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Asian Journal of Advanced Multidisciplinary Researches

ISSN: 2782 - 9057

Antimitotic Property of Different Rhizome Extracts

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ABSTRACT

Cancer is a condition in which a cell's mitotic division is uncontrolled. Cancer is the leading cause of death among all diseases, and because it is a complex disease, drug research for cancer has long been a promising subject in recent years. In this study, three least explored medicinal plants – black turmeric (*Curcuma caesia),* turmeric (*Curcuma longa* L.) and white ginger (*Hedychium coronarium*) was investigated for their antimitotic potential as a blocking agent that inhibits cell division with the aim of developing a new anticancer drug from natural plant extracts. An *Allium cepa L. Root Assay* was conducted to determine the significant difference on the mitotic indices of the *Allium cepa* root tip cells as affected by the different rhizome extracts. The treatments used are the following: Treatment zero (T_0) distilled water, Treatment one (T_1) black turmeric extract, Treatment two (T_2) turmeric extract, Treatment three (T_3) white ginger extract; and Treatment four (T_4) combination of all extracts. The *A. cepa* root tips were treated with the different treatments and undergone fixation, incubation, dehydration, staining, squashing, and then examining the specimen under a light microscope. Data were collected through manual *A. cepa* root tip cell counting. Based on the results of the study, there is a highly significant difference on the antimitotic indices of the *A. cepa* root tip cells as affected by the different rhizome extracts and that the black turmeric extract showed the highest antimitotic property. This is due to the presence of tannins, terpenoids, flavonoid, alkaloid, phenol, phytosterol quinones and saponins that were found in black turmeric (Pakkirisamy, M. et al, 2017). Hence, it is recommended that black turmeric extract could be used as a blocking agent that inhibits cell division.

Keywords: antimitotic property, rhizome extracts, antimitotic index (AMI), *A. cepa* L. root assay

1. INTRODUCTION

Plants have long been used as a good source of medication, laying the groundwork for traditional medicine. Traditional medicinal plants play an increasingly important role in meeting global health-care demands, and their use is expected to rise in the future (Sharma, 2016). The use of medicinal plant extracts for the treatment of human ailments has expanded dramatically in recent decades, owing to the negative side effects of chemical medications. Due to the presence of numerous bioactive substances such as phenolics, flavonoids, alkaloids, terpenes, steroids, and saponins, plants have antimitotic, antidiuretic, antidiabetic, antiarthritic, antidepressant, analgesic, antipyretic, antioxidant, antibacterial, and other effects. Plants have been utilized as a source of medicine for thousands of years because their phytochemicals work as medicine (Aggarwal et al., 2003). Exploration of traditionally medicinal plants is significant on two levels: first, as a source of potential chemotherapeutic medications, and second, to assess the safety of using medicinal plants indefinitely (Verschaeve et al., 2004). Based on their use in traditional medicine, most mainstream anticancer medicines are derived from natural sources (Agbafor & Nwachukwu, 2011). Cancer is a condition in which a cell's mitotic division is uncontrolled. Cancer is the leading cause of death among all diseases, and because it is a complex disease, drug research for cancer has long been a promising subject in recent years. According to the World Health Or-

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ganization, cancer claimed the lives of 9.6 million people globally in 2018 (Bray et al., 2018). More than half of the medicines with anticancer action come from natural sources or are related to them. One of the most important parts of cancer treatment is the use of antimitotic agents. Antimitotic agents are a prominent class of cytotoxic medicines that will continue to be used in cancer chemotherapy for the foreseeable future (Fonrose et al., 2007).

For this study, three least explored medicinal plants – black turmeric (*Curcuma caesia)* turmeric (*Curcuma longa* L.) and white ginger (*Hedychium coronarium*) had been selected to investigate their antimitotic potential as a blocking agent that inhibits cell division. Black turmeric (*Curcuma caesia*), often known as kali haldi, is a member of the Zingiberaceae family. This herb can be found in the north-east, central India, the East Godavari, West Godavari, and Andhra Pradesh Papi Hills. The plant's rhizomes have a pleasant scent. Due to the presence of essential oil, the interior part of the rhizome is bluish black in color and exudes a distinct pleasant odor. Fresh and dried rhizomes of *Curcuma caesia* are used to treat leucoderma, asthma, tumors, piles, bronchitis, and bruises in traditional medicine (Das et al., 2013). Turmeric (*Curcuma longa* L.; syn.: *Curcuma domestica* Valeton) is a Zingiberaceae plant that is widely grown in Asia's tropical regions (Gupta et al., 2013). Turmeric has been used as a tonic for a long time. It is also used to treat dyslipidemia, gastrointestinal issues, arthritis, and hepatic ailments, among other things (Delgado-Vargas & Paredes-López, 2002). Curcumin is a polyphenol produced from turmeric 1,7-bis-4-hydroxy-3-methoxyphenyl-1E, 6E-heptadiene-3,5-dione or diferuloyl methane. Curcumin is a yellow tincture made from the rhizome of the turmeric plant (Ireson et al., 2002). White ginger (*Hedychium coronarium)* is a perennial monocotyledon herb of the Zingiberaceae family. Jhon Koenig described the coronarium species for this genus (Hedychium) in 1783. Because its bloom resembles a fluttering butterfly, it is also known as white ginger or butterfly ginger. *Hedychium coronarium* is an aromatic rhizomatous plant with major therapeutic characteristics, and its many sections have been employed in both traditional and modern medicine (Vaidyaratnam, 2006).

A literature review revealed that despite the anticancer potentials of these plants in traditional medicine, the plants were yet to be validated for its anticancer property. Hence, in the present study, a researcher had been investigated the antimitotic property of black turmeric (*Curcuma caesia)*, turmeric (*Curcuma longa* L.) and white ginger (*Hedychium coronarium*) with the aim of developing a new anticancer drug from natural plant extracts for the global market.

2. METHODOLOGY

Preparation of the Different Rhizome Extracts

An amount of five hundred (500) grams each rhizome of black turmeric, turmeric and white ginger gathered from the researcher's locality in Purok- 8 Datu Panas, Buug, Zamboanga Sibugay Province has been washed thoroughly with water and been cut into halves. The rhizome of black turmeric has been chopped in a cube size then were pounded with the use of mortar and pestle and then put in a cheese cloth and squeeze so that the extracts would separate from the sap of the rhizome. Same procedure has been done to the rhizome of turmeric and white ginger. The black turmeric, turmeric and white ginger extracts has been measured at exactly 200 ml and then set aside for the treatments.

Figure 1. Sample Collection. (**A**) rhizome of black turmeric, (**B**) rhizome of turmeric, and (**C**) rhizome of white ginger.

Exposure to the Rhizome Extracts

The descaled *Allium cepa* bulbs (approx. 15 bulbs) has been germinated in water on top of plastic test tubes for 72 hours at room temperature in a dark condition. Then *Allium cepa* bulbs were removed from the water and placed on a layer of tissue paper to remove excess of water. The bulbs were divided into five groups with three replicates each. In the first group, the *Allium cepa* roots has been submerged in a 15 ml distilled water on each replicate which served as a control. Second group: *Allium cepa* roots were dipped in the black turmeric (*Curcuma caesia)* rhizome extract in 15 ml. Third group: *Allium cepa* roots were

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dipped in the turmeric (*Curcuma longa* L.) rhizome extract in 15 ml. Fourth group: *Allium cepa* roots were dipped in the white ginger (*Hedychium coronarium*) rhizome extract in 15 ml. Fifth group: *Allium cepa* roots were dipped in the combined three rhizome extracts (5 ml in each of the three extracts). All the groups have been further planted from its corresponding treatments for another 48 hours in a closed laboratory room (see figure 2). For each onion, the roots were counted as well as their lengths after its exposure to the treatments. After 2 days, it was then ready for the *Allium cepa* L. root assay.

Figure 2. (**A**) Growing of the *Allium cepa* roots in distilled water for 72 hours and (**B**) exposure of the *Allium cepa* roots in distilled water (control) and different rhizome extracts in 48 hours.

Antimitotic Activity - *Allium cepa L.* Root Assay

The submerged roots of *Allium cepa* in each group have been collected. Onion roots were being cut about 5 mm on its root tips at 11:00-12:00 a.m. or p.m. since these were the observed times for active mitotic division. Then, the root tips were placed in a fresh mixture of Farmer's Fluid (1.5 ml 95% ethyl alcohol and 0.5 ml glacial acetic acid) in the vials for 2 days. Two days after, the sections were being put with 45% glacial acetic acid and were being incubated in an oven at 60 degrees centigrade for 15-30 minutes until the roots became transparent (see figure 3).

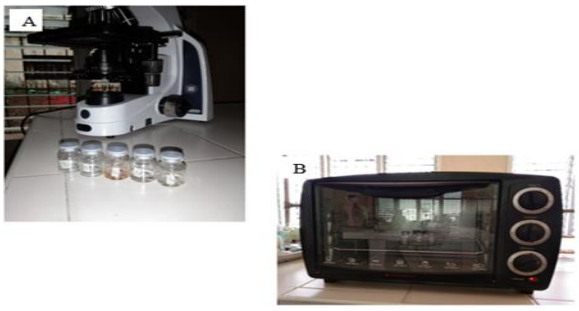

Figure 3. (**A**) Fixation of *A. cepa* root tips for 48 hours and (**B**) incubation of root tips at 60°C in 15 minutes.

Prepared root tips have been placed on a clean slide and put a drop of acetocarmine stain and passed to a low flame for about 5 minutes to intensify staining. After flaming, root tips have been added two to three drops of the stain and cover the slide with a cover slip. Squashing has been achieved with a bouncing movement by striking the cover glass with a tip of a cover of a pen to disperse the cells equally on the surface of the slide or adding a pressure using a thumb of the hand. Sealing the cover slip was done using transparent nail polish (Amoroso C. & Amoroso C., 1994). It was then examined under a light microscope at 100× magnification and 400× magnification and photographs were taken (see figure 4).

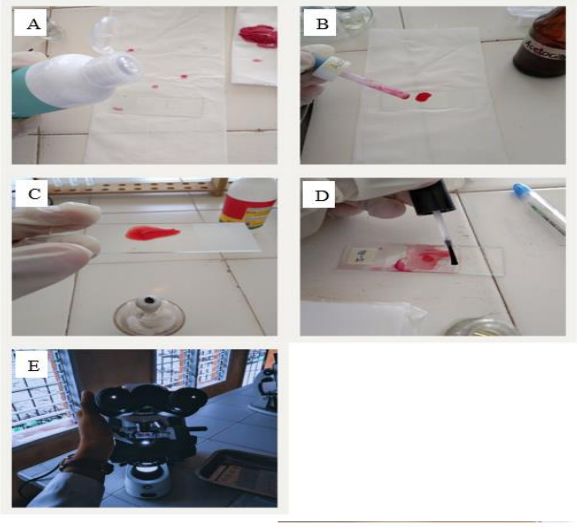

Figure 4. (**A**) Clearing and dehydration of root tip samples; (**B**) stain application; (**C**) heating of the stain; (**D**) squashing and sealing of the prepared slide; (**E**) microscope viewing of the specimen of *A. cepa* root tip cells.

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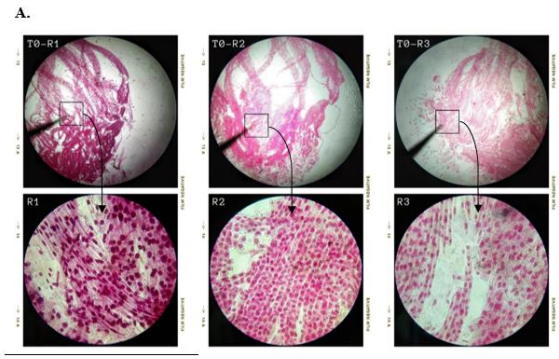
Antimitotic index (AMI) has been calculated as divided cells/total cells multiplied by 100. The acquired data has been subjected to statistical and descriptive analysis.

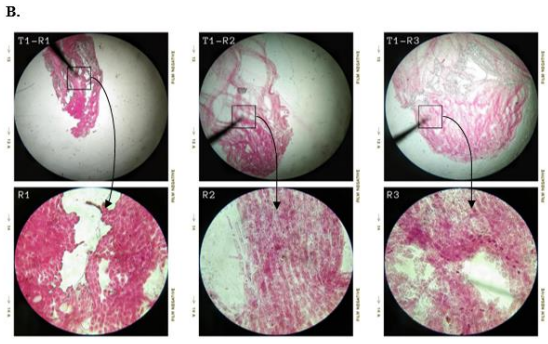
Antimitotic Assay: Microscopic Observation

After staining, the *A. cepa* root tip cells were observed under light microscope by using acetocarmine stain.

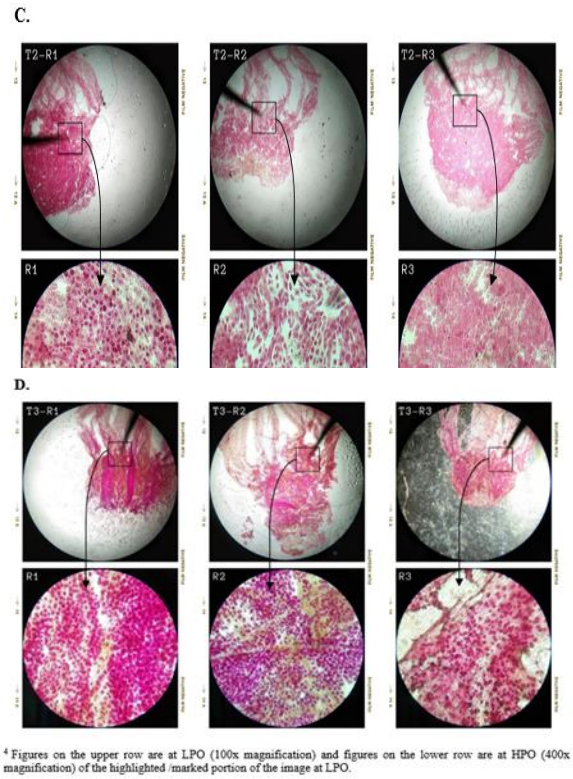
The microscopic view of stained root tip cells at LPO with 100× magnification and at HPO with 400× magnification are shown in figure 5.



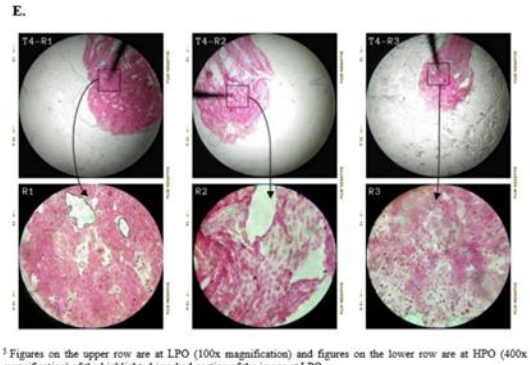
[1] Figures on the upper row are at LPO (100x magnification) and figures on the lower row are at HPO (400x magnification) of the highlighted /marked portion of the image at LPO.



[2] Figures on the upper row are at LPO (100x magnification) and figures on the lower row are at HPO (400x magnification) of the highlighted /marked portion of the image at LPO.



[4] Figures on the upper row are at LPO (100x magnification) and figures on the lower row are at HPO (400x magnification) of the highlighted /marked portion of the image at LPO.



[5] Figures on the upper row are at LPO (100x magnification) and figures on the lower row are at HPO (400x magnification) of the highlighted /marked portion of the image at LPO.

Figure 5. Photomicrograph of *A. cepa* root tip cells

Figure 5 showed the photomicrograph of *A. cepa* root tip cells at 100× magnification and 400× magnification under a light microscope. Treatment of *Allium* cepa root tip cells with (A) distilled water (control), (B) black turmeric (*Curcuma caesia)*

extract, (C) turmeric (*Curcuma longa* L.) extract, (D) white ginger (*Hedychium coronarium*) extract, and (E) combination of *C. caesia, C. longa* L. and *H. coronarium* extracts were shown in the figure above.

Statistical Treatment Analysis

The significant difference on the number, length (cm) of *Allium cepa* roots and antimitotic indices of the *Allium cepa* root tip cells as affected by the rhizome extracts of black turmeric (*Curcuma caesia),* turmeric (*Curcuma longa* L.) and white ginger (*Hedychium coronarium*) have been investigated using mean and one-way analysis of variance (ANOVA). The significance level was set at $P < 0.05$. The Duncan's Multiple Range Test (DMRT) has also been used to further determine the significant difference on the antimitotic indices of the *Allium cepa* root tip cells as affected by the different rhizome extracts.

3. RESULTS AND DISCUSSION

The study on the antimitotic property of black turmeric (*Curcuma caesia),* turmeric (*Curcuma longa* L.) and white ginger (*Hedychium coronarium*) extracts has led to the given results below. The study focused on the number of *Allium cepa* roots as shown in figure 6 and length (cm) of *Allium cepa* roots as revealed in figure 7 as well as the antimitotic indices of the *Allium cepa* root tip cells as affected by the different rhizome extracts which is unveiled in figure 8.

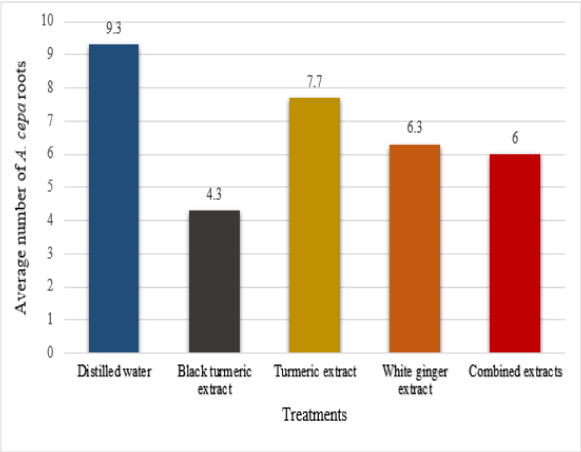


Figure 6. Average number of *A. cepa* roots

Figure 6 showed the average number of *Allium cepa* roots after two days of exposure to the treatment by the different rhizome extracts. Treatment zero (T_0) distilled water showed the highest number of *A. cepa* roots with a mean of 9.3, followed by Treatment two (T_2) turmeric (*Curcuma longa* L.) extract with 7.7, Treatment three (T_3) white ginger (*Hedychium coronarium*) extract with 6.3, Treatment four (T_4) combination of all extracts with 6.0 and Treatment one (T_1) which is the black turmeric (*Curcuma caesia)* extract showed the least average number of *A. cepa* roots with a mean of 4.3. This is due to the natural components of black turmeric extract which hindered root development by stopping mitosis.

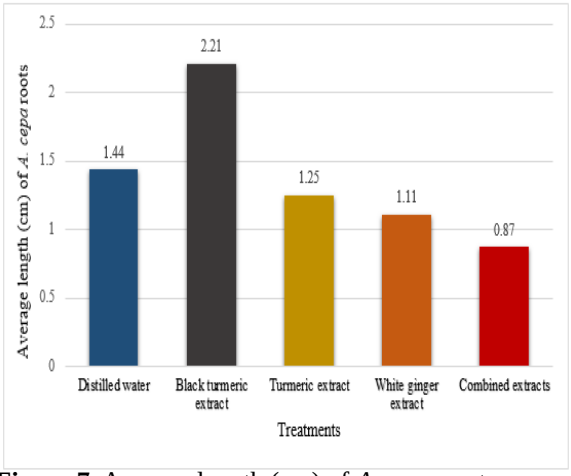


Figure 7. Average length (cm) of *A. cepa* roots

Figure 7 showed the average length (cm) of *Allium cepa* roots after two days of exposure to the treatment by the different rhizome extracts. Treatment one (T_1) black turmeric (*Curcuma caesia*) extract showed the highest average length of *A. cepa* roots with a mean of 2.21 cm. Black turmeric may promote differentiation through cell elongation but halts meristematic activity. Treatment zero (T_0) distilled water had an average length of *A. cepa* roots with a mean of 1.44 cm, Treatment two (T_2) turmeric (*Curcuma longa* L.) extract with 1.25 cm, Treatment three (T_3) white ginger (*Hedychium coronarium*) extract with 1.11 cm and Treatment four (T_4) combination of all extracts showed the least average length of *A. cepa* roots with a mean of 0.87 cm. The roots remain its length except in the water treatment where some are swelling and separated from its root caps due to the presence of toxic nature of substances.

Figure 8. Antimitotic indices (AMI) of *Allium cepa* root tip cells.

Figure 8 showed the antimitotic indices (AMI) of *Allium cepa* root tip cells in corresponding treatments on distilled water (control) and rhizome extracts of black turmeric (*Curcuma caesia)* , turmeric (*Curcuma longa* L.), and white ginger (*Hedychium coronarium*). The graph showed the average mean of the antimitotic indices of *Allium cepa* root tip cells as affected by the different treatments. Treatment one (T_1) black turmeric (*Curcuma caesia)* had the highest AMI mean with 2.47, followed by Treatment four (T_4) combined extracts which had an AMI mean with 10.18, Treatment two (T_2) turmeric (*Curcuma longa* L.) with 32.78, Treatment zero (T_0) distilled water with an AMI mean of 69.79 and Treatment three (T_3) white ginger (*Hedychium coronarium*) with the lowest AMI mean of 73.87.

Analysis of Variance (ANOVA) Results on the Parameters

Table 1. Analysis of Variance (ANOVA) Results on the Parameter

Parameters	F-stat	P-value	Decision
Number of *A. cepa* roots	0.2145	0.9244	Accept H_0
Length of *A. cepa* roots	0.5977	0.6727	Accept H_0

The computed F value on the number of *A. cepa* roots is 0.2145 and has a probability value of 0.9244 which provide a strong basis for the acceptance of the null hypothesis. The computed F value on the length of *A. cepa* roots is 0.5977 with a probability value of 0.6727 which warrants the acceptance of the null hypothesis. Thus, the Analysis of Variance (ANOVA) test revealed that there was no significant difference on the number and length of *Allium cepa* roots as affected by the different rhizome extracts.

Analysis of Variance (ANOVA) test revealed that the computed F value is 12.28 with a P-value of 0.0007 which provides a strong statistical evidence to accept the alternative hypothesis. Hence, there was a highly significant difference on the antimitotic indices of the *Allium cepa* root tip cells as affected by the different rhizome extracts. The Duncan's Multiple Range Test (DMRT) further revealed that Treatment one (T_1) black turmeric (*Curcuma caesia)* extract, Treatment four (T_4) combination of all extracts and Treatment two (T_2) turmeric (*Curcuma longa* L.) extract showed high antimitotic property among the five treatments. Of the three best treatments, Treatment one (T_1) black turmeric (*Curcuma caesia)* extract ranked first, followed by Treatment four (T_4) combination of all extracts and Treatment two (T_2) turmeric (*Curcuma longa* L.) extract but were statistically the same. Thus, this revealed that black turmeric (*Curcuma caesia)* extract showed the highest antimitotic property.

4. CONCLUSIONS

Based on the findings of the study, there was no significant difference on the number and length of *Allium cepa* roots as affected by the different rhizome extracts as revealed by the Analysis of Variance (ANOVA) test. However, there was a highly significant difference on the antimitotic indices of the *Allium cepa* root tip cells as affected by the different rhizome extracts. Treatment one (T_1) black turmeric (*Curcuma caesia)* extract, Treatment four (T_4) combination of all extracts and Treatment two (T_2) turmeric (*Curcuma longa* L.) extract showed high antimitotic property among the five treatments. Of the three best treatments, Treatment one (T_1) black turmeric (*Curcuma caesia)* extract ranked first, followed by Treatment four (T_4) combination of all extracts and Treatment two (T_2) turmeric (*Curcuma longa* L.) extract but were statistically the same. Thus, this revealed that black turmeric (*Curcuma caesia)* extract showed the highest antimitotic property.

This is due to the natural constituents of *C. caesia* which were primarily responsible for its medicinal value. Alkaloids, amino acids, carbohydrates, flavonoids, flavones, steroids, and proteins were found in black turmeric (V. John Dennis, 2021). As revealed by GC-MS and FT-IR, tannins, terpenoids, flavonoid, alkaloid, phenol, phytosterol quinones, and saponins were detected in *Curcuma caesia* (black turmeric). The methanolic extract also contained alpha-santalol (46.90 percent), retinal (10.72 percent), ar-tumerone (10.38 percent), alloaromadendrene (5.93 percent), megastigma-3, 7 (E) (4.80 percent), and many other low-level components. *Curcuma caesia* has thus been determined to be a feasible herbal alternative as well as a functional and medicinal food for a number of diseases (Pakkirisamy, M. et al., 2017).

5. RECOMMENDATIONS

The researchers have recommended the following:

1. Application of black turmeric extract as viable alternative organic anticancer drug.

2. A study can be conducted using other parts of the plants on black turmeric that would be used as a blocking agent that inhibits cell division.

3. Different plant assays must be utilized to evaluate a wide range of genotoxic/antigenotoxic substances from black turmeric extract.

4. A study can be conducted in developing effective and selective methods for the extraction and isolation of natural prod-

ucts from black turmeric.

5. An experiment to undergo of *Curcuma caesia* (black turmeric) showing its cytotoxic effect against EAC in vitro and antitumor activity in Ehrlich's ascites carcinoma (EAC) – treated mice.

6. A detailed study of the mechanism of action to have a better understanding of the therapeutic efficacy and to avoid adverse reaction and side effects when used clinically in humans.

ACKNOWLEDGMENTS

The researchers are grateful to Prof. Jether P. Sumpo, Prof. Nor-Aina E. Salipada, Prof. Jameyla A. Ladias, and Prof. Omar Bryan M. Hampong for allowing us to conduct this research and for providing valuable guidance and suggestions throughout the process. Working and studying under their supervision was a great privilege and honor. Also, to the Mindanao State University-Buug Campus for providing required facility for the study and most of all to the all-inclusive and all-sufficient Jesus Christ, the Author and Perfecter of our faith.

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