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Phytochemical Screening, Cytotoxicity, Antimicrobial, Mutagenicity, Antimutagenicity, and Protective Effects of the Various Extracts from the Leaves of *Syzygium brevistylum*

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ABSTRACT
Syzygium brevistylum, locally known as Sagimsim, is a tree that belongs to family Myrtaceae of genus Syzygium found growing in Philippines. The leaf extract of the plant is commonly used by some faith healers in Mindanao for treatment of cough and colds. Phytochemical analysis of the crude methanolic extract revealed the presence of saponins, flavonoids, tannins and polyphenols. Brine shrimp lethality test of hexane extract (HE), ethyl acetate extract (EAE) and aqueous extract (AE) show that EAE has the highest toxicity observed after 24 hours and considered to be the most bioactive extract. The antimicrobial assay shows that EAE has the highest potential on inhibiting the growth of Escherichia coli, Staphylococcus aureus and Candida albicans compared to HE and AE. Highest inhibition of EAE was observed against CA. One-way Analysis of Variance showed that there is a significant difference (P value<0.05) on the effect of increase EAE concentration with the zone of inhibition against the two bacteria. The mutagenicity, antimutagenicity, and protective effects of EAE were assessed using peripheral blood micronucleus test. Occurrence of micronucleated polychromatic erythrocytes were observed at EAE administered alone, and was observed highest at dose of 500 mg/kg. Based on the result, it shows that EAE lowers the genotoxic activity of methyl methanesulfonate and hence it can be considered to have an antimutagenic and protective potentials against chemically induced mutation.
Keywords: *Syzygium brevistylum*, antigenotoxic, cytotoxicity, antimicrobial

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INTRODUCTION

The continuing economic progress of the human race have been accompanied by a continuing increase in the numbers of chemical entities to which man is intentionally or unintentionally expose. These chemical entities may have beneficial as well as harmful effects to human body [1]. In addition to these chemicals, microorganisms are also of primary concern. The emergence of multi-resistant microorganisms has led to many microbial infections, which are difficult to control. Furthermore, improper use of many antibiotics was reported with various side effects to human biological system. With these into consideration, it is apparent that harmful chemicals and microorganisms place a major threat to public safety.

Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases has led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments [2]. For many people of low economic status, an alternative medicine provides a great support to deal with illnesses. Therefore, it is of great interest to carry out a screening of the plants in order to validate their use in traditional medicine and to understand their mode of action by isolation and characterization of their constituents.

Philippines, blessed with rich natural ecosystem, is a potential source of medicinal plants. *Syzygium brevistylum*, locally known as Sagimsim, is a tree that belongs to family Myrtaceae of genus *Syzygium* found growing in the country. The leaf extract of the plant is commonly used by some faithhealers in Mindanao for treatment of cough and colds. Related species within *Syzygium* genus like *Syzygium aromaticum* (clove), *Syzygium cumini* (lumboy), *Syzygium samarangensi* (makopa), has been known with various medicinal purpose. As a member of *Syzygium* genus, sagimsim could be a potential source of

phytochemicals, which could be the reason for its medicinal effects. Presently there are no found literatures on researches relating to the pharmacological activity and phytochemical constituents of *Syzygium brevistylum*.

MATERIALS AND METHODS

Preparation of extracts

The *Syzygium brevistylum* leaves were collected from Barangay Kalawit Conception, Zamboanga del Norte. The *Syzygium brevistylum* leaves were washed thoroughly with running water and air-dried. The leaves were cut into smaller pieces and weighed using a top loading balance. Six hundred eighty five grams (685) of dried leaves were soaked in 95% methanol for 96 hours and concentrated *in vacuo* using a rotary evaporator. The concentrated crude methanolic extract was refrigerated for storage.

Solvent partitioning

Approximately 15 grams of the crude methanolic extract was weighed using a top loading balance. Then, the weighed crude extract was dissolved with 50 mL of 10% methanol. The dissolved portion was transferred to a separatory funnel. Then, 50 mL of hexane was used to dissolve the remaining portion and then transferred to the separatory funnel together with the previously dissolve portion. The mixture was shaken gently with occasional opening of the cartridge to dissipate the evolved gases. Afterwards, the solution was allowed to stand for several hours until complete separation of layers was observed. The organic portion was observed at the top layer while the aqueous portion was at the bottom layer. The top organic layer was taken out of the separatory funnel, using a pipette, and placed in a beaker labeled with hexane extract (HE). The procedure was repeated until a clear top organic layer was observed. The remaining aqueous portion was partitioned with ethyl acetate following the procedure used in the partitioning with hexane. The resulting solution from partitioning with ethyl acetate was labeled ethyl acetate extract (EAE). The remaining portion after partitioning with ethyl acetate was labeled aqueous extracts (AE). After the partitioning process, the acquired extracts were then concentrated *in vacuo* using rotary evaporator.

Phytochemical screening

The crude methanolic extract was screened for its phytochemical constituent using the procedure described by Saidu and Garba [3].

Test for flavonoids

Two mL of 0.1M NaOH was added to 2.0 mL of the extract. The appearance of a yellow color indicates the presence of flavonoids.

Test for alkaloids

Five mL of 2 M HCl was added to 3 mL of the extract. Then it was placed in a boiling water bath for 5 minutes and was cooled. After the solution cooled, 0.50 g of NaCl was added and filtered. It was washed with 2 M HCl to bring the volume to 5 mL. About 2-3 drops of Dragendorff's reagent was added. Turbidity indicates the presence of alkaloids.

Test for tannins and polyphenols

To 3 mL crude methanolic extract, 25 mL of hot distilled water was added. The solution was mixed, cooled and decanted. Then, 10% NaCl solution was added and filtered. The filtrate was placed into 3 test tubes. One portion was treated with gelatin- salt reagent. Formation of jelly precipitate indicates the presence of tannins. The other portion was added with 3 drops of ferric chloride solution. A blue-black color indicates the presence of hydrolysable tannins; white brownish green color indicates the presence of condensed tannins.

Test for saponins

Two milliliter of the crude methanolic extract and 10 mL of distilled water were mixed in a test tube, capped and shaken for thirty minutes. The solution was permitted to set for thirty minutes. Persistent honeycomb froth greater than 1.5 cm above the surface of the liquid indicates the presence of saponins.

Brine shrimp lethality test

Approximately 0.05 g of the partitioned extracts were weighed separately and dissolved with 5 mL methanol (stock solution). From the stock solution, 0.5 mL, 0.05 mL and 0.005 mL were placed into separate tubes, using a pipette, and allowed to dry at room temperature. The dried solutions were diluted to 5 mL with artificial seawater to obtain a final concentration of 10 ppm, 100 ppm, and 1000 ppm respectively. Five replicates for each concentration were prepared.

A shallow rectangular dish was filled with artificial seawater. Plastic divider with several holes was placed in the dish to divide it into two unequal compartments. The brown brine eggs were sprinkled into the large compartment, covered to keep away from light, leaving the small compartment open and

illuminated with light bulb. After 48 hours, the hatched brownish orange nauplii were pippeted from the illuminated compartment of the dish.

With the use of 3-inches Pasteur pipette, ten nauplii were transferred into each sample test tube at different concentration. Ten nauplii were also transferred to the control tube, containing 5 mL artificial seawater. A drop of yeast was added as food in each test tube. The test tubes were kept illuminated for 24 hours. The number of live nauplii was counted after 24 hours [4-6].

Antimicrobial assay

For the antibacterial assay, *Escherichia coli* a Gram negative bacterium and *Staphylococcus aureus* a Gram positive bacterium was inoculated, aseptically and separately, in a nutrient broth and was incubated for 24 hours. For antifungal assay *Candida albicans* was inoculated, aseptically, in a potato-dextrose broth and incubated for 48 hours. All the materials that were used in the antimicrobial assay were sterilized.

The assay plate was prepared by following aseptic measures. Nutrient agar was used as culture medium for the bacterial strains while potato-dextrose agar was prepared for the fungal strains. To 30 mL test tubes, approximately 15 mL of agar medium was placed. The test tubes containing the agar medium were sterilized in an autoclave for 15 minutes at 15 psi at 121^{0}C. Then, the warm and sterile agar was inoculated, aseptically by direct seeding, with 200 μL of the microorganism and poured aseptically into the pre-labeled sterile petri dishes and was allowed to solidify at room temperature. After the agar medium was cooled and solidified at room temperature, a sterile paper disc of 6 mm diameter was saturated with the test extracts and antibiotic standards of concentrations of 10000 ppm, 20000 ppm and 30000 ppm. The saturated discs were deposited fairly into the plate. The top of the disc was pressed gently to ensure firm contact with the agar. After that, the plates were incubated at 37^{0}C for 24 hours. After incubation the diameter of inhibition was measured in millimeters using a ruler [6]. Analysis of the data was done using One-way ANOVA with DMRT.

Micronucleus test

Micronucleus test for chromosomal aberration was conducted using peripheral blood. All procedures performed were according to the guidelines described by OECD [7-8] draft TG474 with minor modifications.

Albino mice were used as test animal. Approximately 7-9 weeks old mice were acclimatized in the laboratory condition for 2 weeks. They were fed with commercial pellets and water throughout the acclimatization and experimental period. The animals were placed in a room at ambient temperature and subjected to regular light/dark cycle.

Maximum tolerable dose

From the result of the antimicrobial and cytotoxicity assay the most bioactive extract was chosen. A concentration of 1000 ppm was prepared as a starting concentration. Five mice per group were chosen for the determination of the maximum tolerable dose of the extract in the test mice. Then the extract was administered to the mice orally via gavage with dose of 0.2 mL/20g body weight and was observed for 24 hours. A sign of toxicity to 50% of the total population determines the maximum tolerable dose of the extract from the test mice. The concentration of the extract administered to the test was increased until a sign of toxicity to at least 50% of the group was observed [6]. The mice were grouped into eight treatments with three replicates.

Peripheral blood micronucleus test

Male and female mice of 9-11 weeks old were randomly chosen for each experimental group. A total of 48 mice were divided into twelve groups with each group consisted of four mice. The mice were treated for 6 days with 24 hour treatment interval. Prior to administration, the mice were fasted from food and water for 16 hours. Two hours after administration, food and water *ad libitum* was given to the experimental mice. Methyl methanesulfonate was used as positive control and distilled water and DMSO as negative control. The twelve groups are treated as mentioned in table 1.

All treatments were administered at volume of 0.2 mL/20 mg BW. Methyl methanesulfonate was administered at dose of 50 mg/kg BW and EAE was administered at dose of 100, 300, and 500 mg/ kg BW, respectively.

Peripheral blood was collected 24 hours after the last treatment. Blood samples were collected from the tail of the mouse and a drop of blood was placed on a pre-cleaned and coded glass slides, smeared with a cover slip and then air-dried. The prepared blood films were fixed with 90% methanol and then air-dried. The smeared preparations were stained with acridine orange [9].

Table 1: Micronucleus assay treatment set-up

Group	Treatment	Test
1	6 days distilled water	Negative control
2	6 days DMSO	Negative control
3	4 days distilled water + 2 days MMS	Positive control
4	2 days distilled water + 4 days 10000 ppm EAE	Mutagenicity
5	2 days distilled water + 4 days 30000 ppm EAE	Mutagenicity
6	2 days distilled water + 4 days 50000 ppm EAE	Mutagenicity
7	2 days MMS + 4 days 10000 ppm EAE	Antimutagenicity
8	2 days MMS + 4 days 30000 ppm EAE	Antimutagenicity
9	2 days MMS + 4 days 50000 ppm EAE	Antimutagenicity
10	4 days 10000 ppm EAE + 2 days MMS	Protective
11	4 days 30000 ppm EAE + 2 days MMS	Protective
12	4 days 50000 ppm EAE + 2 days MMS	Protective

Micronucleus scoring

Micronuclei (MNi) are morphologically identical to but smaller than nuclei. They also have the following characteristics [10]:

a. The diameter of MNi in human lymphocytes usually varies between 1/16th and 1/3rd of the mean diameter of the main nuclei, which corresponds to 1/256th, and 1/9th of the area of one of the main nuclei in a binucleated cell, respectively.

b. MNi are non-refractile and they can therefore be readily distinguished from artefact such as staining particles;

c. MNi are not linked or connected to the main nuclei;

d. MNi may touch but not overlap the main nuclei and the micronuclear boundary should be distinguishable from the nuclear boundary;

e. MNi usually have the same staining intensity as the main nuclei but occasionally staining may be more intense.

Slides were coded and examined without the knowledge of the treatment (blind scoring) to avoid personal bias. To ensure that a cell was counted only once, the slide was carefully moved from top to bottom and left to right using the mechanical stage clip. Cells were scored by using 1000x (10x eyepiece and oil immersion objective) magnifications of Ken α-Vision Light Microscope. Five slides per mouse were prepared and scoring was done from 1000 polychromatic erythrocytes (PCE) per slide. Statistical analyses of the results were undertaken using One-way Analysis of Variance (ANOVA) with DMRT.

RESULT AND DISCUSSION

Phytochemical screening

Phytochemical components such as alkaloids, flavonoids, saponins, tannins and polyphenols were determined in *Syzygium brevistylum* crude methanolic extract. Phytochemical analysis revealed that important plant secondary metabolites flavonoids, saponins, tannins and polyphenolic compounds were present in the methanolic extract of *Syzygium brevistylum* leaves.

Table 2: Phytochemical constituents of the methanolic extract from the air-dried leaves of *Syzygium brevistylum*.

Phytochemical	Test	Result	Indication
Alkaloids	Dragendorff's	No reaction	(-)
Flavonoids	NaOH test	Yellowish brown	(++)
Saponins	Froth	Persistent Froth	(+++)
Tannins/Polyphenols	Gelatin	Jelly-precipitate	(+++)
	Ferric chloride	Blue-black coloration	(+++)

Legend: (+) trace, (++) moderately present, (+++) copiously present, (-) absent

Brine shrimp assay

Preliminary assessment of toxicity was conducted using brine shrimp lethality test (BSLT). A higher percentage of mortality indicates higher toxicity and bioactivity. Calculations on the Lethal concentration (LC_{50}) were done using Probit analysis develop by Finney and Tattersfield [11].

Table 3: Number of dead nauplii and percentage mortality in each partitioned extracts after 24 hours.

Extracts	Conc. (ppm)	Mean	% Mortality	LC_{50} after 24 hrs. (ppm)
Hexane	10	7.6	76	1.00
	100	8	80	
	1000	9.4	94	
Ethyl acetate	10	7.8	78	0.312
	100	9	90	
	1000	9.4	94	
Aqueous	10	4.8	48	29.1
	100	7.6	76	
	1000	8.6	86	
Blank		2.0	20	

Legend: Blank = artificial seawater

The average percent mortality of *Artemia salina* in ethyl acetate extract (EAE) at concentration of 10, 100, and 1000 ppm were 78%, 90% and 94%, rating the highest among three extracts. The hexane extract (HE) also showed similar percent mortality of 94% with that of EAE at 1000 ppm concentration rating 94%. The aqueous extract (AE) shows the lowest average mortality of *Artemia salina* at concentration of 10 ppm, 100 ppm and 1000 ppm rating 48%, 76%, and 86%, respectively. Compared to the blank, which has an average of 20% mortality in brine shrimp, the organic extracts from the leaves of Syzygium *brevistylum* is toxic to *Artemia salina*. The data suggest that the extracts from the sample has high bioactivity against *Artemia salina*, the EAE has the highest toxicity, and hence the bioactive among the three extracts.

The toxicity of the organic extracts can also be determined using the lethal concentration (LC_{50}) and the extract with the lowest LC_{50} value analyzed in 24-hour interval determines the most toxic and bioactive extract. The most toxic extract to *Artemia salina* is the EAE having a LC_{50} value of 0.312 ppm. The extract with lowest toxicity is AE (LC_{50} = 29.1 ppm) followed by HE (LC_{50} = 1.00 ppm). It has been discussed that there is correlation between the toxicity and bioactivity with BSLT (12). Thus, the most toxic and bioactive extract against *Artemia salina* observed at 24-hour interval is the EAE.

Antimicrobial assay

This assay was done to evaluate the inhibiting capacity of the hexane, ethyl acetate and aqueous extracts on specific bacterial and fungal strains. *Escherichia coli* and *Staphylococcus aureus* were used as test microorganisms for antibacterial assay and *Candida albicans* for antifungal assay. For paper disc diffusion method, a substance is considered bioactive against microorganisms if a minimum of 10 mm diameter of inhibition is observed [6].

Antifungal assay

The HE and EAE showed bioactivity against *Candida albicans* at all concentration levels but AE only showed bioactivity at 30000 ppm.

EAE have the highest inhibiting capacity against the microorganism, compared with the other two extracts at concentrations of 10000, 20000, and 30000 ppm, respectively. AE has the lowest inhibiting capacity followed by HE.

Table 4: Antifungal effects of hexane, ethyl acetate, and aqueous extracts against *Candida albicans* at specific concentration.

Treatment	*Candida albicans*		
	Mean zone of inhibition (mm)		
	10 000 ppm	20 000 ppm	30 000 ppm
Ketoconazole	13.33^A^	15.83^A^	19.17^A^
Hexane	10.7^B^	12.5^B^	12.0^B^
Ethyl acetate	15.3^A^	17.3^A^	17.3^A^
Aqueous	0.0^C^	7.67^B^	12.8^B^
Distilled water	0.0^C^	0.0^C^	0.0^C^

Legend: Means having the same letters are not significantly different at α = 0.05. A-C = highest - lowest

At 10 000 ppm, EAE which is comparable with Ketoconazole, exhibited the highest inhibition against the fungi, followed by HE. The AE has the lowest inhibition, which is comparable with that of distilled water. At 20 000 ppm, the EAE has the highest inhibiting capacity which is also comparable with Ketoconazole, followed by the HE. The HE and AE has similar inhibiting capacity by One-way ANOVA and DMRT. At

30000 ppm, EAE has also the highest inhibiting capacity followed by AE and HE. The EAE has comparable inhibitory capacity with ketoconazole. Distilled water has no inhibitory capacity against the fungi.
Statistical analysis of the results show no significant difference on the diameter of inhibition of EAE and HE with the varying concentrations against *Candida albicans*. However, for the AE and Ketoconazole, the effect of concentration in the inhibiting capacity is significantly different from each other by ANOVA.

Antibacterial assay

For the antibacterial capacity against *Escherichia coli*, HE and EAE showed no bioactivity against the bacteria since a minimum of 10 mm diameter of inhibition [6] was not reached. Conversely, the AE is bioactive at concentration of 30000 ppm but shows a very low inhibiting capacity compared with Ampicillin.

Table 5: Antibacterial effects of hexane, ethyl acetate, and aqueous extracts against *Escherichia coli* at specific concentration.

Treatment	*Escherichia coli* Mean zone of inhibition (mm)		
	10000 ppm	20000 ppm	30000 ppm
Ampicillin	23.83	26.33	28.33
Hexane	7.2	7.2	8.5
Ethyl acetate	7.3	8.7	9.8
Aqueous	0.0	7.2	13.7
Distilled water	0.0	0.0	0.0

Legend : Distilled water = negative control, Ampicillin = positive control.

For the antibacterial activity against *Staphylococcus aureus*, the three extracts showed low inhibiting capacity compared with ampicillin. Of the three extracts, only the HE and EAE showed zones of inhibition against *Staphylococcus aureus* which is less than 10 mm in diameter. The EAE extract showed the highest inhibiting capacity among the three extracts at all concentration range.

Table 6: Antibacterial effects of hexane, ethyl acetate, and aqueous extracts against *Staphylococcus aureus* at specific concentration.

Treatment	*Staphylococcus aureus* Mean zone of inhibition (mm)		
	10000 ppm	20000 ppm	30000 ppm
Ampicillin	30.17	31.67	33.33
Hexane	7.2	7.7	8.7
Ethyl acetate	7.2	8.3	9.0
Aqueous	0.0	0.0	0.0
Distilled water	0.0	0.0	0.0

Ampicillin has the highest inhibitory capacity against *Staphylococcus aureus* followed by EAE then HE at all concentration levels. The aqueous extract, which is comparable with distilled water, has no inhibitory capacity against the bacteria. At 10000 ppm, the EAE and HE have the same inhibition of 7.2 mm. At 20 000 ppm and 30 000 ppm the EAE has the highest inhibition compared with HE.
The results show that there is a directly proportional relationship between the diameter of inhibition and concentration. The inhibition against the bacteria increases with concentration.

Table 7: Susceptibility of the test microorganisms towards the different treatments

Test microorganism	Hexane extract	Ethyl acetate extract	Aqueous extract
Candida albicans	A	A	A
Escherichia coli	B	B	A
Staphylococcus aureus	B	B	B

Legend: Means having the same letters are not significantly different at $\alpha = 0.05$. A-B = highest-lowest

In terms of sensitivity of microorganisms to specific extracts, it is demonstrated that the inhibiting capacity of the extracts is greatest against *Candida albicans* compared with *Escherichia coli* and *Stapylococcus aureus*. The sensitivity of *Escherichia coli* and *Candida albicans* in aqueous extract is comparable with one another. All the partitioned extracts exhibit less inhibiting capacity against *Staphylococcus aureus*.
It is discussed from the literatures that some plant secondary metabolites exhibits antimicrobial effect. The results showed that the extracts of *Syzygium brevistylum* leaf greatly inhibit the growth of the fungi

compared with the two bacteria. The extracts from the plant sample is only bioactive against *Candida albicans*. This effect may be attributed to the presence of saponins, tannins and polyphenols which are the highly present components of the crude methanolic extract [12-13].

Micronucleus assay

The most bioactive extract, based on the results of BSLT and antimicrobial test, was ethyl acetate extract (EAE). The peripheral blood micronucleus assay was employed to test the mutagenicity, antimutagenicity and protective effect of the ethyl acetate extract of *Syzygium brevistylum* leaves. The maximum tolerable dose (MTD) was 500 mg/kg and was used as highest dose for the experiment. The occurrence of micronucleated polychromatic erythrocyte in mouse peripheral blood was examined using the criteria set by Fenech [10]. The reports were tabulated presenting the mean frequency of MNPCE.

It is demonstrated (Table 8) that MNPCE is observed in all treatments. The result showed a high significant difference (P value < 0.05) in the occurrence of MNPCE on three treatments compared with MMS (positive control), and DMSO and distilled water (negative control), which implies that the effect of the treatments is different with the controls.

Table 8: The mean micronucleus frequency in mice peripheral blood of each treatment compared with the positive and negative controls by One-ANOVA with DMRT.

Treatment	Concentration of EAE (ppm)	Dose (mg/kg)	MNPCE frequency		
			Mean	P values	Remarks
Mutagenicity	10000	100	15.25	0.00	Highly Significant
	30000	300	21.1	0.00	Highly Significant
	50000	500	30.35	0.00	Highly Significant
Antimutagenicity	10000	100	21.5	0.00	Highly Significant
	30000	300	23.7	0.00	Highly Significant
	50000	500	28.8	0.00	Highly Significant
Protective	10000	100	18.75	0.00	Highly Significant
	30000	300	20.45	0.00	Highly Significant
	50000	500	22.55	0.00	Highly Significant
Distilled water			2.35		
DMSO			1.9		
MMS	5000	50	51.95		

Legend: Positive control = MMS, Negative control = Distilled water and DMSO

For the mutagenicity test, occurrence of MNPCE was observed in mice peripheral blood at three doses. One-way ANOVA demonstrated that negative controls, DMSO and distilled water, were not significantly different from each other. For the mutagenicity test, the occurrence of MNPCE in all dose level of EAE is significantly different from the negative control. This result implies that the EAE extract significantly increase the occurrence of MNPCE. Nevertheless, compared with MMS administered at 50 mg/kg, the occurrence of MNPCE in mice treated with EAE at the three doses is low for it to be considered mutagenic. The frequency of MNPCE on mice treated with EAE alone was dose dependent as analyzed by One-way ANOVA and increases at higher dose levels.

For the antimutagenicity test, results showed a high reduction of MNPCE compared with the positive control (MMS). The observe effect is dose dependent as analyzed by One-way ANOVA and decreases at higher dose levels. A trend is also observed a highest decrease in the frequency of MNPCE at the lowest dose (100 mg/kg) with a mean MNPCE frequency of 21.5 compared with 51.95 mean MNPCE frequency in the positive control.

The test for protective effect of the EAE it shows a high significant reduction of MNPCE compared to MMS. The effect is also dose dependent. The reduction of MNPCE is observed to be highest at 100 mg/kg with mean MNPCE frequency of 18.75 compared with 51.95 mean MNPCE frequency of the positive control, MMS.

According to Loomis [1], all chemicals can be toxic to biologic system within a dose. Thus, the observe effect on the mutagenicity test of EAE is dose related. The minimum dose of the extract to which the EAE is toxic to mice peripheral blood is reached. It is suggested that test for mutagenicity of the extract be done at lower dose. In addition, it has been discussed in the literature that some phytochemicals are toxic to some cell line [15-16]. Other factors may also be responsible for the effect (heavy metals, residual solvents etc.).

The discussion of Loomis [1] in biotransformation mechanism involves the role of microsomal enzymes in the increase and decrease of toxicity of a compound. Although this study cannot pinpoint the exact reason for the protective effect of the compound, it is presumed that the observed activity may be due to

microsomal enzyme induction and antioxidant property of phytochemicals [17]. The antimutagenicity effect may be attributed to the presence of dietary phytochemicals, which possess radical scavenging activity. It also noteworthy that the observe results is still inconclusive and may be subjected to other test to verify its results.

CONCLUSION

The analysis of phytochemical constituent, cytotoxicity, antimicrobial capacity, mutagenicity and antimutagenicity of the *Syzygium brevistylum* leaf extract was carried out. Based on the results, the crude methanolic extract of the leaves of *Syzygium brevistylum* is positive of the presence of flavonoid, saponins, tannins and polyphenols. Various organic extracts from the sample showed high toxicity in brine shrimp *Artemia salina*. The extracts also showed antifungal activity against *Candida albicans*. The antifungal activity of AE against *Candida albicans* significantly increases with concentration. For EAE and HE there is no significant increase in the activity with concentration. Results for antibacterial effect showed that the extracts from the plant sample has no bioactivity against *Escherichia coli* and *Staphylococcus aureus*. Micronucleus test of the EAE, administered alone, showed an increase in the frequency of micronucleated polychromatic erythrocytes (MNPCE) compared with the negative control. The increase of MNPCE showed highest at 500 mg/kg bw. The EAE has low mutagenic activity to test animals compared with MMS at the three dose level used. The EAE, treated before and after the treatment of MMS, showed a significant reduction in the frequency of MNPCE in mouse peripheral blood which is observed highest at dose of 100 mg/kg. Thus, the EAE of *Syzygium brevistylum* is considered to have antimutagenic and protective effect against chemically induce mutation.

CONFLICT OF INTEREST

No conflict of interest.

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