Antifungal Activity Of Phenolic Extract Of *Triticum aestivum* L. Against *Phytophthora* sp. Infection In *Murraya koenigii* L. Spreng.

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ABSTRACT

The present study was carried out to identify the antifungal properties of *Triticum aestivum* L. It is the freshly sprouted first leaves of the common wheat plant (*Triticum aestivum* L.). It is used as a food, drink, or dietary supplement. Wheatgrass is rich in iron, calcium, amino acids, magnesium, chlorophyll, and vitamins A, C, K, B, and E. Scientists believe that it is helpful in many health problems. In addition, it is used as antioxidants, antibacterial and anti-inflammatory agent. It is one of the best herbal remedies in healing and treating of many diseases.

In this present study the antifungal properties of wheatgrass (*Triticum aestivum* L.) were used against the white spot disease caused by *Phytophthora sp.* in *Murrya koenigii* L. Spreng. Phenolic compounds were extracted from the wheatgrass by using soxhlet apparatus. Leaf disc culture is used to get pure culture of *Phytophthora* sp. in PDA culture medium and well diffusion method was used to find out the antifungal properties. The wheatgrass extract shows high antioxidant activity, so it can act as an antifungal agent. Fungicide is prepared with the wheatgrass extract and applied directly to *M. koenigii* affected with *Phytophthora sp*. Field application of fungicide shows positive results within 20 days. So the phenolic extract of wheatgrass can be used as an effective fungicide in *Murraya* sp.

CONTENTS

INTRODUCTION

Poaceae is the large and nearly ubiquitous family of monocotyledonous flowering plants commonly known as grasses. It includes the cereal grasses bamboos and grasses of natural grassland and species cultivated in lawns and pasture. They commonly referred to as grasses with around 780 genera and around 12000 species. The poaceae is the fifth largest plant family following asteraceae, orchidaceae, fabaceae and rubiaceae. Poaceae are the world's single most family which provide important source of food, so that they are important family of earths flora. They grow in all continents in desert to fresh water and all habitats. They are annual, biennial, or perennial plants that are usually herbaceous but may be woody in some genera. The nearly 800 genera of grasses fall into three distributional patterns. Nearly three quarters are confined to one of the seven basic centres of distribution; Africa, Australia, Eurasia north of Himalayas, South and South east Asia, North America, and tropical America. About one fifth of the genera encompass even broader distribution patterns throughout temperate and tropical regions of the world.

Wheat is one of the oldest and most important of the cereal crops. Of the thousands of varieties known, the most important are common wheat (*Triticum aestivum* L.), used to make bread; durum wheat most importantly used for making pasta such as spaghetti and macaroni; and club wheat (*T. compactum*); a softer type, used for cake, crackers, cookies, pastries, and flours. Additionally, some wheat is used by industry for the production of starch paste, malt, dextrose, gluten, alcohol, and other products.

Wheatgrass is the name of the young grass of a wheat plant. It is a thick, dry grass that looks like hay or straw but is bright green and it can be used as a food.

It’s regarded as a super potent health food with amazing benefits. It’s usually consumed as a fresh juice, but it also comes in powdered form. Fresh wheatgrass juice is considered to be a “living food.” While many farmers are cultivating wheatgrass for animal feed, and many peoples were started to purchase it and also cultivating for including in their diet. It gets harvested early in its development, typically 7 to 10 days after harvesting. It may look like the grass in our front yard, but the young shoots of the wheat plant are packed with nutrients that may provide important health benefits.

Due to its high antioxidant content, wheatgrass may help to kill cancer cells. According to one test-tube study, wheatgrass extract decreased the spread of mouth cancer cells by 41% (Rucha Diwakar Gore *et al*. 2017). In another test-tube study, wheatgrass induced cell death and reduced the number of leukemia cells by 65% within three days of treatment (Noorjahan Banu Alitheen *et al*. 2011). So research indicates that wheatgrass juice may also help to reduce cancer cells. And it is also known to lower cholesterol levels, which helps to lower the risk of heart attack (J Sethi *et al*. 2010). Wheatgrass has high levels of enzymes that aid in digestion by helping our body to break down food and absorb nutrients and there by helps in digestion. They have anti-inflammatory properties it may help to ease some of the symptoms of arthritis, such as stiffness, pain, and swelling (Satyavati Rana *et al.* 2011*)*. Wheatgrass may improve overall mental function and relieve anxiety. Its neuroprotective effects allow for better cognitive function and also used to treat Alzheimer’s disease (Jung-Hee Jang *et al*.2010.*) S*tudy found that wheatgrass juice decreased the risk of impaired bone marrow function, a common complication of chemotherapy. Many people have started adding wheatgrass juice to their diet as a quick and convenient way to boost weight loss. wheatgrass has been shown to improve blood sugar levels in rats with Type II diabetes (Garima Shakya *et al*. 2012).

The research found that wheatgrass can be used for treating antibiotic resistant infections or people who allergic to specific antibiotics.

Practitioners of traditional medicine have used wheatgrass to reduce stomach pain and also for minor gastrointestinal problems, such as diarrhea or digestive problems. Wheatgrass has a high fibre content, and fibre helps to keep the gut healthy. Ping Wan *et al.* (2014) suggested that wheatgrass helps in the treatment of ulcerative colitis, an inflammatory condition that affects the large intestine.

Wheatgrass is a source of potassium, dietary fibre, vitamin A, vitaminC, vitaminD, vitaminK, thiamin, riboflavin, niacin, vitaminB6, pantothenic acid, iron, zinc, copper, manganese, and selenium. It is also a good source of protein it contains 17 forms of amino acid which helps to boots our immune system. It is available fresh as produce, in tablets, frozen juice, and powder. Wheatgrass is also sold commercially as a spray, cream, gel, massage lotion and liquid herbal supplement.

The curry tree, *Murraya koenigii* L. Spreng. or *Bergera koenigii* L. is a tropical to sub-tropical tree in the family Rutaceae and is native to Asia. India is the largest producer and consumer of curry leaf. The southern state of Tamil Nadu is one the major curry leaf producing area. The plant is also sometimes called sweet neem, though *M. koenigii* L. Spreng. is in a different family to neem. Curryleaves are the foliage of the curry tree (*M. koenigii*.) and its leaves are used for both medicinal and culinary applications. They’re highly aromatic and have a unique flavour.

Curry leaves are rich in protective plant substances, such as alkaloids, glycosides, and phenolic compounds, that give this fragrant herb potent health

benefits. The tree is native to the Indian subcontinent. Commercial plantations have been established in India, and more recently Australia. The leaves are indispensable part of Indian cuisine and Indian traditional medicines. They are most widely used in southern and west coast Indian cooking. The leaves of *M. koenigii*. are also used as herb in Ayurvedic and Siddha medicine in which they are believed to possess anti-disease properties.

Compounds found in whole part of curry tree include cinnamaldehyde, and numerous carbazole alkaloids, including mahanimbine, girinimbine, and mahanine. The leaves are a rich source of carotenoids, beta-carotene, calcium, iron, vitamin A, vitamin B, vitamin C, and vitamin B2 apart from a its distinctive odour and pungent taste.

It contains 108kcal energy; 63.8gm moisture; 18.7gm carbohydrate; 6.1 gm protein; 6.4 gm dietary fibre; 1gm of total fat;4mg vitamin C; 2.3 mg of niacin; 0.21mg of riboflavin; 830 mg calcium; 0.93mg iron; 0.1mg copper; 44mg of magnesium & 57mg phosphorus.

The leaves have found its way in many holistic treatments like Ayurveda, Sidda, Unani, and traditional Chinese treatments. It is also used for treating diarrhea and gastro intestinal disorders etc. The extract from the curry leaves had active constituent which is antidiabetics in nature and hence are widely used for the treatment of diabetes. The paste from the dried grounded leaves also had anti-helminthic, anti-fungal and anti- bacterial properties which are potent against infections and skin disorders.

And they have played a significant role in oral hygiene. The tin twigs were used as a datum. In modern ayurvedic treatments the powder of leaves is being

used as a tooth powder for preserving good oral health. Curry leaves play a vital role in the field of weight loss. It is extensively used in the treatment of anaemia, indigestion, obesity, kidney problems, hair and skin problems. And women who suffer from calcium deficiency, osteoporosis etc. can find an ideal natural calcium supplement in curry leaves. Fresh juice of curry leaves, with lime juice and sugar, is an effective medicine in the treatment of morning sickness, nausea and vomiting due to indigestion (Sinha Parul *et al.* 2012).

Curry leaf is susceptible to powdery mildew and curry leaf spot. In *M. koenigii*. small white spots are present in the upper surface of the leaves. It is a fungal disease caused by *Phytophthora* sp. In the early stages of disease, it is seen in some leaves. In severe case it fully affects the plant and reducing chlorophyll content and there by decrease the aroma and flavour of the curry leaves. And also a few pests that may attack curry leaf plants, including scale, aphids, mealybugs, and citrus psyllid. Insects that are likely to attack our lemon and orange trees are also capable of infesting curry leaf plant since curry leaf plants belong to the citrus family. Insects and aphids both harm plants by sucking fluid from plants and also making a silvery appearance on the lower surface of the leaves. As the little bugs feed on foliage and sap, turn leaves yellow, wilt, and fall off.

REVIEW OF LITERATURE

Phytochemical quantitative screening showed a good range of primary and secondary metabolites in *Triticum aestivum* L. (wheat grass). The plant was endowed with molecules such as vitamin, terpinoids, phenolics, lignin, tannin, flavanoids quinines, and other metabolites. So the plant shows antioxidant activity, antiinflammatory, antidiabetic and antimicrobial activity, the quantification of alkaloids and phenols indicates the quantum store of valuable secondary metabolites in wheatgrass (Suriyavanthana *et al.* 2016).

A study was mainly designed to screen various solvent extract of the leaves of wheat grass to show the potent antioxidant activity in order to find possible sources for novel antioxidants in wheat grass plant by analysing flavonoids and phenol content. (Seyed hosen zendehbad *et al.*2014). The study conducted by Varalakshmi Durairajet *et al*. (2014) found that the aqueous extract of wheatgrass contains bioactive compound such as phenol, flavonoids and poly phenols content. The varying percentage of squalene, caryophyllene and amyrins compounds from gas chromatography and mass spectrometry analysis proved that the plant has an effective free radical scavenger activity. The phytochemical, nutritional and therapeutic potential on chronic diseases such as cancer, ulcer and haemolytic anaemia was done in wheatgrass juice (M. Chauhan 2014). The dried and fresh wheatgrass extract were dissolved in different solvents such as methanol, acetone, ethanol, and water and the results by conducting various experiments for secondary metabolites showed its presence (Megha *et al*. 2016).

The phenolic content and flavonoid content were determined by using the solvent extraction of wheatgrass. From this study it was found that the wheatgrass is an immune booster and an antimicrobial agent. (Towkir Ahmed Ove *et al*. 2012).

The research on wheatgrass conducted by Neethu S Kumar (2016) proved that the plant is an herbal drug and it can be used for the treatment of anaemia, eczema, kidney swelling and common cold.

Polyphenol extract was taken from *Phyllanthus emblica* by Soxhlet extraction method using solvents like ethanol, methanol and acetone. (AS Periasamy Manikandan *et al*. 2019.). Phenolic compounds were determined by taking the extract from soxhlet apparatus of medicinal plants using different solvents such as methanol, ethanol, acetone and their mixtures (Agnieszka Arceusz *et al*. 2013). By using the method of Agnieszka, Phenolic compound extract was taken from *Vernonia cinerea* using 80% ethanol as solvent (Oluwaseun R Alara, 2018). A methodology was used to get extract of phenolic compounds from licorice root by using 80% ethanol as solvent (80:20) for about 6 hours (Karami *et al*. 2015).

From the above review, the quantitative and qualitative analysis were carried out by using the extract from soxhlet apparatus extraction and chromatography method in wheat grass (P S Sutar Kapashikar *et al*. 2018).

The comparative study on antioxidant activity of coloured wheat grains and its phenolic compounds shows that the antioxidant activity is more in Phenolic extract (Qin liu *et al*,2010). The solvent extract of *Lantana camera* L. shows antifungal properties against some pathogenic fungal strains (Modasir Fayaz *et al*. 2017).

Wheatgrass is the most commonly used herb in India. This plant is believed to have many nutritional values; it has been shown that the plant has anti-inflammatory, antioxidant, anti-carcinogenic, immune-modulatory, laxative, astringent, diuretic, antibacterial and anti-ageing properties. Wheatgrass extracts being natural medicine can be extremely valuable for treating various sicknesses

from minor scratches and blazes to genuine infections (Dr. Amit Kumar Dutta and Dr. Wasim Raja, 2016).

The chlorophyll content of 10 different medicinal plants such as *Melothira maderaspatana, Clitoria ternate, Boerhavia diffusa, Pongamia pinnata, P. pierre, Aegle marmelos, A. correa, Phyllanthus fraternus, Mimosa pudica, Pinsonia grandis* and *Acalypha indica* are estimated by using acetone (Rajalekshmi K $N Banu 2014).

The qualitative difference of chlorophyll a (Chl.a) and chlorophyll b (Chl.b) content between young and adult leaves of ten plant species such as Mango (*Magnifera indica),* Hibiscus (*Hibiscus rosa-sinensis*), Gavua (*Psidium guajava*), Almond (*Prunus dulcis*), Bryophyllum (*Bryophyllum pinnatum*), Sapodilla (*Manikara zapota*), Neem (*Azadiracta indica*), Ashoka (*Polyalthia longifolia*), Ficus *(Ficus benjamina*) and Datura (*Datura stramonium*) showed that the adult leaves showed higher chlorophyll content in comparison to young leaves. This has been proved that the age of leaves was an important factor for chlorophyll content. (Pramod N *et al.* 2015). From this, we can understand that the adult wheat grass contains more chlorophyll content.

Phytochemical and pharmacological screening of wheatgrass juice shows that the plant can be used as an immunomodulator, antidiabetic agent and anastrigent (Shirude Anup Asokh, 2011). P. Tirgar and B. Desai in 2011 detected the presence of iron chelator in wheat grass.

The effect of wheatgrass length on antioxidant activity and total phenolic content in wheatgrass was studied by Yusuf *et al*. 2020. Nutritional quality and antioxidant activity of wheatgrass was discovered by proteome profiling, DPPH

and FRAP assay. Total of 297 proteins were identified by nano-LC-MSE in all stages of wheatgrass (Santosh B. Parit *et al*. 2008).

The wheatgrass extract shows antifungal activity on *Asperigillus niger, Asperigillus flavus, Trichorma viride* (S.G. Vishnu Prasanna *et al.* 2016). The anthocyanin content, antioxidant potential and antimicrobial activity of black, purple and blue coloured wheat flour and wheatgrass juice helps to fight against common human pathogens (Natasha Sharma *et al.* 2020).

Study on quantitative profiling of total phenolic content (TPC) and antioxidant activity (AOA) in seeds, sprouts, and grasses of corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.) showed that the grasses between 10 and 13 days has highest TPCs and AOA. The AOA in sprouts and grasses were a fifth and two-thirds of ascorbic acid (AA) standard, so these are natural antioxidants. (Anuj Niroula *et al.* 2019)

Wiwat Wangcharoen and Suthaya Phimphilai (2016) studied the chemical properties of processed wheatgrass drinks and the results explains that the processed wheatgrass drinks contain less chlorophyll contents and more antioxidant potential. But the consumption of the drink was very low due to its taste.

The study conducted to evaluate the phytochemical profile of green grasses including barley and wheat with special reference to antioxidant properties and the result indicates that the antioxidant activity and free radical scavenging activity of barley grass is higher than wheatgrass (Aiza Qamar *et al.* 2018).

HPLC analysis revealed the presence of major bioactive compounds such as rutin, chlorogenic acid, tocopherol, chlorogenic acid, and gallic acid in *T. aestivum* L. grass and it can be used as an antimicrobial agent for pathogenic bacteria including *Salmonella typhi, Staphylococcus aureus* and *Vibrio cholerae*. These

findings confirm that *T. aestivum.* grass containing medicinally important bioactive compounds and it may have significant potential to be used in traditional medicine system for the treatment of various diseases caused by pathogenic microorganisms (Anand Rajoria *et al*. 2005). The study conducted to evaluate the therapeutic potential of wheatgrass in the treatment and prevention of chronic and acute diseases such as gout, osteoarthritis, thalasemia, skin disease such as eczema, ache, disease related to digestive system, blood and circulatory system, reproductive system, respiratory system, tooth and gum, migraine (Keshari Roshan *et al*. 2016).

The study on effect of wheatgrass juice in supportive care of terminally ill cancer patients showed that it is an alternative of blood transfusion and also its use by cancer patients get better results (S Day *et al.* 2006). And Gil Bar-Sela *et al.* in 2007 find out the effect wheatgrass in improving the haematological toxicity related to chemotherapy in breast cancer patients.

The effect of photoperiod durations (16 h light:8 h dark vs 22 h light:2 h dark) and different doses of Murashige and Skoog medium on the yield and antioxidant characteristics of wheatgrass from hard, medium-hard and soft wheat varieties were analysed. An increase in total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) of wheatgrass in different strengths of MS media under normal photoperiod was observed (Amardeep Singh Virdi *et al.* 2021).

A preliminary screening showed that the curry leaf extracts from different locations exhibits significant anticarcinogenic effects inhibiting the growth of breast cancer cell line (MDA-MB-231) and maximum inhibition of MDA-MB-231 cell was observed in the curry leaf extract from Kelantan. Based on these results, the Malaysian curry leaf collected from the North (Kelantan) might be potential

source of potent natural antioxidant and beneficial chemo preventive agents. (Ali Ghasemzadeh *et a*l. 2014.)

Phytophthora diseases are among the most important diseases in sub-Saharan Africa which result in severe socio-economic consequences. Roots and tubers crops are important staples and foreign exchange earner crops in Ghana which are significantly challenged by the incidence and severity of *Phytophthora* diseases. To ensure food availability, safe ecosystem and protection of the environment, innovative and sound management practices are needed to destroy the different *Phytophthora* diseases on crops, more specifically with cocoa and taro as case studies. (Benedicta Nsiah Frimpong *et al*.2021)

The study conducted by Sheji *et al*. in 2019 find the sequence analysis and the secondary structure of RNA of *Phytophthora* and it revealed that black pepper isolate of *Phytophthora* shares characteristics of both *P. capsici* and *P.tropicalis* whereas coconut and cardamom isolates matched that of *P. palmivora* and *P. meadii.*

Numerous pesticides, herbicides and chemical fertilizers are being applied by the farmers to deal with the plant disease but leave very disastrous and undesirable after effects on ecosystem as non-degradable molecules. Botanical agents can be utilized as an eco-friendly and effective alternative against chemical as they are of natural origin. In this context, two synthetic fungicides namely Manzate and Nystanin in three different concentrations namely 500ppm, 1000 ppm and 1500 ppm were evaluated against *Sclerotium rolfsii*, *Alternaria alternata*, *Fusarium monilifrome, Rhizoctonia solani* and *Aspergillus niger* in vitro to compare them with ethanolic botanical extracts of spices (clove, cinnamon, thyme) and weeds (parthenium and calotropis) at 5%, 10%, 15%, 20% and 25%. The Results revealed

that the botanical extract of clove extracts showed maximum inhibition (100%), followed by reduced inhibition in cinnamon, thyme on Parthenium and Calotropis treated plates. Hence the herbal products can be applied as a potent, eco-friendly and economical substitute to chemical pesticides. (Jyoti Singh *et al.* 2019).

Carbazole alkaloids which are abundantly present in the leaves, fruits, roots and bark of the *M. Koengii* L. Spreng. So the plant shows antidiabetic, anticancer, antibacterial, anti-nociceptive and antioxidant activities. Besides these activities, the plant is described to have a wide array of therapeutic activities. (Prasan R Bhandari, 2012)

M. koenigii belongs to the Rutaceae family, which is commonly used as a medicinally important herb of Indian origin in the Ayurvedic system of medicine. Previous reports have demonstrated that the leaves, roots, and bark of this plant are rich sources of carbazole alkaloids, which produce potent biological activities and pharmacological effects. Rengasamy Balakrishnan *et al*.in 2020, studied the Medicinal Profile, Phytochemistry, and Pharmacological Activities of *Murraya koenigii* L. Spreng. and its Primary Bioactive Compounds. This research also focused on the molecular basis of such activities in various cellular and animal models to validate the efficacy of *M. koenigii* and its derivatives as potent therapeutic agents.

The study conducted by Nivetha *et al.* 2019. shows that the *Murraya koenigii* L. Spreng. contains phytochemicals such as saponins, proteins, steroids, tannin, carbohydrates, alkaloids, flavonoids and glycoside. It has antimicrobial, antifungal, antidiarrheal, anticancer, antidiabetics and anti-inflammation property. It also has the skin improving effect.

According to in vitro and in vivo models, the leading constituents of curry leaves play a crucial role in diabetic and anticancer management by regulating various molecular pathways, including Bcl-2, Bax, NF-κB, and TNFα. So the study conducted by D T Abysinghe *et al*. in 2021 explained the phytoconstituents, their structures, biological activities, and pharmacological actions with clinical studies of curry leaves.

The study conducted to review the ethnobotanical properties, pharmacognostic, phytochemical and pharmacological properties of *M. koenigii.* plant showed that the various parts of this plant are widely used by different tribal communities. The leaves of plant are use as tonic, stomachic, carminative, internally in dysentery, vomiting. It is also used as an anti-helminthic, analgesic, anti-inflammatory agents. (Harish K Handral and Anup Pandith, 2012).

Medicinal plants have been used in traditional healthcare system throughout human history and are considered as a source of healthy human life. Different parts of the plants like roots, leaves, stem, bark, fruits and seeds have been used in combating infection and strengthening the immune system. *M. koenigii*. is a potential medicinal plant highly valued for its characteristic aroma and bioactive compounds. The leaves and roots in different forms have great therapeutic potential and it is used for the treatment of night blindness, dysentery, diarrhoea, vomiting, bites of poisonous animals, bruises and eruption. Leaves are often used in curries for flavouring and seasoning due to their aromatic nature.

Phytocompounds like koenimbine, koenine, mahanimbine, murrayazolidine, murrayazoline, murrayacine, girinimbine, mukoeic acid, etc. have also been isolated and characterized from the curry leaves plant. These bioactive compounds possess antioxidant, antimicrobial, anthelmintic, analgesic, anti-

inflammatory, antidiarrheal, hepatoprotective and antitumor properties. So the isolated compounds can be used for the development of viable drugs for the treatment of varieties of ailments. (Dheeraj K.Gahlawat *et al*. 2014)

AIMS AND OBJECTIVES

AIM

Find out the effect of phenolic extract of wheatgrass (*Triticum aestivum* L.) against the *Phtophthora* sp. in *Murraya koenigii* L. Spreng.

OBJECTIVES

- Cultivation of wheatgrass in appropriate field.
- To collect 13 days old wheatgrass (*Triticum aestivum* L.).
- To identify *Phytophthora* sp. affected *Murraya koenigii* L. Spreng. (with the help of microscope).
- Soxhlet extraction of phenolic compounds from wheatgrass (*Triticum aestivum* L.).
- Testing of the antifungal activity of phenolic extract.
- Preparation of fungicide using phenolic extract of wheatgrass.
- Application of fungicide directly to the infected *Murraya koenigii* L. Spreng.

MATERIALS AND METHODS

Cultivation of *Triticum aestivum* L.

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Monocots

Clade: Commelinids

Order: Poales

Family: Poaceae

Subfamily: Pooideae

Genus: Triticum

Species: *Triticum aestivum* L.

Wheat grains are allowed to soak in water for about 30 minutes, after the water is drained and allowed to dry overnight in a cotton cloth. The wheat grains were sawn in soil and allow to grow the seeds. Grains were evenly distributed in the soil as much possible.

Collection of wheat grass

The 13th day grass of *T. aestivum* was collected and washed thoroughly in water in order to remove the soil and other dirt from the grass and chopped in to small pieces with the help of knife. It was dried in shade and the dried grasses are powdered with mechanical grinder and stored in air tight containers for studies.

Extraction of plant material

Wheat grass Powder was subjected to Soxhlet extraction by using 80% ethanol (300ml) for about 6 hours. The extract obtained by this method are evaporated to dryness for removing the solvents and kept in a container for further study (Karami *et al*. 2015).

Preparation of fungicide

The extract obtained from Soxhlet extraction of wheat grass were dissolved in solvent (distilled water). Using this solvent stock solution prepared at the concentration of 1g /100ml.

Fungal culture preparation

Potato dextrose agar (PDA) is used for the cultivation of fungi.

Principle of potato dextrose agar (PDA)

Potato dextrose agar (PDA) is composed of dehydrated potato infusion and dextrose that encourage luxuriant fungal growth. Agar added as a solidifying agent. Dextrose as carbohydrate source. Specified amount of sterile tartaric acid (10%) to lower the PH of this medium to 3.5 +/-0.1 inhibiting bacterial growth. Chloramphenicol acts as selective agent to inhibit overgrowth of competing

microorganism from mixed specimen, while permitting the selective isolation of fungi. Care should be taken not reheat the acidified medium, heating in the acidic medium will hydrolyse the agar which can render the agar unable to solidify.

Composition of potato dextrose agar

Potato infusion :20g

Dextrose :2g

Agar :2g

Distilled water :100ml

Procedure

To prepare potato infusion, boil 20g slices peeled potatoes in 100ml distilled water for 30 minutes. Filter through cheese cloth saving effluent which is the potato infusion. Mix with dextrose (2g), agar (2g) and distilled water and boil to dissolve.

The medium is autoclaved for 15 minutes at 120degree. Adjust the pH 6. Dispense 20-25 ml portion into sterile 15/100 mm petri dishes.

Isolating fungus from leaves and leaf disc culture

Cut small sections of 5×3 mm square size from margin of infected lesions. After that place the pieces of leaves into surface sterilant solution make sure that the whole surface was immersed in it. After,15 - 30 seconds the sections are taken out aseptically. Then the sections are washed in sterile water blotted dry as clean sterile paper towels and placed on nutrient medium.

Inoculate 3-5 bits of leaf disc per petridishes and the petridishes are placed in plastic tray and also place four cottons with alcohol in each corner of the tray. Incubate at 250 degree celsius for 3-5 days After 5 days the leaf disc culture is sub cultured to produce pure culture in potato dextrose agar medium.

Fungal culture

Phytophthora sp. which causing white spot disease in curry leaves are isolated and used to test the antifungal effect of wheat grass extract using different solvents (distilled water, chloroform and acetone) by well diffusion method.

Antifungal activity of wheat grass extract

Antifungal activity is accessed by agar well diffusion method (Dubey R C *et al*,2002). Steps involved in the screening are,

1. 8 sterile petriplates are maintained.

2.Prepare agar medium (20g potato infusion,2g dextrose,2g agar dissolved in 100ml distilled water.) and sterilize the medium in autoclave.

3.Pour the agar medium to the petriplates with thickness of 0.5 cm, allow it to solidify.

4.Prepare the well using well cutter in 0.6 cm diameter.

5.Swab the fungal culture on the agar plate.

6.Pour the extract (separately dissolved in water, chloroform and acetone solution) to the well.

7.Incubate the extract filled with petriplates for 3-5 days

8.Observe the inhibition.

Application of fungicide directly on *Murraya koenigii* L. Spreng.

affected with white spot disease

The wheatgrass extract dissolved in water with a concentration of 1g/100ml and it is applied on curry leaf plant affected with white spot disease once in day for 20 days.

DPPH free radical scavenging activity

The DPPH FREE radical scavenging activity was determined by the method of shimba (1992).0.1Mm DPPH (2,2-diphenyl-1-picrylhydrazil) was prepared by methanol solution.0.5g of sample was homogenized using 5ml of methanol and centrifuged the contents. The supernatant was collected, different aliquots (0.5and 1ml) were prepared and final volume was made up to 1ml using methanol. To this mixture added 2 ml of 0.1mM DPPH solution (control)and the reaction mixture was measured at 517nm against methanol as blank. The assay was carried out in triplicates. Lesser values of absorbance of the reaction mixture indicate higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using formula

$$\text{Antioxidant activity} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Abs control = absorbance control, Abs sample = absorbance sample

Estimation of chlorophyll

weighed 1g of finely chopped leaf sample and ground the tissue into fine pulp with the addition of 20ml of 80% acetone. Centifuge it at 5000 rpm for 5 minutes. transfer the supernatant to 100ml volumetric flask. Ground the residue with 20 ml of 80% acetone. Centrifuged and transferred the supernatant to the same volumetric flask. Repeat the procedure until the residue is colourless washed the mortar and pestle thoroughly with 80% acetone and collect the washings and centrifuged. Makeup the volume to 100ml using 80% acetone. Read the absorbance of solution at 645 and 663nm.

$$\text{Chlorophyll a (mg/g tissue)} = \frac{12.7(A\ 663)-2.69(A645)}{1000} \times V \times W$$

$$\text{Chlorophyll b (mg/g tissue)} = \frac{22.9(A645)-4.68(A663)}{1000} \times V \times W$$

A645 = Absorbance; A663 = Absorbance at 663

RESULTS AND DISCUSSION

Table :1 and 2 shows the chlorophyll content in normal, affected and treated plant.

	Chlorophyll a	Chlorophyll b
Normal plant	**1.43**	**0.34**
Affected plant	**0.513**	**0.544**

Table 1

Plant A (mg/g tissue)

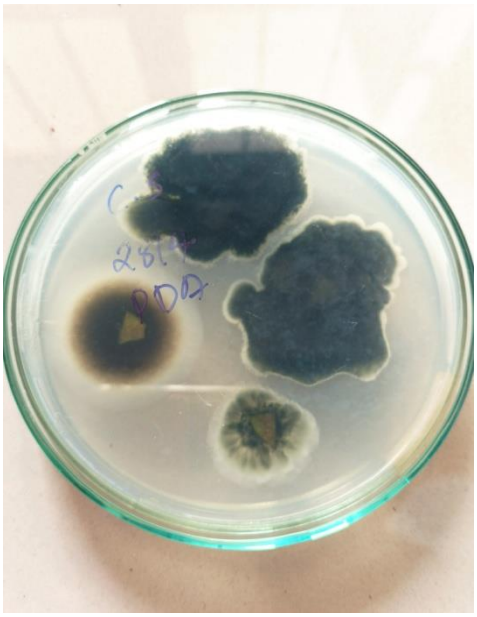
Day	Chlorophyll a	Chlorophyll b
5th day	**0.346**	**0.288**
10th day	**1.282**	**0.752**
15th day	**1.344**	**1.044**

Table 2

In the present study the antifungal properties of wheatgrass (*Triticum aestivum* L.*)* Were used against white spot disease caused by *Phytophthora* sp. In Murraya koenigii L. Normal *Murraya koenigii* L. Spreng. plant with 1.43 Mg/g chlorophyll a and 0.34 chlorophyll b. In *phytophthora* sp. affected Murraya plant the chlorophyll ais reduced to 0.513 and chlorophyll b is reduced to 0.544. After the application of extracted fungicide (concentration; 1g/100ml) the chlorophyll a and chlorophyll b are gradually increased to 1.344 Mg/g and 1.044 Mg/g respectively. The antioxidant activity of wheatgrass powder and wheatgrass extract were estimated by DPPH method, which shows that the wheatgrass extract has highest antioxidant activity.

Potato Dextrose agar culture

Using *Phytophthora* sp. infected leaves of *M. koenigii* leaf disc culture is done in potato dextrose agar medium for 3-5 days, through sub culturing pure culture is obtained.

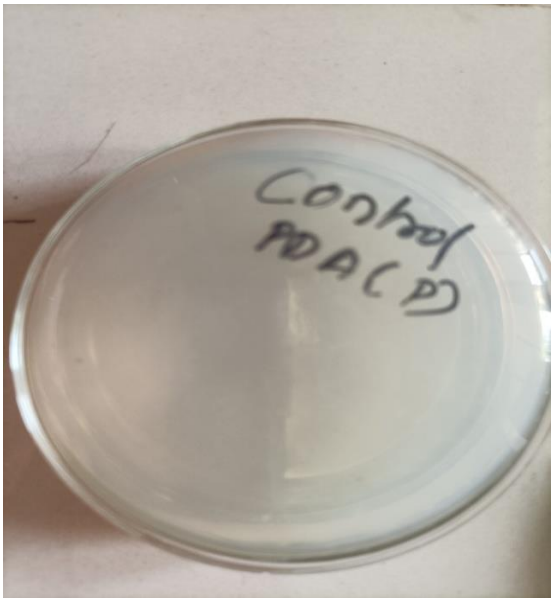


Leaf disc culture of Phytophthora

subculture

Antifungal activity of wheat extract against *Phytophthora*

The antifungal activity of wheat grass extract in chloroform solvent shows high antifungal activity.



Control

Solvent (chloroform 0.1ml)

Application of fungicide in curry leaves

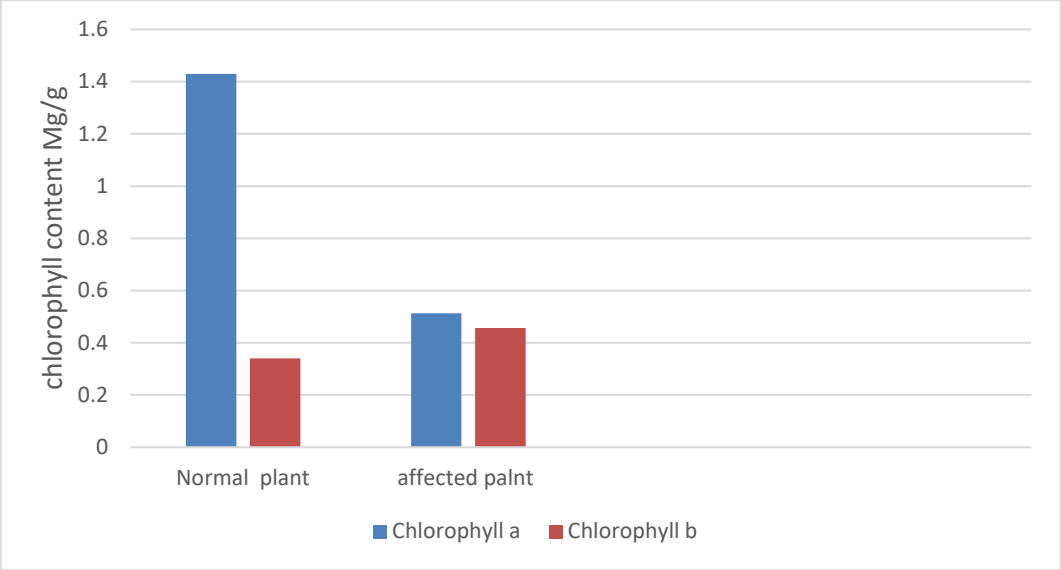
The fungicide with a concentration of 1g /100ml (fig;1.1A, 1.2A, 1.3A) is applied directly to the plant in which maximum results at 20th day against white spot disease in *M. koenigii.*

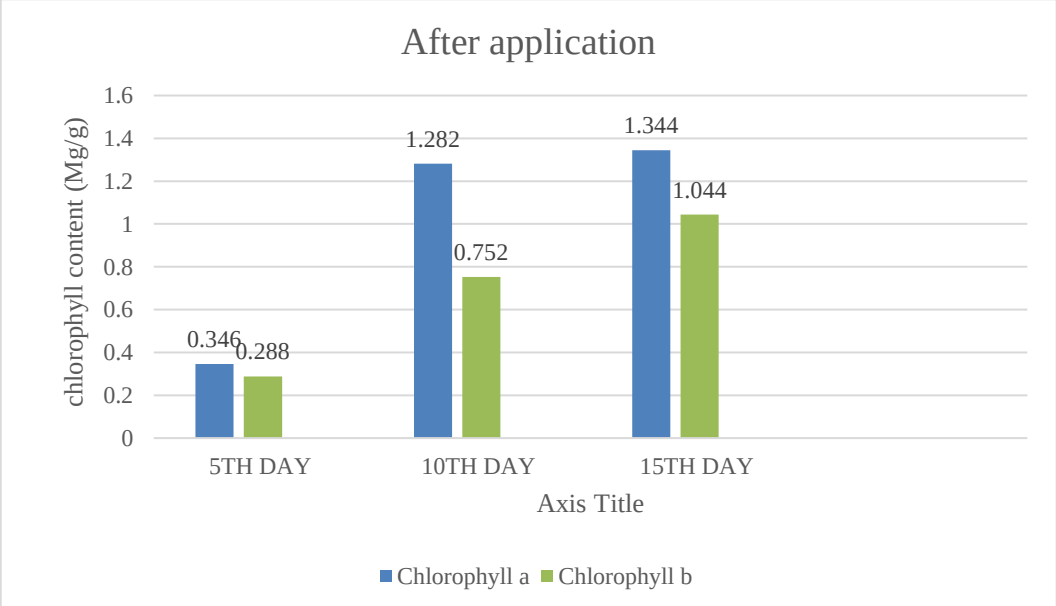
Before application (1.1A)

10th day (1.2A)

20th day (1.3A)

Chlorophyll content in *Murrya koenigii* L. Spreng.



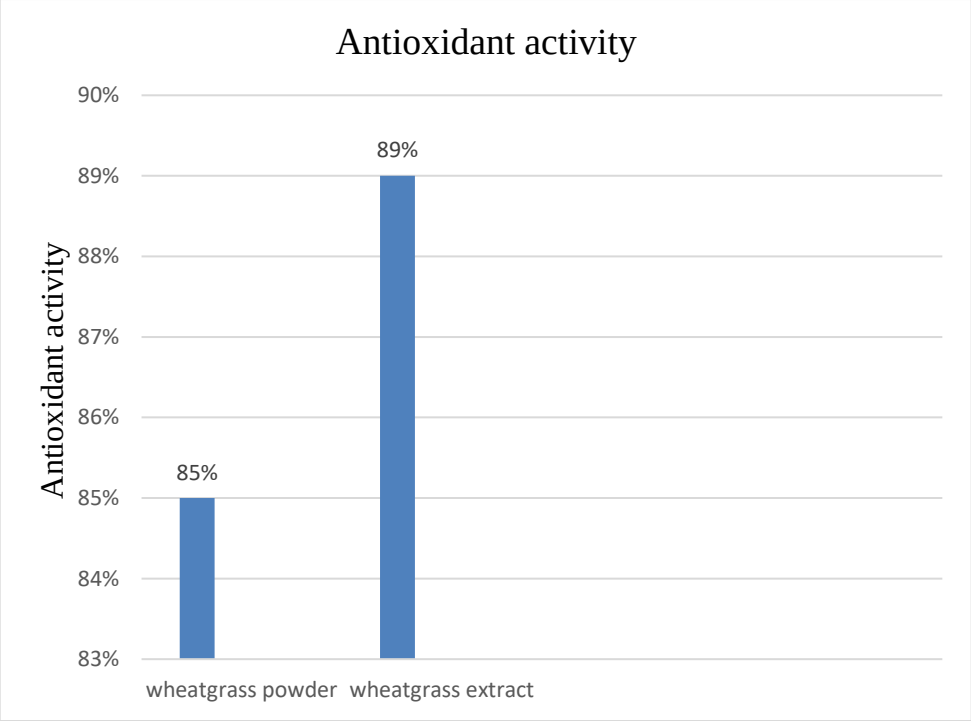


Wheatgrass extract

Total of 4.89g of phenolic extract of wheatgrass (*T. aestivum*) was obtained by soxhlet extraction method.

DPPH Radical scavenging activity of wheat grass

The graph showing antioxidant activity of wheat grass extract and wheat grass powder, in which wheat grass extract have high antioxidant activity.



DISCUSSION

Wheatgrass refers to the seedling of common wheat plant *Triticum aestivum L*. it has been collected for the preparation of effective fungicide especially for the treatment of *Phytophtora* sp. in *Murraya koenigii* L. Spreng.

In this present study the antifungal properties had been identified through well diffusion method. It takes place through the culturing of affected leaf. The antifungal properties of the extract are tested through well diffusion method.

The antifungal activity is very high in wheatgrass extract. It is tested through analysis of chlorophyll content in normal plant, affected plant and treated plant. In normal plant, the concentration of chlorophyll a is 1.43Mg/g and chlorophyll b is 0.34 Mg/g. In affected plant the concentration of chlorophyll a is 0.513 Mg/g and chlorophyll bis 0.544 Mg/g. But in treated plant the concentration of chlorophyll a is 1.344 Mg/g and chlorophyll b is 1.044 Mg/g.

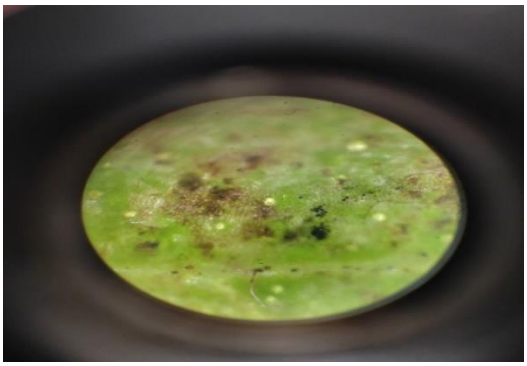
The result reveals that the fungus *Phytophthora* directly affect the chlorophyll synthesis and photosynthetic rate of *M. koenigii*. Phytophthora is an endoparasite that affect the cellular metabolism through the spreading of their mycelium in the intercellular spaces of mesophyll cells and the absorption of water and nutrients. That also affect the flavour and aroma of *M. koenigii*.

The application of fungicide controlled the vigorous growth of *Phytophthora sp.* fungus and promotes the activities of *Murraya koenigii* L.Spreng. The concentration of 1g/100ml is more effective than other concentrations, that is because the concentration of phenolic compound is very effective for the regulation of fungal growth. The present study clearly reveals that the phenolic compounds

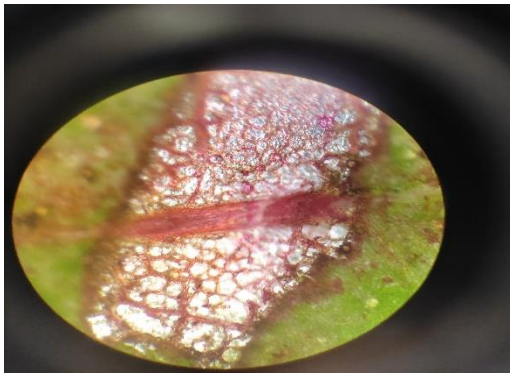
from the higher plants are very effective for the treatment of fungal infections in *Murraya koenigii* L. Spreng.

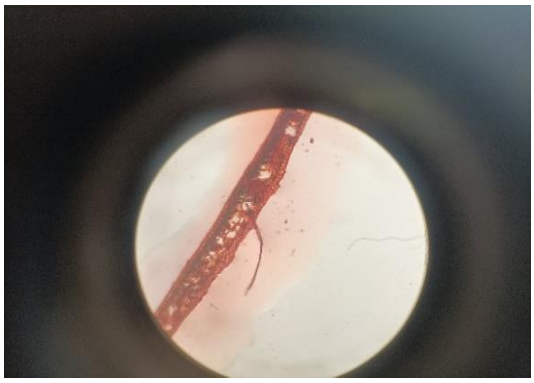
Phytophthora sp.* infected *Murrya koenigii* L. Spreng. *Infected plant

Infected leaf



leaf under microscope





***Phytophthora sp.* infected leaf**

Pure culture

SUMMARY AND CONCLUSION

Wheat is a grass widely cultivated for its seed, a cereal grain that is a worldwide staple food. Wheat is the main cereal crop in India. The total area under the crop is about 29.8 million hectares in the country.

The present study focuses on the antifungal properties of wheatgrass *(Triticum aestivum* L.). The phenolic extract of wheatgrass is used for the treatment of *phytophthora* sp. infection in *Murrya koenigii* L.Spreng .Through well diffusion method the antifungal properties of the extract is determined . wheatgrass extract have high antioxidant activity than wheatgrass powder. And also the wheatgrass extract contains high phenolic content.

The extracted fungicide is applied once in a day for 20 day on the *Phytophthora sp.* affected *Murraya koenigii* L.Spreng. The antifungal activity of wheatgrass extract is tested through the analysis of chlorophyll content in affected *Murraya koenigii* L. Spreng. The affected plant with 0.513Mg/g of chlorophyll a and 0.544Mg/g of chlorophyll b. But after treating with extracted fungicide the concentration of chlorophyll a chlorophyll b is increased to 1.344 Mg/g and 1.044 Mg/g respectively .

Plants are used for the treatment of various diseases due to the phytochemical compounds present in it.Our study reveals that wheatgrass contains phytochemical just like higher plants. So the phenolic extract of wheatgrass has antifungal activities. Screening of antifungal studies reveals that whaetgrass (*Triticum aestivum* L.) shows maximum activity against *Phytophthora* sp. on its chloroform extract . So it can be used as a fungicide in agriculture .

The present study is justified and claimed the uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antifungal agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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