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Cytotoxic Property and Influence in the Estrous Cycle of *Mus Musculus* and Mating Behavior of *Danio Rerio* of *Tinospora Rumphii* Boerl. Extract

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This study evaluated the influence of extracts of the bark of *Tinospora rumphii* Boerl. on the estrous cycle of mice and the mating behaviour of zebra fish, as well as its cytotoxic properties. The phytochemical profile of *T. rhumpii* was determined using standard protocols.. Cytotoxicity of the ethanolic extract of the *T.rhumpii* was tested using brine shrimp lethality assay and MTT assay analysed using human lung adenocarcinoma (A549) cells Antifertility assay was conducted by finding out the influence of feeding *T. rhumpii* in the estrous cycle of albino mice for 15 days and estrous cycle was monitored through vaginal cytology. The effect on mating behavior of male *Danio rerio* exposed for 12 hours to different concentrations of *T. rhumpii* aqueous extract was also assessed.
Phytochemical profiling revealed that *T. rhumpii* vines contained saponins, alkaloids, and tannins but tested negative for flavonoids, phobatannins and cardiac glycosides. Mice fed with *T. rhumpii* feed replacements had irregular estrous cycle patterns with prolonged diestrus stages. Inhibition in the mating behavior of *D. rerio* was observed. *T. rhumpii* ethanolic extract showed to be moderately toxic as tested in brine shrimp lethality assay. On the other hand, MTT assay revealed that the extract was non-toxic to human lung adenocarcinoma (A549) cells.

Keywords: antifertility, cytotoxicity, mating behavior, phytochemicals, *T. rumphii*

Introduction

Utilization of plant products for medicine is widely spread and encouraged that continuing researches are being conducted. Bioactive compounds and phytochemicals of the plants may have relative importance in the development of alternative treatment to various diseases.

According to the World Health Organization, cancers figure among the leading causes of morbidity and mortality worldwide, with approximately 14

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million new cases and 8.2 million cancer related deaths in 2012 while lung cancer rated as one of the top killer diseases in the Philippines (DOH). Many treatment options for cancer exist, with the primary ones including surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and palliative care. These treatments are undeniably costly and for many, unaffordable. This gives way to people considering alternative medicines like traditional herbal medicines introduced by neighboring countries getting significant attention in global health debates (Tilburt and Kaptchuk, 2008).

Plant products may also be used to control population by targeting the reproductive function of organisms, as well as in human beings. Increasing population has started to get some attention and concern in many countries. According to Population Reference Bureau, global human population growth amounts to around 75 million annually, or 1.1% per year. The knowledge of use of plants for traditional antifertility and abortifacients are most often from folklore that there is a need for further study on their effect. The search for safer reproductive suppressing agents from medicinal plants as alternative for estrogen and progesterone containing compounds is global and considered to impact the family economics and health, especially in developing countries (Nayaka *et al*., 2014; Bala *et al.*, 2014; Raj, 2011.). Even the World Health Organization (WHO) includes studies on traditional medical practices for temporary suppression of reproductive function in its program. (Kaur *et al*., 2011)

Tinospora rumphii Boerl., a deciduous climbing shrub, is among the traditionally claimed plants that have therapeutic properties. The leaves, barks and roots contain various bioactive compounds such as alkaloids, glycosides, lactones, steroids, polysaccharides and aliphatic compounds having various medicinal importance (Pandey, 2012). These bioactive compounds are found to have toxic effect to cells which can inhibit the growth or terminate the cells. In the Philippines, it is also known to have abortive properties. Aside from cytotoxic and reproductive suppressing effects, it is also used for various medicinal purposes such as diarrhea, indigestion, rheumatism, typhoid fever, pneumonia and some heart problems as well as for malaria, fever, stomach ache, diarrhea, dysentery, toothache, rheumatism, and for weaning babies from breastfeeding (Stuart, 2013).

Developing natural alternative treatment for cancer and alternate contraception with lesser side effects will bring a huge impact to the medical or health related industry. The alternative medications are now well accepted by the society and evidences to the alternative treatment are well needed to support the different claims practiced by different countries.

This study was carried out to determine the influence of *T. rumphii* extract in the reproductive functions of selected animals. This study evaluated the influence of extracts of the bark of *Tinospora rumphii* Boerl. on the estrous cycle of mice and the mating behaviour of zebra fish, as well as its cytotoxic properties.

Materials and Methods

Preparation of *T. rumphii* Powder and Extracts. Healthy stems of *T. rumphii* were obtained from Nueva Ecija, Philippines. Stems were thinly sliced; sun dried and pulverized using food processor. Powder was stored in a sterilized container.Three hundred (300) ml of distilled water was added to twenty grams of powdered dried leaves and was stirred in a 1000 ml capacity flask. The mixture was placed into a double boiler water bath for two hours at 80-90 ℃. The extracts were filtered using Whatman filter paper No. 1. Aqueous extractwas stored in sterilized amber bottle until use.

Ethanol extraction of *T. rumphii* was based on the procedure of Olowa and Nuneza, (2013). Twenty grams of powdered sample was soaked with 200 ml of 95 % ethanol, giving the ratio of 1:10. The mixture was placed in an Erlenmeyer flask wrapped with an aluminum foil and was left for 48 hours with occasional swirling of the mixture for the first six hours. Extracts were filtered using Whatman filter paper (No. 1) and the residual solvents were removed under reduced pressure at 40 ℃ using a rotary evaporator and extract was stored in sterilized amber bottle.

Phytochemical Assay. Phytochemical profile was tested for the presence of saponins, alkaloids, flavonoids, tannins, phlobatannins and cardiac glycosides. Chemical tests were conducted on the aqueous extract and on powdered forms of makabuhay (*T. rumphii*) using standard methods by Edeoga *et al.* (2005).

Cytotoxicity Assay. Brine shrimp lethality assay. The crude extract was diluted into different concentrations (10 ppm, 100 ppm, 1000 ppm and 10,000 ppm). Eggs of Artemia was obtained from Bureau Of Fisheries and Aquatic Resources, Central Luzon State University, Science City of Muñoz, Nueva Ecija and was allowed to hatch as nauplii for 24-48 hours in artificial seawater which was made by dissolving 38 g of rock salt in 1 liter of distilled water. (Mclaughin et al., 1998) When the nauplii were ready, 5 ml of artificial seawater was added to each plate and 10 newly hatched naupli were introduced in each, hence, 30 shrimps per dilution. The volume was then adjusted with different concentrations of extracts up to 10 ml per plate. The extract concentrations tested were left uncovered under the lamp. The number of dead

nauplii as monitored on the 6^{th}, $12^{th,}$ 18^{th} and 24^{th} hours. Percent mortality was documented and LC_{50} was determined by Probit Analysis.

MTT Assay. Test was performed in Mammalian Cell Culture Laboratory, Institute of Biology, University of the Philippines, Diliman, Quezon City, Philippines. Culture of lung adenocarcinoma cells (A549) was done on a liquid medium in a culturing flask supplemented with Heat Inactive Fetal Bovine Serum, F12 Nutrient Mixture HAMS, Antibiotic-Antimycotic (100x) formulation and 7.5 % Sodium Bicarbonate. The MTT cytotoxicity assay performed was adapted from Mosmann (1983). In detail, A549 were seeded at 4 x 10^4 cells/mL in a sterile 96-well microtiter plates and was incubated overnight at 37˚C and 5% CO_2 concentration. Four concentrations were prepared as treatment: 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL. Doxorubicin served as the positive control while dimethyl sulfoxide (DMSO) served as the negative control. After incubation, cells were treated with 10 µL of each dilution and were incubated again for 72 hours at 37˚C and 5% CO_2 concentration. The media was then removed and 20 µL of MTT at 5 mg/mL PBS was added. The cells were incubated again at 37˚C and 5% CO_2 concentration. Finally, 150 µL DMSO was added to the well. Absorbance was read at 570 nm. The cytotoxicity index was determined using the untreated cells as negative control. The percentage of cytotoxicity was calculated using the background corrected absorbance. The 50% inhibitory concentration was computed through linear equation from regression analysis.

Antifertility Assay in Mice. Powdered dried *T. rumphii* was added to the diet of mice as pellets. A total of 30 female white mice ranging from 22 - 32g of sexually mature age (23 - 25 days old) were acclimatized for one week in cages with free access to water and commercial feeds. The cages were placed in room temperature with a 12-hour light – 12-hour darkness cycle during the course of the study (Rahman *et al.*, 2005). The mice were randomly divided into three treatments with each treatment consisting of 4 replicates with 3 mice per replicate. Mice were fed *ad libitum* with feed replacement (T1:10%,T2: 20%and T3:0%) of *T. rumphii* for 15 days.

Vaginal smears were collected daily from each mouse at 12-hours interval between 10:00 to 13:00 and between 22:00 to 01:00. The vaginas of the mice were flushed with 10 µl saline solution 0.9% NaCl (Caligioni, 2009; Goldman *et al.*, 2007) three to five times with same saline solution. Final flush containing vaginal fluid was smeared, fixed in 95% ethanol for 2-3 minutes, stained with crystal violet for 1-3 minutes (McLean *et al.*, 2012) and observed a compound light microscope.

Number of cells on smears was obtained by counting manually three to six (3-6) microscope fields until total number of cells reached 500 (personal

communication Duran, 2014). The proportion of each type of cell found in the smear was computed. The characterization of each phase of the estrous cycle was based on the proportion (percentage) among leukocytes and the three types of cells to be observed in the vaginal smear (Omar *et al.*, 2007; Mettus and Rane, 2003; McLean *et al.*, 2012): parabasal cells, intermediate cells and cornified cells. Parabasal cells have a large, predominant nucleus; intermediate cells have a smaller nucleus; and cornified cells have a keratinized non-visible nucleus. The determination of phases was upon the following criteria: Proestrus – predominance of intermediate epithelial cells; Estrus – when there is predominance of cornified epithelial cells; Metestrus – less than 20% cornified cells and reappearance of intermediate and parabasal cells, and Diestrus – predominant leukocytes with few cornified cells and parabasal cells.

The study was laid out using the Complete Randomized Design (CRD). Analysis of Variance was done to determine significant differences and Duncan Multiple Range Test to compare means. Spearman rho was used to establish correlations. Level of significance was set at 5%. Statistical Analysis was done using Statistical Package of Social Sciences (SPSS 20).

Assessment of Mating Behaviour of *D. rerio*. Aqueous extract was diluted with distilled water to prepare different concentrations (2%, 4%, 6%, 8%). Pure distilled water was used as the control. Healthy and sexually matured *D. rerio* were bought from commercial suppliers. Male were separated from females and were acclimatized for four days at 26 ± 1℃ (room temperature) condition before treatment application. They were fed with flakes and left-over food was daily removed for the maintenance of water cleanliness and quality. In separate plastic containers, 200 ml of each concentration, including control, were placed in triplicates. A pair (male and female) of zebra fish was transferred to each container and were arranged in a shelf for observation. Prior to assessment, the treatments were left for 12 hours in a dark condition. After treatment, mating behaviors were observed and frequency was quantified accordingly for a span of 5 minutes and recorded. Behaviors identified and analyzed were chase, circle and pin as described by Spence *et al*. (2008). Data gathered were analyzed using ANOVA. (Analysis of Variance)

Results

Phytochemical Profile of *T. rumphii*. The phytochemical profile of *Tinospora rumphii* were identified in this study and results are shown in Table 1. Results revealed that makabuhay stems have saponins, alkaloids, and tannins; but tested negative for flavonoids, phobatannins, and cardiac glycosides.

Table 1. Phytochemicals present in *T. rumphii*

Phytochemicals	Makabuhay
Tannins	Positive (+)
Alkaloids	Positive (+)
Saponins	Positive (+)
Phobatannins	Negative (-)
Flavonoids	Negative (-)
Cardiac Glycosides	Negative (-)

Cytotoxic Effect of *T. rumphii* using Brine Shrimp Lethality Assay. Brine shrimp lethality bioassay is a simple, high throughput cytotoxicity test of bioactive chemicals. It is based on the killing ability of test compounds on a simple zoological organism – brine shrimp (Harwig & Scott, 1971). The concentrations of *T. rumphii* extracts used ranged between 10 – 10,000 ug/ml. The ethanol extracts of plants tested showed significant brine shrimp mortality activity. The degree of lethality was directly proportional to the concentration of the extract as shown in Figure 1. Highest mortality rate (70%) was observed at 10,000 µg/ml concentration. At 10 µg/ml concentration, 27% of the nauplii died which indicates that in such small amount of extract, there was still high mortality rate. Based on the results, the brine shrimp lethality of the extract was found to be concentration-dependent wherein the higher the concentration, the higher the mortality rate. It was also evident basing on the table that 50% lethality concentration is at the range of 100 – 1000 µg/ml, given that the concentration 100 µg/ml has mortality rate of 40 % and 1000 µg/ml resulted to 57 percent deaths.

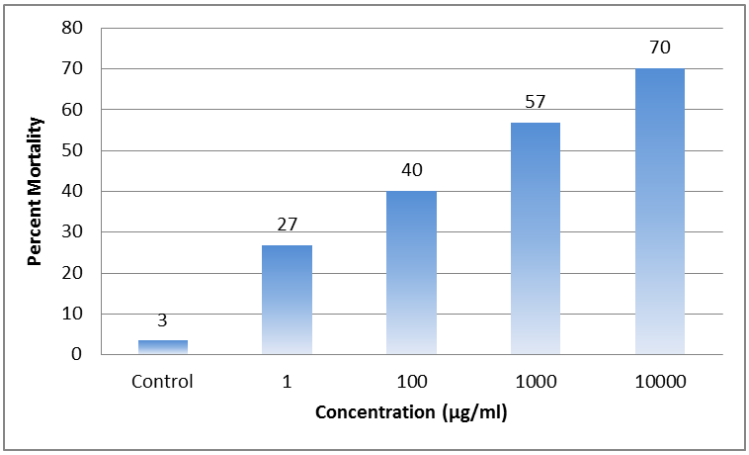

Figure 1. Mortality Rate of Nauplii 24 hours post-treatment of *Tinospora rumphii* Extracts.

Using probit analysis, Figure 2 shows the linearity of the mortality rate and computed value of LC_{50} using the linear equation is 365.31 µg/ml. The observed lethality of the extract to brine shrimps indicated that small amount of the extract is needed to perform cytotoxic activity. According to the rating of Alhadi et al. (2015): LC50 of ≤ 249 µg/mL as highly toxic; LC50 of 250 - 499 µg/mL is moderately toxic; and LC50 of 500-1000 µg/mL is mildly toxic and values above 1000 µg/mL are non-toxic (McLaughlin and Rogers, 1998), therefore, the computed LC_{50} value 365.31 µg/ml is considered moderately toxic.

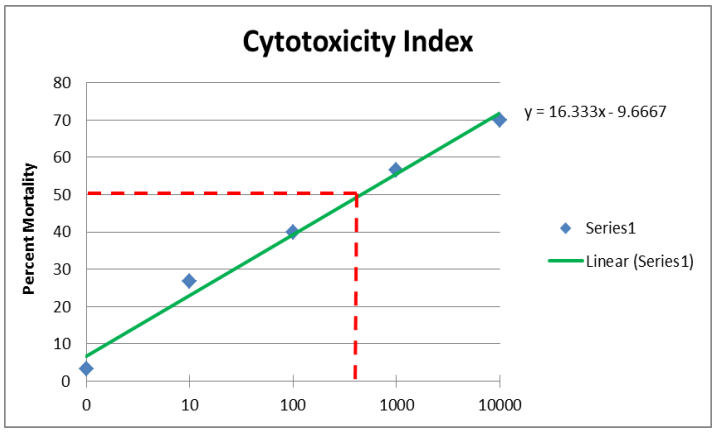

Figure 2. LC_{50} determination Using Probit Analysis

Cytotoxic Effect of *T. rumphii* using MTT Assay. Cytotoxic property of *T. rumphii*. was assayed in vitro through MTT using lung adenocarcinoma (A549) cells. Figure 3 shows the absorbance value of cells exposed to the negative control (Doxorubicin). The highest absorbance was observed in 20 µg/mL concentration with a value of 0.279 while the least absorbance was observed at 3.125 µg/mL with a value of 0.192. Results indicate that concentration of Doxorubicin is proportional to the absorbance value wherein, higher concentrations of Doxorubicin provide higher absorbance value. This implies that higher concentrations of the positive control provide more viability to cells compared than the lower dilutions.

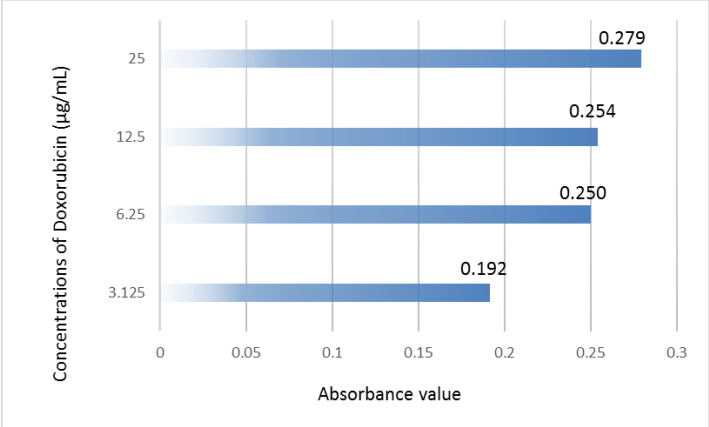

Figure 3. Absorbance value of cells exposed to positive control (Doxorubicin).

Figure 4 shows the means absorbance value of cells exposed to DMSO and different concentrations of extracts of *Tinospora rumphii*. Cells exposed to DMSO provided an absorbance value of 0.806 while the different concentrations of *T. rumphii:* 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL had an absorbance value of 0.830, 0.891, 0.833 and 0.778, respectively. Highest absorbance was observed at 25 µg/mL concentration of the extract while the least absorbance was observed in a lower concentration of 6.25 µg/mL. Results also showed that there was higher absorbance value in concentrations 50 µg/mL, 25 µg/mL and 12.5 µg/mL compared to cells exposed to the negative control (DMSO). This implies that there was higher cell proliferation and viability in the concentrations of extracts tested than the negative control.

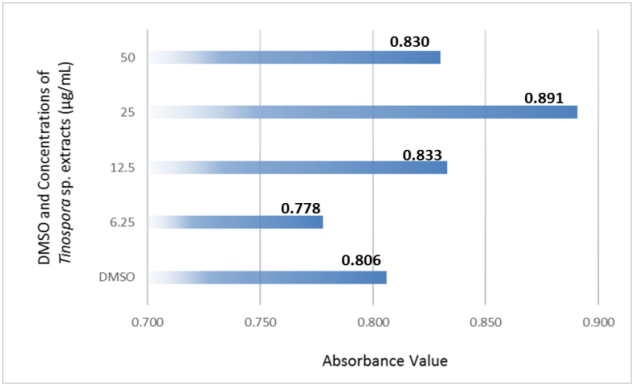

Figure 4. Absorbance value of cells exposed to DMSO and *T. rumphii*.extract. (50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL)

Cytotoxicity index was determined and Figure 5 shows that *Tinospora* extract at 6.25 µg/mL had the highest toxicity having 40.76 % mortality compared to other concentrations. Least cytotoxic effect was observed at 25 µg/mL having 15.94% mortality. Results implied that cytotoxicity is best observed at lower concentration of extract compared to higher doses which is hypothetically believed to provide higher toxicity rate. After computing for the 50 % inhibitory concentration using the linear equation from regression analysis, IC_{50} is at approximately 0.133 µg/mL at one end, and 221.46 µg/mL at the other end. The screening protocol based on National Cancer Institute of America has indicated that plant extracts with IC_{50} less than 30 µg/mL were considered to be cytotoxic and non-cytotoxic if otherwise. (Roslen et.al, 2014) Since the trend of the results is curvilinear and 50% inhibition concentration was not within the tested range, it is none conclusive that *Tinospora rumphii* extract is cytotoxic to lung adenocarcinoma cells or not.

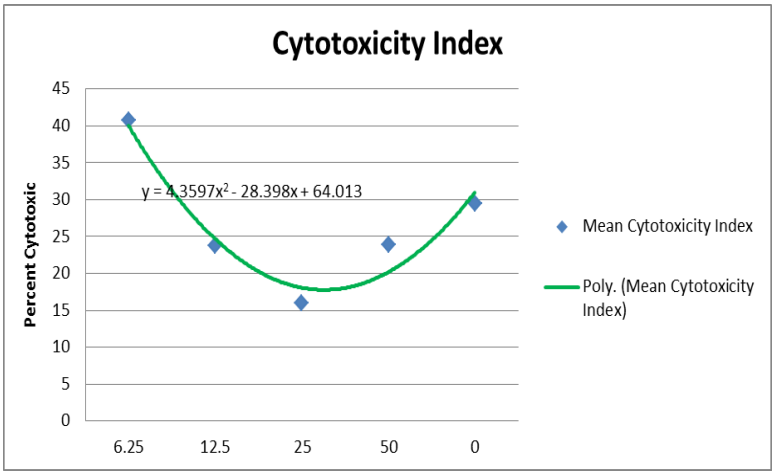

Figure 5. Cytotoxicity index of different concentrations of *Tinospora*

Influence of *T. rumphii* on the Estrous Cycle of White Mice. The estrous cycles of the *Mus musculus* were determined using vaginal cytology analysis and confirmed by examining the vaginal appearance. The different stages of the estrous cycle were characterized by identifying the percentage of parabasal, intermediate and cornified epithelial cells and leukocytes from the vaginal smears of mice. Figure 6 shows the appearance of the different types of cells under the compound light microscope at High Power Magnification and the vaginal appearance at different stages of estrous. The vaginal opening of mice in proestrus is characterized by swollen, moist, pink tissue; the opening is wide and there are often wrinkles along the dorsal and ventral edges. In estrus,

the vaginal opening is less pink, less moist and less swollen and has a wide opening. Metestrus is characterized by a vaginal opening that shut or closed and not swollen. And in diestrus, the vaginal opening is small with no tissue swelling (Byers *et al*., 2012).

The estrous cycle of the mice without any feed replacement showed regular pattern of estrous cycle of 4 to 5 days showing all stages of proestrus, estrus, metestrus, and diestrus each stage changing on a daily basis (Goldman *et al.*, 2007, Caligioni, 2009 and Omar *et al.* ,2007). Mice were given *T. rumphii* replacement diet showed more irregular patterns in contrast with the control showing irregularities such as persistent diestrus in some mice (Figure 7).

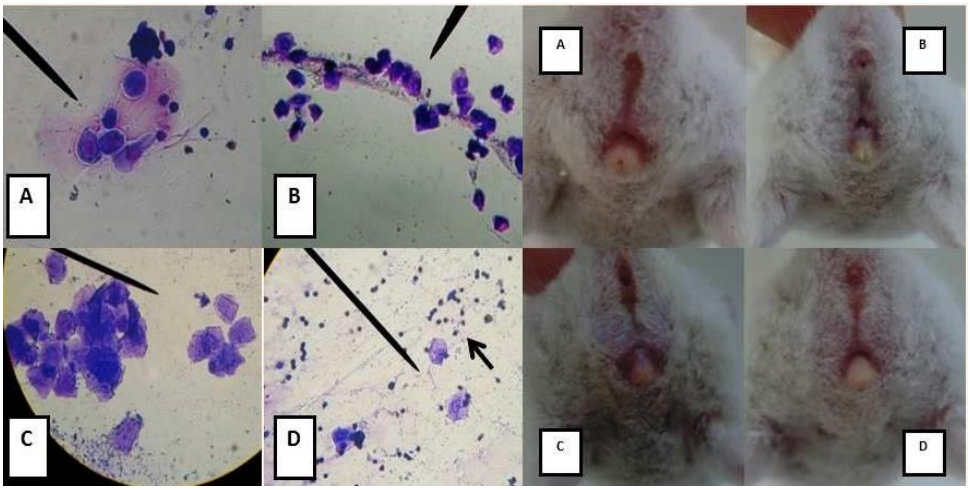

Figure 6. Types of cells found on different (A) Parabasal (nucleated) cells, (B) Intermediate cells, (C) Cornified cells, and (D) Leukocytes and corresponding of vagina of mice during (A) Proestrus, (B) Estrus, (C) Metestrus, and (D) Diestrus

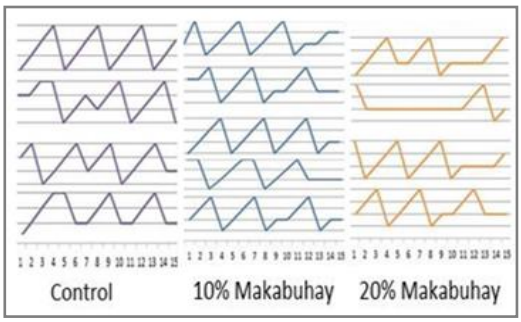

Figure 7. Estrous cycle of mice given different *T. rumphii* replacement concentrations for 15 days. Lower peak points indicate the stage of metestrus, upper peak points indicate the stage of estrus and middle lines indicate the stage of diestrus.

Results further showed that by the end days of the administration of treatments, feeding with *Tinospora* replacement had altered the estrous cycle of mice by significantly decreasing the number of estrus stage on the third cycles as compared to the control (Table 2). It appeared that feeding with *T. rumphii* feed replacement interfered with the normal cycling of mice causing irregularities in the estrous cycle exhibiting antifertility effects. The prolongation of diestrus phase may explain the remote chance of the rats to get pregnant (Nayaka *et al.,* 2014). Thus, an elongated period of diestrus means that the estrous cycle has been altered and the ovulation has been delayed so the feed replacement has anti-ovulatory effects.

Table 2. Mean number of days of metestrus, diestrus, proestrus and estrus stages of mice for 15 days under different treatments

Treatments	Cycle 1				Cycle 2				Cycle 3			
	Metestrus	Diestrus	Proestrus	Estrus	Metestrus	Diestrus	Proestrus	Estrus	Metestrus	Diestrus	Proestrus	Estrus
Control	.90a	1.10a	1.10a	1.00a	.60a	1.40a	1.00a	1.00a	.90a	1.10a	1.00b	1.00b
10% Makabuhay	.67a	1.33a	1.00a	1.11a	1.00a	1.33a	1.00a	1.00a	.78a	2.78b	.78a	.44a
20% Makabuhay	.87a	5.17b	1.12a	1.00a	.75a	1.62a	.75a	.87a	.87a	3.00b	.37a	.12a

*means with the same letter on each column are not significantly different from each other at 5% DMRT probability level

Influence of *T. rumphii* on the Mating Behavior of *Danio rerio*. Fish at the age of approximately 7 months was put into mating pairs separately with

other pairs in the setup. Each container received different concentrations of plant extract. Observations were analyzed quantitatively by observing behavior patterns.

Shown in Table 3 is the mean number of mating behavior responses at different concentration at 5 minute observation. The extract was found to inhibit the mating behavior of *D. rerio* at concentrations from 0.5% to 4%. *T. rumphii* extract was found to be active against this organism. Among the four different concentrations, 4% concentration showed the highest inhibition rate in mating behavior. Circling and pinning behaviors were totally inhibited at 2 and 4 % concentration. It is also evident that there is a significant difference in the concentrations and the control in all the observed behaviors. The decreasing trend in all the concentrations shown in Figure 8 supports the claim that extract of *Tinospora rumphii* has inhibiting capabilities.

Table 3. Mean number of mating behaviour at different concentration of leaf extract

Concentration (%)	Mean number of behaviour after 12 hour exposure at 5 minutes observation		
	Chase	Circle	Pin
0.5	9.67^{b}	4.00^{b}	1.33^{b}
1	7.33^{c}	2.00^{b}	0.33^{b}
2	4.67^{d}	0.33^{c}	0.00^{b}
4	3.33^{d}	0.00^{c}	0.00^{b}
Control (-)	12.67^{a}	9.33^{a}	3.00^{a}

*Means in a column that have different letters are significantly different at .05 significant diff.

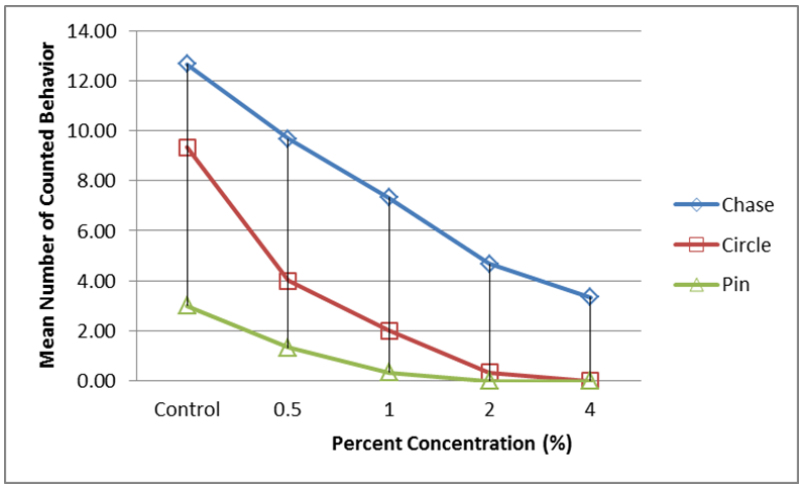

Figure 8. Mating behaviour at 12 hour exposure at 5 minute observation

Discussions

Plants have been used for medical purposes since the beginning of human history and are the basis of modern medicine. Plants and their extracts can be used to produce drugs for treatment and contraceptives. The active ingredients present in plants that would be helpful in obtaining drugs could be phytochemicals such as alkaloids, glycosides, saponins, tannins, terpenoids, and isoflavonoids (Bala *et al.*, 2014; Nayanatara *et al.* (2012). Phytochemicals are non-nutritive plant chemicals but provide definite physiological action on the body and are widely used in the human therapy, veterinary studies, agriculture, and scientific research (Yadav and Agarwala, 2011).

Female cyclicity, characterized by vaginal changes as observed in estrus cycle, is an index of good functioning of the neuroendocrine – reproductive system and ovarian activity, loss of normal estrus cycle indicates the disruption of ovarian progesterone and estrogen balance (Marcondes *et al.*, 2002; Dela Cruz *et al.*, 2008). Estrogenic chemicals are known to cause infertility by shortening the time of transport of egg, disrupting estrous cycle, lowering the plasma progesterone. Furthermore, the works of Hazarika and Sarma (2007) and Tamura (1997) cited that vaginal smear to monitor the estrous cycle of albino rats also indicated an alteration of estrous cycle and disruption of ovarian endocrine function.

Diestrus is the last phase of the cycle where unfertilized eggs are eliminated, the vagina and vulva are at a minimum size, and new follicles begin to undergo a rapid growth for the next ovulation (McLean *et al.*, 2012; Caligioni, 2009). Thus, an extended diestrus may alter the capability of the mice to be fertilized by delaying the estrus phase where the mice are ready for mating. Prolonged diestrus may be a sign of pseudopregnancy, this refers to an endocrine state characteristic of a pregnancy but without implantation end embryonic development (Goldman, *et al.*, 2007). The presence of irregularities in the estrous cycle in both 10% and 20% *T. rumphii* feed replacement implies that it may have substances that interfered with the normal estrous cycle. Results show that ingestion of *T. rumphii* feed replacement has affected the pattern of cycling thus it could alter the fertility of mice.

Inhibition of mating behavior may be due to a specific or a combination of phytochemical present in the extract. Saponins are a special class of glycosides which have soapy characteristics (Banso, 2009). It has also been shown that saponins are active antifungal agents and anti-prolific activity (Sodipo *et al*., 1991).

The biological action of saponins in animal system: a review (Br J Nutr 2002) described saponins as steroid or triterpenoid glycosides, commonly present in a large number of plants and plant products that are important in

human and animal nutrition. Extensive researches were carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals. Saponins were found to have both positive and negative effects on the viability of human sperm cells in vitro. Some ginseng saponins increase motility as well as progression of sperm (Chen *et al.* 1998) while *Sesbania sesban* saponins were found to be spermicidal at 1 0–1 3 mg/ml (Dorsaz *et al*. 1988). Saponin-rich extracts of *Turnera diffusa* and *Pfaffia paniculata* improved the copulatory performance of sexually slow or impotent rats while being ineffective in sexually potent rats (Arletti *et al.* 1999), which the authors attributed to increased central noradrenergic and dopaminergic tone, and possibly oxytocinergic transmission. Researchers found inhibitory effects of dietary Quillaja saponins on mating behavior and egg production in Nile tilapia (Francis *et al*. 2001). Saponins also reportedly affect functioning of the male sexual reproductive behavior (Njiwa *et al*., 2004). The negative effects of saponins on animal reproduction have long been known and have been ascribed to their abortifacient, antizygotic and anti-implantation properties (Tewary *et al*. 1973; Stolzenberg & Parkhurst, 1976).

In summary, alkaloids, tannins and saponins are present in *T. rumphii* that could enhance cytotoxicity capabilities and could affect reproductive function of organisms. *T. rumphii* extract is moderately toxic to brine shrimp, however, its toxicity to lung adenocarcinoma cells is non-conclusive, and therefore, further test is needed. *T. rumphii* feed replacements of 10% and 20% disrupt estrous cycle of white mice (*Mus musculus* Linn.) by prolonging the diestrus stage. The feed replacements had anti-ovulatory effects on mice lengthening and changing to irregular pattern of the estrous cycle. Likewise, extract of *T. rumphii* can inhibit the mating behavior of *Danio rerio.*

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