacterial activity was observed against *Bacillus cereus* with a zone of inhibition of 7mm.

Extract – 5 showed antifungal activities against selected fungal strains. Among two fungal strains the highest antifungal activity was observed against *Trichoderma viride* with a zone of inhibition of 3mm whereas the lowest antifungal activity was observed against *Aspergillus niger* with a zone of inhibition of 2mm.

DNA damage was visualized under UV-transilluminator and the image revealed that the Extract - 5 cleaved the sample DNA and showed the DNA damage. Extract – 5 showed highest inhibitory activity on Alpha-amylase of  $63.53 \pm 0.00\%$  and lowest inhibitory activity on Alpha-glucosidase of  $57.46 \pm 0.02\%$ .

Extract - 5 enhance the glucose uptake by  $33.99 \pm 0.00\%$ ,  $42.32 \pm 0.02\%$  and  $31.35 \pm 0.05\%$  at 50  $\mu\text{l}$ , 100  $\mu\text{l}$  and 150  $\mu\text{l}$  concentrations over control. 100  $\mu\text{l}$  showed maximum glucose uptake activity while 150  $\mu\text{l}$  showed minimum glucose uptake activity. Insulin used as the standard anti diabetic drug (1IU/ml) enhances the glucose uptake by  $77.63 \pm 0.03\%$  over control. The results showed Extract - 5 did not confer any cytotoxicity and showed better glucose uptake potential.

MTT assay is based on the metabolic reduction of MTT into formazan crystals on treatment with cancer cell lines. The inhibitory activity of Extract - 5 was observed in He La cancer cell lines. The cancer cell viability percentage was found to be at different concentration of extracts. Anticancer activity at the different concentrations of 5  $\mu\text{l}$ , 10  $\mu\text{l}$  and 15  $\mu\text{l}$  showed effective inhibition against cancer cell lines such as  $24.68 \pm 0.00\%$ ,  $33.36 \pm 0.03\%$  and  $43.95 \pm 0.07\%$ . Increased percentage of Cell line inhibition by suppressing viability was observed in 15  $\mu\text{l}$ . Minimum inhibitory concentration was observed based on the percentage of cell viability was  $24.68 \pm 0.00\%$  at 5  $\mu\text{l}$ .

On phytochemical analysis, exclusive extracts namely Extract – 1 and Extract – 2 contained eight and seven phytochemicals while combination extracts namely Extract –

3, 4 and 5 contained six, five and seven phytochemicals respectively. Hence based on this result Extract – 4 was excluded and Extract – 1, 2, 3 and 5 were selected for further in- vitro antioxidant analysis. Based on the result of in-vitro antioxidant analysis Extract – 5 (TAC:  $190.26 \pm 0.64$  mg/g, TFC:  $0.52 \pm 0.02$  mg/ml, DPPH:  $51.13 \pm 0.23\%$ , TAA:  $94.03 \pm 0.05$  mg/ml and SOD:  $232 \pm 0.00$  mg/ml) was selected for further assays in order to identify the synergistic potential of selected extract concentration against He La cell lines. Extract – 5 on synergistic studies revealed the increased efficacy on therapeutic potential and enzymatic antioxidants than the antioxidant contents and therapeutic potential of exclusive extracts of *Triticum aestivum* and *Andrographis paniculata*. Glucose Uptake Activity showed maximum activity at 100  $\mu$ l concentration of Extract – 5. Hence it can be concluded that the synergistic potential of Extract – 5 is found to be effective in antioxidant contents, antimicrobial, DNA Damage, antidiabetic, anticancer activity and in glucose uptake.

The present study thus proves that the aqueous Extract - 5 did not cause any adverse effect and hence could be considered nontoxic and safe. The results of the present study confirm that it can be safely consumed. Hence it can be concluded that the synergistic potential of Extract – 5 is found to be effective in antioxidant contents, antimicrobial, antidiabetic, DNA Damage, anticancer activity and in glucose uptake.

### **Recommendations**

- Evaluate the therapeutic effect of supplementation of (3:1) synergistic combinations of *Triticum aestivum* and *Andrographis paniculata* on animal and human models.
- Evaluate the therapeutic effect of supplementation of (3:1) synergistic combination of *Triticum aestivum* and *Andrographis paniculata* on Cancer and Diabetes mellitus.
- Formulate and standardize nutritious and acceptable recipes with this extract combination.
- Increase awareness among the population regarding the therapeutic potentials of *Triticum aestivum* and *Andrographis paniculata*.

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सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2018/Tech. / 3266

दिनांक/Date: 9<sup>th</sup> March 2018

सेवा में / To

Ms. Poorni Kirthi. V  
II M. Sc. Food Science and Nutrition  
Department of Food Science and Nutrition  
Avinashilingam Institute for Home Science & Higher Education for Women  
Coimbatore - 641 043

महोदया / Madam,

The plant specimen brought by you for authentication is identified as *Andrographis paniculata* (Bum.f.) Nees - ACANTHACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

धन्यवाद / Thanking you,

भवदीय / Yours faithfully,

डॉ सी मुरुगन / Dr. C. Murugan  
वैज्ञानिक 'डी' एवं कार्यालय अध्यक्ष /  
Scientist 'D' & Head of Office

वैज्ञानिक 'डी' एवं कार्यालय अध्यक्ष  
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9/3/18