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POTENTIAL OF DUHAT (SYZIGIUM CUMINI) LEAVES AND BARK EXTRACT

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ABSTRACT

Fruit bearing trees are potential sources of chemicals called mitotic inhibitors. These chemicals can be used as ingredient to anticancer medecines which have the effect of inhibiting mitosis and deterring the growth of cancer cells. The mitotic inhibitory property of Duhat also known as Java plum *(Syzigium cumini)* have motivated the researcher to conduct his investigatory project , to discover the inhibiting action of the duhat leaves and bark against mitosis in onion and mung beans. The primary aim is to determine the phytochemical components of duhat that can be used as mitotic inhibitors. The phytochemical analysis revealed that duhat leaves extract contains carbohydrates, reducing sugars, flavonoids, tannins, saponins, phytosterols and anthraquinone. In addition, the duhat bark extract was found to contain carbohydrates, reducing sugars, flavonoids, tannins, saponins, glycosides and phytosterols and anthraquinone. In contrast, glycosides are present in duhat leaves extract only and it is absent in duhat bark extract. The phytochemicals present are the chemicals effective as mitotic inhibitors. The 50 % and 100 % duhat leaves extract are the two most effective mitotic inhibitors in onion, whereas the 50 %, 75 % and 100 % duhat bark extract inhibited mitosis in onion at same rate. The 75 % and 100 % duhat leaves extract are the two most effective mitotic inhibitor in mung bean, while the 100 % duhat bark extract is the most effective mitotic inhibitor in mung bean. Therefore, instead of synthesizing mitostic inhibitory drugs, Duhat leaves and bark should also be considered as an effective source of natural mitotic inhibitors.

Keywords: Dahut, Bark Extract, Mung Bean,

I. INTRODUCTION

*"Philippines is an ecological paradise"*If one thing is true about our country, it is the unending catalogue of animal and plant species. It is an ecosystem with an assorted life forms.This archipelago is blessed with flora and fauna and other natural resources waiting to be discovered and be used. Bulacan, the southernmost province of Central Luzon lies on a fertile land area. One of its municipality- *San Ildefonso*, is an agricultural town and famous for being dubbed as the "vegetable capital of Bulacan". Unknowingly, this town is not only vegetable-producing but also prominent for its fruit bearing trees. Locals are aware that once you plant a seed in a good soil it will grow. Name a Tagalog fruit and the town have plenty of it. During the summer season many of these sweet delights from nature ripens. Cashews, star apples, mangoes, siniguelas, camachile and watermelons, aratilis, cantaloupes and carambolas *(balimbing)* are familiar summer fruits. Fruit trees are also well known for their leaves. In Philippine folk medicine, leaves from fruit trees are used as decoction and tea substitute. Among them, the boiled guava, atis and caimito leaves are the most familiar drinks. Leaves are natural. Phytochemicals obtained from them are free from synthetic chemicals. There are so much to discover from plant leaves (Prakash, 2013).

One fruit tree that is very familiar to barrio children is the Java plum, also known in Luzon as *Duhat.* The fruit started to mature once the month of May arrives. The Java plum fruit is a luscious barrio dessert, but its leaves often fall in the ground and withered just like any other common leaves. The researcher aims to utilize the Duhat leaves in some useful application such as in medical field.

The society is in need of natural medicine for treating various illnesses. 21st century lifestyle brings about many types of diseases. Cancer is a disease that oftentimes is caused by peoples' lifestyle. Cancer is a broad term that encompasses many types depending on the organ that it affects. Cancer involves abnormal cell growth with the potential to invade or spread to other parts of the body. A benign tumor on the other hand is the opposite of cancer because its cells do not spread. Possible signs and symptoms of a cancer include a lump, abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements. While these symptoms may indicate cancer, they can also have other causes. Over 100 types of cancers affect humans (American Cancer Society, 2018).

In 2015, about 90.5 million people had cancer. About 14.1 million new cases occur a year. It caused about 8.8 million deaths (15.7% of deaths).

Cancer remains a national health priority in the Philippines with significant implications for individuals, families, communities, and the health system. Cancer is the third leading cause of morbidity and mortality in our

country after diseases of the heart and the vascular system (Philippine Health Statistics 2009). Among Filipino men, the 6 most common sites of cancer diagnosed in 2010 (Globocan) were lung, liver, colon/rectum, prostate, stomach, and leukemia. Among Filipino women the 6 most common sites diagnosed were breast, cervix, lung, colon/rectum, ovary and liver. Furthermore, 189 of every 100,000 Filipinos are suffering from cancer while four Filipinos die of cancer every hour or 96 cancer patients every day, according to a study conducted by the *University of the Philippines' Institute of Human Genetics, National Institutes of Health.* (Department of Health, 2018).

In 2012, a foreign study stated that about 165,000 children under 15 years of age were diagnosed with cancer. The risk of cancer increases significantly with age, and many cancers occur more commonly in developed countries. Cancer is one of the leading causes of death nowadays. . It was revealed in a research that majority of cancers, which is about 90-95% of cases are due to genetic mutations from environmental and lifestyle factors. The remaining 5-10 % are due to inherited diseases. (Hanahan and Weinberg, 2011). Rates are increasing as people gets older and as lifestyle changes occur in the developing world. If we will change our lifestyle and go for natural food and medicine choices, the risk of getting cancer will become lower (Stefanska et al, 2012).Cancer are made up of cells too. Cancer is fundamentally a disease of tissue growth regulation. Cancer cells spread through mitosis or cell division.

Cell division is a normal process used by the body for growth and repair. A parent cell divides to form two daughter cells, and these daughter cells are used to build new tissue, or to replace cells that have died because of aging or damage. Healthy cells stop dividing when there is no longer a need for more daughter cells, but cancer cells continue to produce copies. They are also able to spread from one part of the body to another in a process known as metastasis. Cancer cells are created when the genes responsible for regulating cell division are damaged (Mukhtar et al, 2014). Cell division in mitosis can be inhibited by certain chemical known as mitotic inhibitor. These mitotic inhibitors are usually drugs that disruot microtubules which are structures that pull chromosomes apart when a cell divides. Flavonoids, alkaloids, tannins and saponins are some phytochemicals used in mitosis inhibition (Wang, 2015). They interrupt cell division ussualy in the phase of cell cycle when two sets of fully formed chromosomes are supposed to separate into daughter cells. Mitotic inhibitors are used in cancer treatment, because cancer cells spread through continuous mitotic division. Thus, cancer cells are more sensitive to inhibition of mitosis than normal cells (Bowman, 2010).

The Duhat or Java plum leaves and bark extracts are viewed by the researcher as possible sources of mitotic inhibitors. Java plum has long been placed in Filipino folkloric medicine, thus scientific studies about its chemical content will validate its healing properties. The Duhat leaves and bark extracts' mitotic inhibition property will be confirmed by testing it to selected organisms. The eggs of zebrafish is a common test animal cells in inhibiting mitotic growth (Spence, 2016). In this research, plant cells especially onion bulb and mung bean were chosen as plant organisms that served as subject for experimental testing. Cell divisions occur rapidly in growing root tips of sprouting seeds or bulbs. Onion bulbs and sprouting mungbeans are the two most suitable subject for this research due to their availability in the community. In addition, the antifungal property of duhat leaves and bark extract will also be tested. This is to find links between the relationship of mitotic inhibition and antifungal property of duhat.

Onion bulbs *(Allium cepa, L.)* grow root tips that are extremely popular for viewing the different phases of mitosis because of its large chromosomes under the light microscope. Onion root tip is a rapidly growing part of the onion and thus contains many cells in different stages of mitosis. The mung bean seeds (*Vigna radiata*) on the other hand are very easy to germinate and mitosis is responsible for its growing sprouts. This characteristics made mung beans appropriate for this study (Oliva, et al, 2012). The potential mitotic inhibitors in duhat's leaves and bark extracts could possibly stop or inhibit the mitosis in onion root tips and mung bean sprouts.

Plant cells will be used in the experimentation of this study to observe the delay of mitosis. However, plants don't get cancer like animals and humans do. Plants on the other hand can get tumors and these tumors do not become cancerous because plant cells do not move around. Rather thay are held in place by cell walls (Pells, 2013). Plant tumors — aggregates of cells that have multiplied excessively — are usually caused by a bacterium, virus or fungus, or may develop as a result of structural damage. Nevertheless, mitosis is still exhibited by growing plant cells and mitotic inhibition has an effect on them.

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The researcher tried to evaluate the potential of Duhat (*Syzigium Cumini*)*)* plant extract as substitute mitotic inhibitor to commercial and synthetic ones. Mitotic inhibitor chemicals will then be used as a potential active ingredient to anticancer medicines.

Statement of the problem. Specifically the researcher aims to;

1. Determine the mitotic inhibitor contents of Duhat *(Syzigium cumini)* leaves and bark extracts;
2. Compare and contrast the phytochemical profiles of Duhat *(Syzigium cumini)* leaves and bark extract;
3. Determine the antifungal property of Duhat (*Syzigium cumini)* leaves and bark extract against *Candida albicans.*
4. Determine the effects of Duhat *(Syzigium cumini)* leaves and bark extract on the mitotic cell growth of onion *(Allium cepa, L.)* Root tips and mung bean *(Vigna radiata)*.

Hypothesis. The researcher follows the null hypotheses;

1. The Duhat *(Syzigium cumini)* leaves and bark extracts have no phytochemical contents that can be considered as mitotic inhibitors;
2. The Duhat *(Syzigium cumini)* leaves and bark extract have no significant differences in their phytochemical profiles;
3. The Duhat (*Syzigium cumini)* leaves and bark extract do not inhibit the growth of the test organism *Candida albicans.*
4. The Duhat *(Syzigium cumini)* leaves and bark extract have no significant mitotic inhibitory effect on the growth of onion *(Allium cepa, L.)* root tips and germination of mung bean *(Vigna radiata)* sprouts;

II. PROCEDURE

1. Authentication of plant

Duhat leaves and bark were gathered, washed, cleaned and dried for proper authentication in UP Diliman, Institute of Biology at Diliman Quezon City.

2. Preparation of plant for rotary evaporation.

Fresh Duhat leaves and fresh duhat bark were gathered and a bottle of alcohol was also prepared. The leaves and bark were chopped and weighed. 1 kg of plant leaves was mixed with 1L 70% of ethyl alcohol then soaked it for 72 hours. Another 1 kg of plant bark was mixed with 1L 70% of ethyl alcohol then soaked it for 72 hours.

After soaking, the leaves and bark were further turned into smaller particles using a blender. The samples were then squeezed using clean piece of cheese cloth and strainer then were set aside in a clean sterilized glass bottle. The extract was submitted to the College of Chemistry Analytical Analysis Division for the rotary evaporation process. After 5 working days the pure extracts of leaves and bark were obtained from the rotary evaporation process. The solvent was already removed from the pure plant extract.

3. Preparation of plant for phytochemical analysis

The pure Duhat leaves and bark extract were submitted to the Phytochemical Analysis Division of University of the Philippines Manila, Ermita, Manila City for Phytochemical analysis.

4. Preparation of plant extract for the Antimicrobial assay *(Antifungal test)*

20 ml pure Duhat leaves extract and another 20 ml of pure Duhat bark extract were submitted to the University of the Philippines Diliman, Natural Sciences Research Institute (Miranda Hall) for the antimicrobial assay using *C. albicans* as the test organism.

For the conduct of the antimicrobial test, the microbial suspension was prepared from 24-hour old culture of the test organism. The suspending medium used was 0.1 % peptone water.The Glucose Yeast Paptone Agar plates are about 3 mm thick. The GYP plates were incubated at 35 degrees Celcius and observed after 24 hours. The clearing zone was measured in millimeters and the average diameter of the clearing zones was calculated. The antimicrobial index (AI) was calculated using the formula;

$$AI = \frac{\text{Diameter Of Clearing Zone} - \text{Diameter Of Well}}{\text{Diameter of well}}$$

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5. Preparation of the Onions and analysis of onion root tips

The onions were weighed and cleaned using a dry cloth. Dead root tips were removed. For the set-up, 20 ml of leaf and bark extracts at different concentrations were separately poured in beakers. There is one onion in each cup and is placed at different concentrations of extract. The onion is suspended using a stick and straw. It is positioned in a way that the onion tip is dipped on the surface of the extract, thus, avoiding the direct contact between the beaker's floor and the onion tip. This set-up will let the onion tip to grow its roots without disruption. The distilled water that promotes growth of onion roots will be used as the negative control and will then become the basis for comparison.

The growth of onion root tips were observed after every 24 hours for five days and the growth of roots were observed as well and counted. The numbers and length of roots were recorded.

6. Preparation of the Mung beans and its growth analysis

The mature mung beans are weighed and cleaned using a dry cloth. 15 seeds of mung bean were placed in each petri dish. A clean and soft tissue is used as protective underlayer of mungbeans to avoid it from having a direct contact with the glass. In doing the treatment, 3 ml amount of the leaf and bark extracts at different concentrations were separately dropped on the beans in each of the set-up. Distilled water will also be used as negative control.

The mung beans were observed after every 24 hours for five days. The growth of the sprouts were observed, its numbers and length were recorded.

III. DATA ANALYSIS

The average number of onion bulbs and mung beans which did not produce roots and sprouts were determined in each treatment. The data were subjected to ANOVA, Analysis of Variance- Two Factor without Replication (Statistics Solution, 2013) for both 0.05 and 0.01 level of significance.

IV. RESULTS

A. Phytochemical Analysis of pure Java plum (duhat) leaves extract

Table 1. Phytochemical profile of duhat leaves

(Analyzed by the Institute of Pharmaceutical Sciences; Phytochemical Analysis Division of University of the Philippines Manila)

	Phytochemical Analysis of Duhat Leaves		
Tests	**Positive results**	**Actual results**	**Indication**
For carbohydrates			
Molisch test	Violet ring at the junction	Violet ring at the junction	Positive
For reducing sugar			
Fehling's Test	Formation of brick red precipitate	Brick red precipitate	Positive
For flavonoids			
Alkaline reagent test	Yellow coloration disappears upon the addition of dilute acid	Yellow coloration disappears upon the addition of dilute acid	Positive
Lead acetate test	Presence of yellow turbidity or precipitate	Yellow precipitate	Positive
For alkaloids			
Hager's test	Yellow precipitate or turbid solution	Clear yellow solution	Negative
Mayer's test	White precipitate or turbid solution	Clear yellow solution	Negative
Wagner's test	Reddish brown or turbid solution	Clear reddish brown solution	Negative

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For tannins			
Ferric chloride test	Blue solution-presence of gallic tannins Green to black solution-presence of catabolic tannins	Black colored solution	Positive
For glycosides			
Keller kilani test	Reddish brown/purple ring at the junction	Brown ring at the junction	Positive
For saponins			
Froth test	Froth greater than 2 cm even after 30 seconds	Froth formation that is persistent after 30 seconds	Positive
For resins			
Test for resins	Turbid solution	Clear light yellow solution	Negative
For phytosterols			
Liebbermann-Burchard test	Brown ring at the junction , green upper layer-presence of sterols/ Deep red- presence of triterpenoids	Purple ring at the junction	Positive
For anthraquinone			
Test for anthraquinone	Red color	Orange-red turbidity	Positive
For proteins (Peptide bonds)			
Buret test	Purple or violet color	Brown turbidity	Negative

B. Phytochemical Analysis of pure Java plum (duhat) bark extract

Table 2: Phytochemical profile of duhat bark

(Analyzed by the Institute of Pharmaceutical Sciences; Phytochemical Analysis Division of University of the Philippines Manila)

	Phytochemical Analysis of Duhat bark		
Tests	**Positive results**	**Actual results**	**Indication**
For carbohydrates			
Molisch test	Violet ring at the junction	Violet ring at the junction	Positive
For reducing sugars			
Fehling's Test	Formation of brick red precipitate	Brick red precipitate	Positive
For flavonoids			
Alkaline reagent test	Yellow coloration disappears upon the addition of dilute acid	Yellow coloration disappears upon the addition of dilute acid	Positive
Lead acetate test	Presence of yellow turbidity or precipitate	Yellow precipitate	Positive
For alkaloids			
Hager's test	Yellow precipitate or turbid solution	Clear yellow solution	Negative

Mayer's test	White precipitate or turbid solution	Clear yellow solution	Negative
Wagner's test	Reddish brown or turbid solution	Clear reddish brown solution	Negative
For tannins			
Ferric chloride test	Blue solution-presence of gallic tannins Green to black solution-presence of catabolic tannins	Black colored solution	Positive
For glycosides			
Keller kilani test	Reddish brown/purple ring at the junction	No ring at the junction	Negative
For saponins			
Froth test	Froth greater than 2 cm even after 30 seconds	Froth formation that is persistent after 30 seconds	Positive
For resins			
Test for resins	Turbid solution	Clear light yellow solution	Negative
For phytosterols			
Liebbermann-Burchard test	Brown ring at the junction , green upper layer-presence of sterols/ Deeo red- presence of triterpenoids	Purple ring at the junction	Positive
For anthraquinone			
Test for anthraquinone	Red color	Orange-red turbidity	Positive
For proteins (Peptide bonds)			
Buret test	Purple or violet color	Brown turbidity	Negative

Table 3: Summary of the Phytochemical contents of duhat leaves and bark.

	Syzigium cumini	
Tests	**Java plum leaves**	**Java plum bark**
For carbohydrates		
Molisch test	+	+
For reducing sugars		
Fehling's Test	+	+
For flavonoids		
Alkaline reagent test	+	+
Lead acetate test	+	+
For alkaloids		
Hager's test	-	-
Mayer's test	-	-

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Wagner's test	-	-
For tannins		
Ferric chloride test	+	+
For glycosides		
Keller kilani test	+	-
For saponins		
Froth test	+	+
For resins		
Test for resins	-	-
For phytosterols		
Liebbermann-Burchard test	+	+
For anthraquinone		
Test for anthraquinone	+	+
For proteins (Peptide bonds)		
Buret test	-	-

C. Experimental Design and Treatments

Table 4: Growth of onion root tips (Allium cepa, L.) in various treatments

Treatments	**Amount of extract**	**No. of days of observation**					**Total roots grew**	**Average length of onion root tip growth** *(after 5 days)*
		1	**2**	**3**	**4**	**5**		
Leaf extract	20 ml	0	0	0	0	0	0	0
50 %	20 ml	4	5	5	5	5	5	1 mm
75 %	20 ml	0	0	0	0	0	0	0
100 % Pure Extract	20 ml	0	0	0	0	0	0	0
Negative control (Distilled water)								
Bark extract								
50 %	20 ml	0	0	0	0	0	0	0
75 %	20 ml	0	0	0	0	0	0	0
100 % Pure Extract	20 ml	0	0	0	0	0	0	0
Negative control (Distilled water)	20 ml	0	0	0	0	0	0	0

Onions are treated with different concentrations (100 %, 75 % and 50 %) of duhat leaves and bark extract. They were observed for five consecutive days and the numbers of onion root tip that grows were counted. The number of roots that grew per day were recorded. The distilled water is used as a negative control bacuse it allows the growth of onion roots.

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Table 5: Inhibition on the Growth of mung beans (Vigna radiata) in various treatments

Treatments	Amount of extract	No. of days of observation					Average growth of mung bean sprouts
		1	**2**	**3**	**4**	**5**	
Leaf extract							
50 %	3 ml	15	13	10	3	1	7.79mm
75 %	3 ml	15	15	15	15	15	0
100 % Pure Extract	3 ml	15	15	15	15	15	0
Negative control (Distilled water)	3 ml	11	4	2	2	2	33.15mm
Total		**56**	**47**	**42**	**35**	**33**	***33 unsprouted beans***
Bark extract							
50 %	3 ml	14	12	8	8	8	10.71mm
75 %	3 ml	15	12	11	8	8	10.57mm
100 % Pure Extract	3 ml	15	15	15	15	15	0
Negative control (Distilled water)	3 ml	11	4	2	2	2	33.15mm
Total		**55**	**43**	**36**	**33**	**33**	***33 unsprouted beans***

The numbers of mung beans that did not sprouted in each treatment (100 %, 75 % and 50 %) were counted. Each concentration of extract were tested in 15 mung beans.

The distilled water is used as a negative control. Distilled water doesn't contain substance that can inhibit mitosis in germinating seeds.

V. DISCUSSION

A. Phytochemical analysis

In this study, the leaves and bark extract of Duhat (*Syzigium cumini)* underwent the process of rotary evaporation and phytochemical screening test.

The result showed that duhat leaves extract contains carbohydrates, reducing sugars, flavonoids, tannins, saponins, phytosterols and anthraquinone. In addition, the duhat bark extract was found to contain carbohydrates, reducing sugars, flavonoids, tannins, saponins, glycosides and phytosterols and anthraquinone.Commercial and processed mitotic inhibitory drugs often contains alkaloids, flavonoids, saponins and tannins. Three of these are present in duhat leaf and bark extracts (flavonoids, saponins and tannins). Meanwhile, alkaloids were not found in both duhat leaf and bark. These phytochemicals which are present in the duhat leaves and bark have different cytotoxic effects and properties related to cell growth inhibition (Raza et al, 2017). It was confirmed after doing an experimental testing using onions and mungbeans and was supported by recent scientific research.Carbohydrates and reducing sugars are naturally occurring phytochemicals usually present in many leaves and bark. They are often found in the cellulose and in cell wall of plants. Flavonoids have been found to scavenge free radicals that can damage macromolecules, including DNA, it regulate proliferation, DNA repair, or activation of pathways leading to apoptosis (programmed cell death) in case of irreversible DNA damage and it inhibit tumor invasion and angiogenesis (Kumar and Pandey 2013). It was discovered in a study that one of the biological effects of flavonoids appeared to be related to their ability to modulate a number of cell-signaling cascades. Flavonoids have been shown to exhibit anti-inflammatory, antithrombogenic, antidiabetic, anticancer, and neuroprotective activities through different mechanisms of action *in vitro* and in animal models (Manach et al 2014).

In a research conducted by Yu et al., (2015) a plant from *Allium chinense* contains steroidal saponins, flavonoid compounds and others. Saponin materials are potential anticancer agents, and different saponins possess different mechanisms of action. Saponins from *Allium chinense* inhibited the cancer cell spreading of the B16 Melanoma and 4T1 Breast Carcinoma Cells. The cytotoxic effects of saponins may be attributed to nonapoptotic cell death stimulation. Saponins have been reported to inhibit cancer cell growth, invasion, and metastasis.

Phytosterol, another chemical present in duhat leaces and bark is proven to reduce intestinal cholesterol absorption, leading to decreased blood LDL-cholesterol levels and lowered cardiovascular disease risk. However, other biological roles for plant sterols have also been proposed. Certain researches gave considerable emerging evidence supports the inhibitory actions of phytosterols on lung, stomach, as well as ovarian and breast cancer. Phytosterols seem to act through multiple mechanisms of action, including inhibition of carcinogen production, cancer-cell growth, angiogenesis, invasion and metastasis, and through the promotion of apoptosis of cancerous cells. Phytosterol consumption may also increase the activity of antioxidant enzymes and thereby reduce oxidative stress. In addition to altering cell-membrane structure and function, phytosterols probably promote apoptosis by lowering blood cholesterol levels (Woyengo et al, 2009). In addition, another study about the mitotic inhibitory property of phytosterols proved that an oxysterol, a type of phytosterol present in a specie called *Myrtillocactus geometrizans* has an inhibitory effect on the mitosis in colon and breast cancer cells, making oxysterols an effective anticancer agent (Carillo et al., 2015).

Anthraquinones, another group of phytochemicals present in duhat leaves and bark are famous also for their antimitosis and anticancer property both in plant and animal cells. A research conducted showed that rhein, a major rhubarb anthraquinone could effectively inhibit the uptake of glucose in tumor cells, caused changes in membrane-associated functions and led to cell death. Interestingly, other types of anthraquinones were reported to have in vitro phototoxic. These results suggests that several bioactive anthraquinones of rhubarb possess promising anti-cancer properties and could have a broad therapeutic potential (Huang et al, 2017).

Phytochemicals present in the duhat leaves and bark extract are effective in inhibiting mitosis in plant cells especially in the growth of onion roots and mungbeans. This findings is supported by the study conducted by Nissar et al. (2016). In their study it was revealed that extract of *Jatropha curcas* seeds exhibited antimicrobial, antioxidant and phytotoxic activities. Higher concentrations of the extract inhibited the growth of radish seedlings. Phytochemicals such as alkaloids, flavonoids and phenols were present in the seeds. It was inferred that alkaloids, flavonoids and phenols are phytochemicals that inhibit plant cell mitosis and could be used in the formulation of drugs to treat various diseases that includes abnormal cell growth in plants. Talking about the duhat leaves and bark, flavonoids are both present in duhat *(Syzigium cumini)* and *J. curcas.* This implied that flavonoid, is the phytochemical content of duhat that is effective in inhibiting the mitosis in onion and mung beans.

In addition, it was discovered that phytochemicals especially glycosides and special types of flavonoids present in *aryltetralin lignans* can be an effective inhibitor against rye (*Lolium multiflorum* L.) and onion (*Allium cepa* L.). Inhibition of root growth was the main developmental response observed on plants tested with the lignans. At the cellular level, the mitotic inhibitors inhibited mitosis by nearly 50 %. The phytochemicals induced abnormal star anaphase chromosomal configurations. Phytochemicals also inhibit cell growth by doing an alteration of the formation of the spindle microtubular organization centers, resulting in the formation of multiple spindle poles and an asymmetrical convergence of the chromosomes (Oliva, 2012).

In terms of mitosis in seed germination, it can be inhibited by some phytochemical components like terpenoids, polyphenoliic compounds, flavonoids, alkaloids and glycosides by the mechanism of inhibiting water uptake system, surface sterilization, reverse mobilization and cell elongation (Samajdar et al, 2017). Flavonoids and glycosides are both present in the duhat leaves.

Saponins interfere with the replication of cellular DNA and they prevent the proliferation of cancer cells. Tannins, on the other hand, are of polyphenolic nature. The features distinguishing tannins from plant-based polyphenols of other types are basically the properties of the binding of the former to proteins, basic compounds, pigments, large-molecular compounds and metallic ions and also the display of anti-oxidant activities. In this review we reported the anticancer activity of different plants having a rich content of saponin and tannin.

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B. Significant differences between the phytochemical profile of Duhat (*Syzigium cumini*) leaves extract and bark extract.

The phytochemical profiles of duhat leaves and bark extract was shown and compared in Table 8.

Table 6: Comparison of the Phytochemical Profiles of Java plum leaves and bark extracts

	Syzigium cumini	
Tests	**Java plum leaves**	**Java plum bark**
For carbohydrates		
Molisch test	Positive	Positive
For reducing sugars		
Fehling's Test	Positive	Positive
For flavonoids		
Alkaline reagent test	Positive	Positive
Lead acetate test	Positive	Positive
For alkaloids		
Hager's test	Negative	Negative
Mayer's test	Negative	Negative
Wagner's test	Negative	Negative
For tannins		
Ferric chloride test	Positive	Positive
For glycosides		
Keller kilani test	***Positive*	***Negative*
For saponins		
Froth test	Positive	Positive
For resins		
Test for resins	Negative	Negative
For phytosterols		
Liebbermann-Burchard test	Positive	Positive
For anthraquinone		
Test for anthraquinone	Positive	Positive
For proteins (Peptide bonds)		
Buret test	Negative	Negative

Note: ** (Different results obtained)

In comparison of the two extracts, both possessed carbohydrates, reducing sugars, flavonoids, tannins, saponins, phytosterols and anthraquinone. Meanwhile, alkaloids, resins and peptide bonds are the three phytochemicals absent in both duhat leaves and bark extract. However, in contrast, glycosides are present in duhat leaves extract and it is absent in duhat bark extract. The presence of glycosides in duhat leaf made a significant difference between the phytochemical profile of duhat *(Syzigium cumini)* leaf and bark.

Glycosides present in the duhat leaf extract are formed between a sugar (saccharide) and another functional chemical group. The glycosidic bond joining these components is usually formed through an oxygen, sulfur, or nitrogen atom. Cardiac glycosides are glycosides from a family of compounds obtained from certain plant such

as *Digitalis purpurea*. In a study conducted, a cardiac glycoside called bufallin induces mitotic entry delay and mitotic arrest in cancer cells (Patel, 2016).

Glycosides can bond to flavonoids. Dietary forms of flavonoids, the flavonoid glycosides have examined if the flavonoid glycosides directly could affect cell division, using the human oral squamous carcinoma SCC-9 cells. The four types of flavonoid glycosides tested and each behaved differently. Genistin, the 7-glucoside of genistein, showed clear and consistent inhibition of cell proliferation, which appeared to be the result of rapid cellular uptake of the glucoside and hydrolysis to genistein. This implies that dietary flavonoid glycosides may exert cellular effects in the oral cavity, but this varies greatly with the nature of the glycoside (Browning, 2015).

C. Antimicrobial analysis

The antimicrobial assay was conducted to test the property of duhat leaves and bark extract in inhibiting the growth of the test organism *C. albicans.*

Table 7: Result of antimicrobial assay

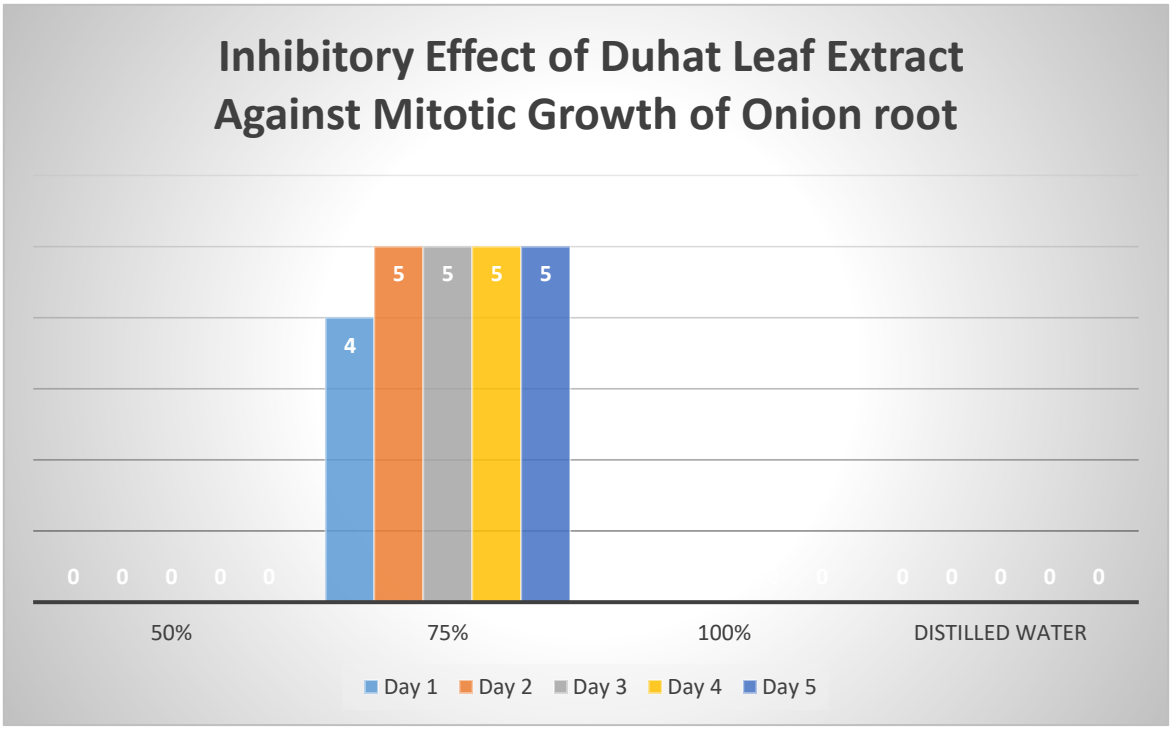
Test organism	Sample	Clearing zone, mm			AI
		1	2	3	
Candida albicans	Duhat leaf extract	-	-	-	0
	Duhat bark extract	-	-	-	0
	Canesten solution, 100 mg	32			2.2

Table 7 shows that there was no clearing zones. It means that the growth of C. albicans was not inhibited by the extract, this is compared to the effect of the positive control, the Canesten solution. It implies that both duhat leaves and bark extract have no antifungal property against *C. albicans.*

D. Experimental Treatment

The discussion of the results of the experimental treatment are as follows;

D.1. Effect of Duhat leaf extract on the mitotic growth of onion root



Graph 1: Inhibitory effect of Duhat Leaf Extract against Mitotic Growth of Onion Root

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Table 8 : Anova: Two-Factor Without Replication.

Summary of results showing the number of onion that grew roots in each treatment for 5 days using duhat leaf extract.

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
50%	5	0	0	0
75%	5	24	4.8	0.2
100%	5	0	0	0
Distilled water	5	0	0	0
Day 1	4	4	1	4
Day 2	4	5	1.25	6.25
Day 3	4	5	1.25	6.25
Day 4	4	5	1.25	6.25
Day 5	4	5	1.25	6.25

Table 9: Computation showing the significant difference in the effect of Duhat leaf extract in onion at varied concentration using ANOVA (Analysis of Variance).

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	*86.4*	*3*	*28.8*	*576*	*3.15E-13*	*3.490295*
Within groups	*0.2*	*4*	*0.05*	*1*	*0.444946*	*3.259167*
Error	*0.6*	*12*	*0.05*			
Total	*87.2*	*19*				

Table 9 shows the result of the first experiment involving onions. The outcomes are presented in Graph 1. After 5 consecutive days of observations, significant data were recorded. The onion in 50 % and 100 % concentration of Duhat leaf extract did not produced roots. The onion in 75 % concentration produced 5 roots with an average length of 1 mm. The onion treated with distilled water did not grow any root also within 5 days.

Table 8 shows the summary of resultsof the number of onions that grew roots in each treatment for 5 days using duhat leaf extract. Anova: Two-Factor Without Replication. The 75 % concentration of Duhat leaf extract has an average of 4.8 and variance of 0.2.

50 % and 100 % Duhat leaves concentrations are effective mitotic inhibitors in the growth of onion root tips. On the other hand the 75% concentration (having an onion that grown 5 roots) is less effective in inhibiting the mitosis in onion. The factors that might caused tis result are the maturity of the onion used in the 75 % duhat leaf concentration and the presence of glycosides in the leaf. More matured onion may lead to more chances of onion root growth. The glycoside chemical on the duhat leaf may have interfere with the mitotic inhibitory property of 75 % duhat leaf extract.

The findings then imply that at 75 % concentration, phytochemical contents of Duhat leaves can still promote mitosis while at 50 % and 100 % Duhat leaf concentration, phytochemicals can possibly delay and inhibit mitosis in onion. Based on Table 8 there is a significant difference in duhat leaf extracts in inhibiting mitosis of onion at different concentration, the ANOVA, Two factor without replication was used and tested at 0.05 level of significance.

On the other hand in Table 9, by looking at the results of the ANOVA computation, it showed that there were significant differences in the level of effectiveness of duhat leaf extract. The 50 % and 100 % leaves extracts both

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inhibit the mitosis in onion producing no roots at all. By looking at the source of variation of between groups, the p value is *3.15E-13.* Since they obtain lower than 0.05 therefore the null hypothesis was rejected.

D.2. Effect of Duhat bark extract on the mitotic growth of onion root

In the onions treated with different concentrations of Duhat bark, 50 %, 75 % and 100 %, all the onions in the Duhat bark treatment did not produce any roots

Table 10: Anova: Two-Factor Without Replication.

Summary of results showing the number of onion that grew roots in each treatment for 5 days using duhat bark extract.

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
50%	5	0	0	0
75%	5	0	0	0
100%	5	0	0	0
Distilled water	5	0	0	0
Day 1	4	0	0	0
Day 2	4	0	0	0
Day 3	4	0	0	0
Day 4	4	0	0	0
Day 5	4	0	0	0

Table 11: Computation showing the significant difference in the effect of Duhat bark extract in onion at varied concentration using ANOVA (Analysis of Variance).

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	*0*	*3*	*0*	*65535*	*#DIV/0!*	*3.490295*
Within groups	*0*	*4*	*0*	*65535*	*#DIV/0!*	*3.259167*
Error	*0*	*12*	*0*			
Total	*0*	*19*				

Table 11 shows no significant difference in the effectivity of duhat bark extract at different concentrations. It happened because all onions in all of the treatment did not produced roots. It implies that the 50 %, 75 % and 100 % duhat bark extracts are equally effective in inhibiting mitosis in onion.

In addition, Table 11 shows no value for the p-value of variation between groups since all the concentrations of duhat bark extract inhibited the growth of onion roots. No comparison shall be made to determine the level of effectiveness of duhat bark as mitotic inhibitor in onion.

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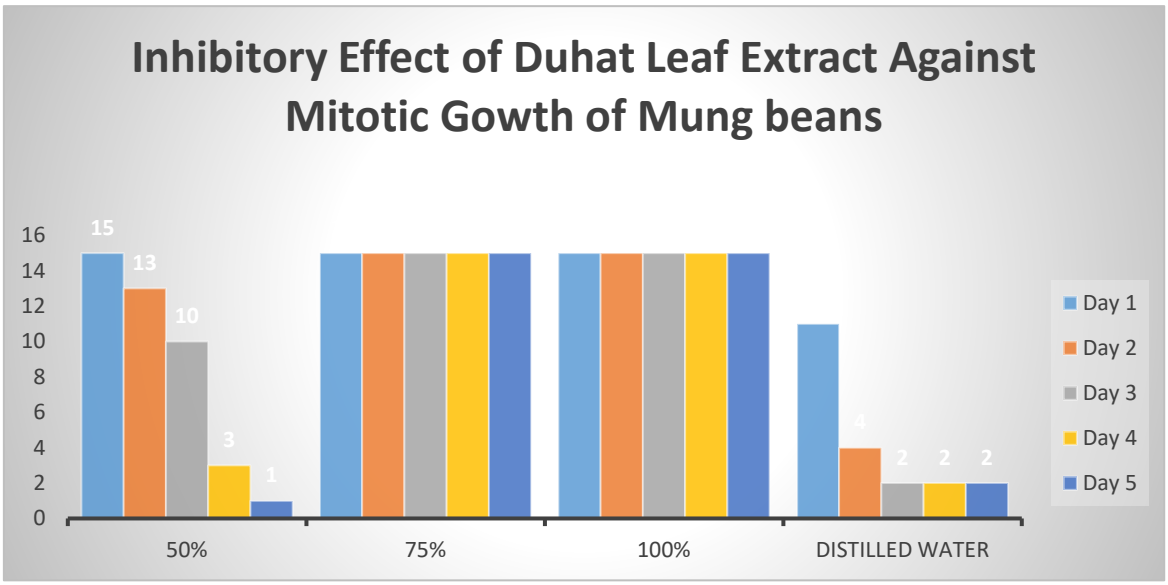
D.3. Effect of Duhat leaf extract on the mitotic growth of mung beans



Graph 2 : Inhibitory Effect of Duhat Leaf Extract Against Mitotic Growth of Mung beans

Table 5 shows the result of the experiment. After 5 consecutive days, the Duhat leaf extract inhibited the sprouting of mung beans having 33 unsprouted beans out of 60 mung beans. Graph 2 presented significant findings.

The 50 % Duhat leaf extract inhibited the germination of 15 out of 15 mung beans on the first day, 13 beans on the second day, 10 beans on the third day, 3 beans on the fourth day and 1 bean on the fifth day. In summary, only 1 bean didn't sprouted.

The 75 % and 100 % Duhat leaves extract both inhibited the germination of 15 out of 15 mung beans. All the 15 beans in both 75 % and 100 % concentration treatments did not sprouted from first to fifth day. The distilled water inhibited the growth of 11 beans on the first day, 4 beans on the second day, 2 beans on the third day, 2 beans on the fourth day and a final number of only 2 beans on the last day. The distilled water allowed the sprouting of 13 beans leaving 2 beans unsprouted.

Table 12: Anova: Two-Factor Without Replication.

Summary of results showing the number of mung beans that did not sprouted in each treatment for 5 days using the Duhat leaf extract.

	Count	*Sum*	*Average*	*Variance*
50%	5	42	8.4	37.8
75%	5	75	15	0
100%	5	75	15	0
Distilled water	5	21	4.2	15.2
Day 1	4	56	14	4
Day 2	4	47	11.75	27.58333
Day 3	4	42	10.5	37.66667
Day 4	4	35	8.75	52.25
Day 5	4	33	8.25	60.91667

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Table 13: Computation showing the significant difference in the effect of Duhat leaf extract in inhibiting mung bean germination at varied concentration using ANOVA (Analysis of Variance).

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	*422.55*	*3*	*140.85*	*13.55413*	*0.000368*	*3.490295*
Within groups	*87.3*	*4*	*21.825*	*2.100241*	*0.14375*	*3.259167*
Error	*124.7*	*12*	*10.39167*			
Total	*634.55*	*19*				

Table 13 presents the summary of results showing the number of mung beans that did not sprouted in each treatment for 5 days using the Duhat leaf extract using the Anova: Two-Factor Without Replication. The 75 % and 100 % duhat leaf extract both obtained an average of 15 and a variance 0. Having two treatments with the least variances, it implies that the 75 % and 100 % duhat leaf extract are the two most effective mitotic inhibitor in mung bean germination since both concentrations were able to inhibit the growth of all 15 mung beans. It is concluded tht two concentrations of leaf extracts were found to be effective due to the presence of glycosides in the duhat leaf. Glycoside is known to contribute inhibition of mitosis in growing cells.

On the other hand in Table 14, by analyzing the results of the ANOVA computation, it showed that there were significant differences in the level of effectiveness of duhat leaf extract between groups, and it has a p value of 0.00368. Since they obtain lower than 0.05. Therefore the null hypothesis was rejected.

D.4. Effect of Duhat bark extract on the mitotic growth of mung beans

Graph 3. Inhibitory Effect of Duhat Bark Extract Against Mitotic Growth of Mung beans

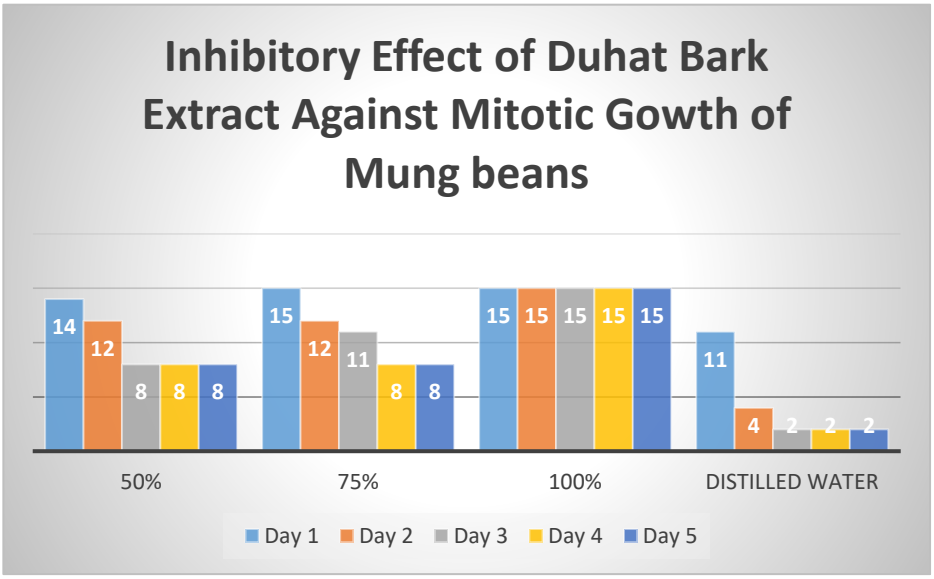

Table 7 also shows the result of the experiment using Duhat bark extract.

After 5 consecutive days, the Duhat leaf extract inhibited the sprouting of mung beans having 33 unsprouted beans out of 60 mung beans.

The 50 % Duhat leaf extract inhibited the germination of 14 out of 15 mung beans on the first day, 12 beans on the second day, 8 beans on the third day, 8 beans on the fourth day and 8 bean on the fifth day. In summary, 8 beans didn't sprouted.

The 75 % Duhat leaf extract inhibited the germination of 15 out of 15 mung beans on the first day, 12 beans on the second day, 11 beans on the third day, 8 beans on the fourth day and 8 bean on the fifth day. In summary, 8 beans didn't sprouted.

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The 100 % Duhat leaves extract inhibited the germination of all 15 out of 15 mung beans. All the 15 beans in 100 % concentration did not sprouted from first to fifth day.

Table 14: Anova: Two-Factor Without Replication.

Summary of results showing the number of mung beans that did not sprouted in each treatment for 5 days using the Duhat bark extract.

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
50%	5	50	10	8
75%	5	54	10.8	8.7
100%	5	75	15	0
Distilled water	5	21	4.2	15.2
Day 1	4	55	13.75	3.583333
Day 2	4	43	10.75	22.25
Day 3	4	36	9	30
Day 4	4	33	8.25	28.25
Day 5	4	33	8.25	28.25

Table 15: Computation showing the significant difference in the effect of Duhat bark extract in inhibiting mung bean germination at varied concentration using ANOVA (Analysis of Variance).

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	*296.4*	*3*	*98.8*	*29.20197*	*8.49E-06*	*3.490295*
Within groups	*87*	*4*	*21.75*	*6.428571*	*0.005283*	*3.259167*
Error	*40.6*	*12*	*3.383333*			
Total	*424*	*19*				

Table 15 presents the summary of results showing the number of mung beans that did not sprouted in each treatment for 5 days using the duhat bark extract through the ANOVA, Two factor without replication. The 100 % bark extract has the highest variance of 10.8 and lowest variance of 0. It implies that the 100 % bark extract is the most effective concentration of duhat bark extract in inhibiting mitosis in mung beans.

Furthermore in Table 16, by analyzing the results of the ANOVA computation, it showed that there were significant differences in the level of effectiveness of duhat bark extract in between groups since they obtain a p value of 8.49E-06 which is lower than 0.05. Therefore the null hypothesis was rejected.

VI. CONCLUSION

The results of this study showed that duhat leaves and bark extracts contain phytochemicals responsible for mitotic inhibition. The two extracts showed significant difference due to the presence of glycosides in the duhat leaf extract, a chemical that is absent in the duhat bark extract. The duhat leaves and bark extracts did not inhibit the growth of *Candida albicans.* It was also concluded that the duhat leaves and bark extracts have significant inhibitory effects on the growth of onion bulb and mung bean. The 50 % and 100 % duhat leaves extract are the two most effective mitotic inhibitors in onion, whereas the 50 %, 75 % and 100 % duhat bark extract inhibited mitosis in onion at same rate. The 75 % and 100 % duhat leaves extract are the two most effective mitotic inhibitor in mung bean, while the 100 % duhat bark extract is the most effective mitotic inhibitor in mung bean.

VII. RECOMMENDATIONS

Based on the findings of this investigation, it is hereby recommended that;

1. Experimentation using duhat leaves and bark extracts should also be tested in animal cells to further identify the mitotic inhibitory effect of the plant in animals;

2. Experimentation in plants should involve plant subjects that are of the same size, weight, variety, maturity and source to avoid bias.
3. Phytochemistry of other plants, maybe of the same genus or family and the duhat fruits and root extracts should also be analyzed;
4. Research about the mitotic inhibitors in duhat leaves and bark extract should also be done in order to discover its usefulness in treating plant cell diseases;
5. Research about the potential anticancer property of the mitotic inhibitors in duhat leaves and bark extracts should also be conducted.

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