CYTOLOGICAL EFFECTS OF MEDICINAL PLANT EXTRACTS USING THE *Allium* TEST

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ABSTRACT

Conducted from April to July 2009, this study determined the effect of hot water extracts of medicinal plants *Centella asiatica*, *Plantago major, Sarcandra glabra* and *Smallanthus sonchifolia* on the cells of the *Allium cepa* L. root tips particularly on mitotic index. The study found out the possible cytological effects of the extracts on the occurrence of chromosome abnormalities, and determined the effects of the extracts on the gross morphological parameters of the roots.Onion root tips treated with *C. asiatica, P. major, S. glabra* and *S. sonchifolia*, plant leaf extracts showed high mitotic indices in the metaphase stage, 101.3%, 113.0%, 139.0% and 144.7%, respectively. Cells failed to divide at telophase as proven by mitotic index, 28.0% (*C. asiatica),* and 27.7% (*P. major),* 26.0% (*S. glabra)* and 18.3% (*S. sonchifolia)* respectively compared to the control with 49.3% indicating inhibition of cell division. Likewise, the plant extract-treated onion cells exhibited some abnormalities in the chromosomes such as: c metaphase or c-mitosis in *S. sonchifolia, S. glabra* and *P. major*-treated cells; and anaphase chromosome bridges in *C. asiatica*-treated cells. Extracts from *C. asiatica* and *P. major* showed lower degree of cytotoxicity as compared to that of *S. glabra* and *S. sonchifolia*. Comparing with the normal cell growth and development in the control (distilled water), macroscopic abnormalities were also observed such as root swellings or c-tumor in *C. asiatica*-treated root.

INTRODUCTION

In pursuance of its responsibility to provide adequate and accessible healthcare to the Filipino, the Department of Health together with local government units promotes the use of traditional herbal medicines as alternative care. It is not uncommon to see traditional and modern medicines practice side by side, not only in rural areas but also in urban centers as well.

Then, it is very important that a proof of herbal medicines safety and efficacy must be studied and proven before any plant can be recommended or distributed by the government.

Indeed, many medicinal plants are widely used around the world and to think that they are ordinarily considered as weeds. One "all-cure herb" plant, *Plantago major* L. (plantain), had been used to treat skin diseases and internal infections.

Further, it also helps to relieve patients from pain caused by infections and also used to prevent cancer (Samuelsen, 2000).

Smallanthus sonchifolia (yacon), the 'wonder root crop', is a cure for diabetes, kidney infection, hypertension, obesity, lung problems, constipation, insomnia and arthritis. Studies showed that its leaves, which capsules are made from, have the ability to reduce glucose in the blood and is a great antioxidant. This can also be used in the diet to prevent chronic illnesses such as arteriosclerosis (Knowledgerush, 1999–2003).

Another plant, *Centella asiatica* (gotu kola) referred to as "food for the brain" functions as a tranquilizer and demonstrates anti-anxiety and anti-stress effects thus, improving mental functions such as concentration and memory. It has a calming effect on the body and is chiefly used to support the central nervous system.

The leaves of *C. asiatica* have been used for centuries to treat leprosy, cancer, skin disorders, arthritis, hemorrhoids, and tuberculosis (Picar *et al.*, 2006).

Sarcandra glabra ('gipas') has numerous medicinal uses that include treatment of pneumonia, influenza, acute gastroenteritis, dysentery, appendicitis, post-operative infections, cellulitis, diarrhea, ulcerating wounds, bleeding wounds, scalds, burns, traumatic injuries, bone fractures, rheumatic arthritis, and stomachache. It is also a good source of several B-complex vitamins including B2 and nicotinic acid which helps in the normal blood circulation (Bu-abbas *et al.,* 1996).

Due to the wide usage of these plants and even other herbals, people use them without even of the appropriate dosage. There is a need to determine if extracts of *C. asiatica, P. major, S. glabra* and *S. sonchifolia,* which are commonly consumed as tea have adverse effects on the cells. An assessment of physiological effect most especially cytotoxic and mutagenic potential is necessary to ensure a relatively safe use of medicinal plants (Teixeira *et al*., 2003).

Locale and Time of the Study

The study was conducted at the Department of Biology Research Laboratory, College of Arts and Sciences, Benguet State University, La Trinidad, Benguet from April - July 2009.

Scope and Delimitation of the Study

This study determined the effect of the plant extracts of *C. asiatica, S. sonchifolia, P. major,* and *S. glabra* on the mitotic activity of the cells, appearance of chromosome aberration, chromosome abnormalities in the metaphase and anaphase bridges employing the *Allium* test.

In addition, observation of the overall structure of the *Allium cepa* including number of roots per bulb, root length, color changes in roots, occurrence of abnormal roots like presence of swellings (c-tumors), twists (crochet hook) and broken tips based on one concentration, the concentration which approximates five grams in 100 ml using only the leaves. The control used was distilled water.

Medicinal Uses of Plants

Thousands of plant species growing throughout the world have medicinal uses, containing active constituents that have direct action on the body. They are used both in herbal and conventional medicine, helping to combat illness and other benefits to regain good health (Chevalier, 1997).

Research has indicated that tea has health benefits because of substances called polyphenols that have antioxidant properties. Polyphenols also show anticarcinogenic, antibacterial and antiviral activity. Scientists believe that polyphenols protect lipids from oxidative degeneration and protect skin damage from ultraviolet light. Two types of polyphenols, catechins and the aflavins, contribute to the antioxidant properties of tea (Capriotti, 1999).

Plantago major , a common and noxious weed, had been reported to cure several human illnesses including urinary/kidney maladies. Its leaves have been used in the treatment of a number of diseases such asthma, emphysema, bladder problems, bronchitis, fever, hypertension, rheumatism and blood sugar control apart from wound healing (Samuelson, 2000). The fresh crushed leaves are applied to wounds, sores, insect bites, bee and wasp stings, eczema, and sunburn because of high allantoin content. All plantains contain high amounts of mucilage and tannin, and have similar medicinal properties. Plantain is high in minerals and vitamins C and K (Broughton & Frey, 2005).

Centella asiatica locally known as 'yahong-yahong' or 'gotu kola' is known to local users due to its potential pharmaceutical attributes (Picar *et al.,* 2006). The plant contains saponins, which have diuretic effect that gradually dissolves kidney stones. Recent studies show that it has a positive effect on the circulatory system by improving the blood flow by strengthening the veins and capillaries. It contains asiaticoside, which is a triterpene glycoside and classified as antibiotic.

It aids in wound healing (Sotheeswaran *et al.*, 1998) and treats leprosy as well as tuberculosis. On the other hand, brahmoside and brahminoside, which are saponin glycosides, are diuretic in nature and have a slightly sedative action in large doses. The plant is also a source of vitamin K, magnesium, calcium and sodium (Herbal Information Center, 2000).

Traditionally, *C. asiatica* is consumed as an herbal tea or the leaves are eaten fresh. The presence of terpentoid is also another reason why *C. asiatica* is used as a rejuvenating agent (Ramlan *et al.,* 2003). Cardiac glycosides were also found to be present, a compound that has been shown to aid in treatment of congestive heart failure and cardiac arrhythmia. Dried *Sarcandra glabra* leaves have been utilized as a beverage tea by many Cordillera folks. It was reported also that even its fresh leaves could be used as a beverage whether dried or using fresh shoots or leaves.

The hypoglycemic action of yacon leaves has been demonstrated in a laboratory study with normal and diabetic rats by researchers. A decoction of yacon leaves was reported to produce a significant decrease in blood sugar levels in normal rats when administered by injection or orally. In glucose tolerance test, a single administration of the leaf decoction lowered the plasma glucose levels in normal rats.

Also, the administration of a lower dosage of leaf tea instead of water for 30 days produced a significant hypoglycemic effect in laboratory-induced diabetic rats. The mechanism of action of these leaf extracts were insulin-like in the way they affected glucose metabolism. An *in vivo* study reports that the leaves also have antioxidant actions as well as a liver protective action by reducing free radical damage in rat liver cells caused by alcohol (Raintree Nutrition, 1996).

Preliminary study by Picar *et al.*, (2006) showed that *C. asiatica* significantly decreased the weight of the kidney stones after four months observation. The plant extract decreased the weight of the stones after four months observation.

The plant extract decreased the weight of the stones by 67.3% compared with another alternative medicine, Sambong at 48.51%. The lowest decrease in weight (25%) was obtained from the standard medicine used which is Rowatinex, the synthetic medicine usually prescribed by the doctors to their patients.

A study showed the high anti genotoxic effect exhibited by the aqueous decoction of *P. major* leaves on mice differ significantly from that of the distilled water (negative control), in terms of micronucleated polychromatic and micronucleated normochromatic erythrocytes (Caldecott, 2008).

The *Allium* Test

The *Allium* test is an easy and sensitive tool for the total toxicity caused by chemical treatments expressed by growth inhibition of the roots of onion bulbs (Fiskesjo, 1985). False negatives on the other hand, have been shown to rarely occur in either the *Allium* test or other similar plant tests (Ennerver *et al.*, 1988) therefore, any compound tested giving a negative result can be reliably considered non mutagenic.

The root tip system of onion, *Allium cepa*, is particularly sensitive to the harmful effects of environment contaminants/pollutants in the environment. Gross effect can be quantified by measurement of inhibition of growth of the newly developing root tip system, whereas examination of the chromosomes of the individual cells of the root tip can indicate mutagenic effects (http:archive.idrc.ca/ aquatox/fr/reasouces/allium.html).

Root growth will be inhibited when the roots are exposed to toxic substances, to a wrong pH, or to undissolved substances that may prevent nutrition uptake. The degree of toxicity of the test chemicals or samples to the allium roots is assessed by measuring root length and computing mitotic index per stages. These test samples may either affect the developing *A. cepa* roots macroscopically or microscopically or both parameters.

These characteristics of the *Allium* test on the onion root tip cells is used as the basis for the potential positive and negative effect of the herbal medicinal extracts on humans because Sadia & Vahidy (1994)

stated that among the test systems for toxicity monitoring, the *Allium* test is well known and commonly used in many laboratories as onions are easy to handle, and the root tip cells constitute a convenient system for macroscopic as well as microscopic parameters.

Effects of Chemicals in Mitotic Index

Meristematic cells of *Allium cepa L*. were used as vegetal test system and bone marrow of Wistar rats as animal test system. Both were treated in vivo to evaluate whether the plants: *Averrhoa carambola L*., *Syzygium cumuni (L.)* Skeels and *Cissus sicyoides L*. presented cytotoxic and mutagenic effects and whether they resulted in cell alterations in their morphology, chromosomes or cell cycles division. Herbal teas were prepared as normally done by the population, albeit in two different concentrations, the usual and a concentration ten times higher.

Results showed that teas did not alter the cell cycles of *Allium cepa* L., with the exception of 24 hours analysis after suspension of treatment (recovery of treatments), with a lower concentration of *A. carambola.* The latter had low mitotic index when compared to control and to the post-treatment analysis, showing an inhibition of cell division. The three herbal teas neither induced an increase in the number of chromosomal damage in bone marrow cells of Winstars rats nor altered the cell division cycle (Camparoto *et al.,* 2002).

According to Badr & Ibrahim (1987), decrease of mitotic index level shows that experimental material had mitodepressive effect resulting in the inhibition of cells access to mitosis. It means that boron disturbs the normal cell cycle process by preventing biosynthesis of DNA and/or microtubule formation (Sadia & Vahidy, 1994).

Bu-Abbas *et al.,* (1996) compared the antimutagenic effect of green, black, and decaffeinated teas and stressed that flavanols are the major tea components responsible for this activity. The aqueous extracts form green tea showed a powerful antimutagenic effect in routinely used concentrations in human's daily diet against major classes of occupational carcinogens. Several authors have reported the antimutagenic effect of black tea extract by various compounds such as aflatoxin B1, benzo [a] pyrene, nitrate derivatives and, most recently, the heterocyclic aromatic amines (Wang *et al.,* 1989; Yen and Chen 1994). Tochu (*Eucommia ulmoides*) teas, a popular beverage in Japan are carcinogenic and mutagenic which was attributed to the presence of polyphenolic compounds (*Wang et al.,* 1989).

Horikawa *et al.,* (1994) assessed the activity of six Chinese medicinal herbs on Salmonella and found that tannin and catechin compounds were responsible for the inhibition of mutagenicity cause by benzo[a] pirene.

Bauhinia candicans contains flavonoids (the second most common groups of metabolites in the vegetable kingdom) and their very low toxicity makes them attractive compounds for use as therapeutic agents (Martins *et al.,* 1995). In a study, Ohtsuka *et al.,* (1995) investigated that the antimutagenic compounds were the saponins and the flavonoids in green tea compared with black tea.

MATERIALS AND METHODS

Materials

Extracts from the four commonly-used medicinal plants/herbs namely: *Centella asiatica* (Plate 1), *Plantago major Linn*. (Plate 2), *Sarcandra glabra* (Plate 3) and *Smallanthus sonchifolia (*Plate 4) were used in the *Allium* test.


Plate 1. *Centella asiatica*


Plate 2. *Plantago major*


Plate 3. *Sarcandra glabra*


Plate 4. *Smallanthus sonchifolia*

Methods

Collection of Test Plants and Preparation of Extract. The fresh mature parts of leaves were collected within the vicinity of BSU where the plants abound and cleaned very well of any adhering debris or dirt. Five grams of each plant species was surface-sterilized with 1% NaOCl for 3 minutes, and then repeatedly rinsed with distilled water. The leaves were boiled in 100 ml distilled water then left to cool down prior to *Allium* test bioassay. Distilled water was used as control. Preparation of the crude extract of each four samples was similarly done as was employed by Lirio (2000).

Procurement of Onion Bulbs

Dried onion bulbs of approximately 1.5 centimeters in diameter were bought at the local market. Bulbs with developing leaves and roots were discarded. Using a small, sharp knife, the yellow-brownish outer scales, were removed, carefully leaving the ring of root primordial intact. The brownish bottom plates of the root primordial were also removed.

Bioassay Employing the *Allium* test

Fifteen test tubes were filled with distilled water and onion bulbs were placed on top of each as shown. If the onion bulb would have root length measuring 1 cm long in 48-72 hours then the onion bulbs would be transferred and subjected to the test samples. Three replications per plant extract were prepared. Each extract was prepared by weighing five grams of leaves. This was placed in a beaker with 100 ml of water and then boiled for ten minutes. The liquid was cooled down before it was poured in the test tubes. The test extracts including the control were changed after 24 hours. The change of liquids was repeated after 48 hours (24 hours + 24 hours). After 72 hours, the root tips were retrieved for microscopic examination as described subsequently. Test plant extracts including the control were changed every 24 hours within the 9- day observation to avoid spoiling the liquids.

Examination of the Microscopic Parameters

After 72 hours, four root tips per bulb per set-up were retrieved for microscopic examination. The roots were removed and placed in FEA (acetic acid-ethanol at the ratio 3:1) for fixation in 15 hours. After washing the root tips with distilled water for a few minutes, these were submerged in 1N HCL for 10 minutes and then washed again with distilled water. Four root tips per treatment were squashed and stained with aceto-orcein, covered with a cover slip, then examined under the microscope. More than three hundred cells were observed to score for mitotic index (number of dividing cells over total number of cells observed). The prepared slides for microscopic examination are shown.

Microscopic abnormalities of the study were identified and labeled using the microscopic results from Da Silva *et al.,* (1999), study on "Mitotic Aberrations in Coffee Leaf Extracts and their Derived Embryogenic Calli".

Examination of the Macroscopic Parameters

The replicates from four plant extract samples and the control (distilled water) were gathered after

nine days for macroscopic examination. Parameters such as number of roots per bulb, number of abnormal roots, and changes in color of the roots were appropriately noted. Root lengths were measured every three days within the nine days observations. The first three roots emerge were measured and the means were computed for later statistical analysis. The extracts obtained from the four tests medicinal plants (*C. asiatica, P. major, S. glabra* and *S. sonchifolia*) represent the four treatments and the control was distilled water.

Data Gathered

1. Microscopic parameter which focused on mitotic index. Mitotic index is one way of quantifying cell division. Quantifying properties of cell division is used to compare differences in cell growth among the different cells and in response to environmental variables. This done by dividing the number of cells in mitosis (interphase, prophase, metaphase, anaphase and telophase-I.P.M.A.T) over the total number of cells observed in the microscope field. Total numbers of cells are obtained by adding all the number of cells counted from the different stages.

$$\text{MI (mitotic index)} = \frac{\text{Number of cells in mitosis (I,P,M,A,T)}}{\text{Total number of cells}}$$

2. Mitotic abnormalities were also observed from the different treatments. This included c-metaphase or c-mitosis, anaphase bridging, binucleated cell, stocky anaphase and double metaphase.

3. The macroscopic parameters taken included the occurrence of abnormal roots, presence of twists (crochet hooks), swellings (c-tumors) and broken tips. Furthermore, the color changes in roots per bulb and root length were observed.

Data Analysis

Data gathered were analyzed for their significant correlation compared to the control (distilled water). One-Way analysis of Variance was employed. DMRT at P=0.05 was used to compare treatment means.

Cluster analysis. The Ward's method (Kaufman and Rousseeuw, 1990) was used for clustering the selected medical plants based on their degree of cytotoxicity. A measure of similarity and dissimilarity was computed using the squared Euclidian (Minkowski Distance Metric) formula.

RESULTS AND DISCUSSION

Effect of Medicinal Plants on Cell Division

A rapidly dividing cell population is expected to have a high proportion of cells in the interphase stage. The significantly low percentages of mitotic indices of the onion root tip cells treated in vitro with the different medicinal plant extracts, 32.0%, 30.7%, 29.6%, and 29.0% in *C. asiatica, P. major, S. glabra* and in *S. sonchifolia* respectively, as compared to the high mitotic index of 75.7% in the control (Table 1), clearly indicates the slow cell division as an effect of the plant extracts. This observation was similarly noted in the prophase stage where the control had relatively higher mitotic index (114.3%) as compared to those in plant extract-treated cells.

Further indication of the slowed cell division (Fiskesjo,1988) has the strikingly high mitotic indices in all plant-extract treated onion cells (101.3% in *C. asiatica,* 113.0% in *P. major,* 139.0% in *S. glabra,* 144.7% in *S. sonchifolia*) during the metaphase stage which was significantly different from that of the control (51.0%). This observation would mean the failure of the cell to divide as most cells just remained in the metaphase stage without proceeding to the telophase stage.

Finally, the above data pointing to the slowed cell division were further supported by the remarkably very low mitotic indices (28.0% in *C. asiatica,* 27.7% in *P. major*, 26.0% in *S. glabra,* 18.3% in *S. sonchifolia*) in the telophase stage as compared to the control (49.3%). This result is the opposite of that observed by Walitang and Lirio (2005) where they reported an increase in mitotic activity (high mitotic index) as the concentration of hot water extract from T*araxacum officinale* was increased, which is usually observed in cancerous cells.

Table 1. Mean mitotic index of the mitotic phases of onion root tip treated *in vitro* with different medicinal plant extracts

	MEAN MITOTIC INDEX (%)				
TREATMENTS	Interphase	Prophase	Metaphase	Anaphase	Telophase
Control (Distilled water)	75.7[a]	114.3[a]	51.0[d]	31.7[b]	49.3[a]
Centella asiatica	32.0[b]	90.7[ab]	101.3[c]	57.0[ab]	28.0[b]
Plantago major	30.7[b]	69.0[b]	113.0[bc]	62.7[a]	27.7[b]
Sarcandra glabra	29.7[b]	79.7[ab]	139.0[ab]	44.0[ab]	26.0[b]
Smallanthus sonchifolia	29.0[b]	73.0[ab]	144.7[a]	46.7[ab]	18.3[b]

Means in the same column having the same letter are not significantly different at P=.05 DMRT.

According to Badr and Ibrahim (1987) a decrease of mitotic level showed that medicinal plants had mitodepressive effect resulting to the inhibition of cells access to mitosis. It means that the four treatments disturbed the normal cell cycle process by preventing biosynthesis of DNA and/or microtubule formation (Sadia and Vahidy, 1994). Hence, this effect could be due to decreased ATP level or suppression of the engine energy production (Jain *et al.,* 1988).

These findings may provide support to the beneficial effect derived from the use of the medicinal plants as potential anticancer agents. The different plant extracts have inhibitory effect on the normal cell division, as compared with the vivid normal development of the *Allium cepa* roots observed in the distilled water-treated cells. This maybe is an indication that the four medicinal plants contain compounds that slowed down the development of the cells or they are toxic to the cells. According to Bianchi *et al.,* (2007) several plant extracts were able to inhibit DNA-protein interactions and they may be a possible source of lead compounds that can alter gene expression. Another study reported *P. polystachyum* infusions present cytotoxic and anti-proliferative activity and therefore have therapeutic potential (Canto- Dorow *et al.,* 2006). The data obtained as to the different mean mitotic index of the different mitotic stages manifest a certain pattern among the four different treatments and the control. The application of Ward's method of classifying the test medicinal plants showed that the different treatments including the control mean mitotic index are of three cluster groups (Figure 1). This statistical manner of analyzing data defines the degree of cytotoxicity effect of the different plant extract treatments. Control has a degree of dissimilarity of 15674.58, which obviously belong to one cluster.

Centella asiatica and *Plantago major* exhibit the same cytotoxicity level with a degree of dissimilarity of 642.05 while *Sarcandra glabra* and *Smallanthus sonchifolia* has another cytotoxicity level with a degree of dissimilarity of 144.45.

This dissimilarity measure implies that the two herbal medicines, *C. asiatica* and *P. major* belong to one cluster and have the same cytotoxicity level effect on the *Allium cepa* roots. *S. glabra* and *S. sonchifolia*, on the other hand, belong to another cluster which allows higher degree of cytotoxicity as compared to *C. asiatica* and *P. major* in the same concentration employed in this particular study. These observations on the failure of the cells to divide normally were evident in Figure 2. Dividing onion root cells show the normal interphase, prophase, metaphase, anaphase and telophase stages (Figure 2a) in the distilled water (control) treatment. While these are how the normal cells would appear, the plant extract-treated onion cells were mostly found in the metaphase stage (Figure 2b-d). High metaphase stages during cell division indicate slow rate of cell division (Fiskesjo, 1985). Camparoto *et al.,* (2003) proved strong inhibition of cell division with the higher concentration of *Psidium guajava* L. infusion, while infusions of *Psidium guajava* L. at the lower concentration and of *Achillea millefolium* L. did not cause significant suppression of cell division in the *Allium cepa* root-tip cells, within the 24-hr treatment period.

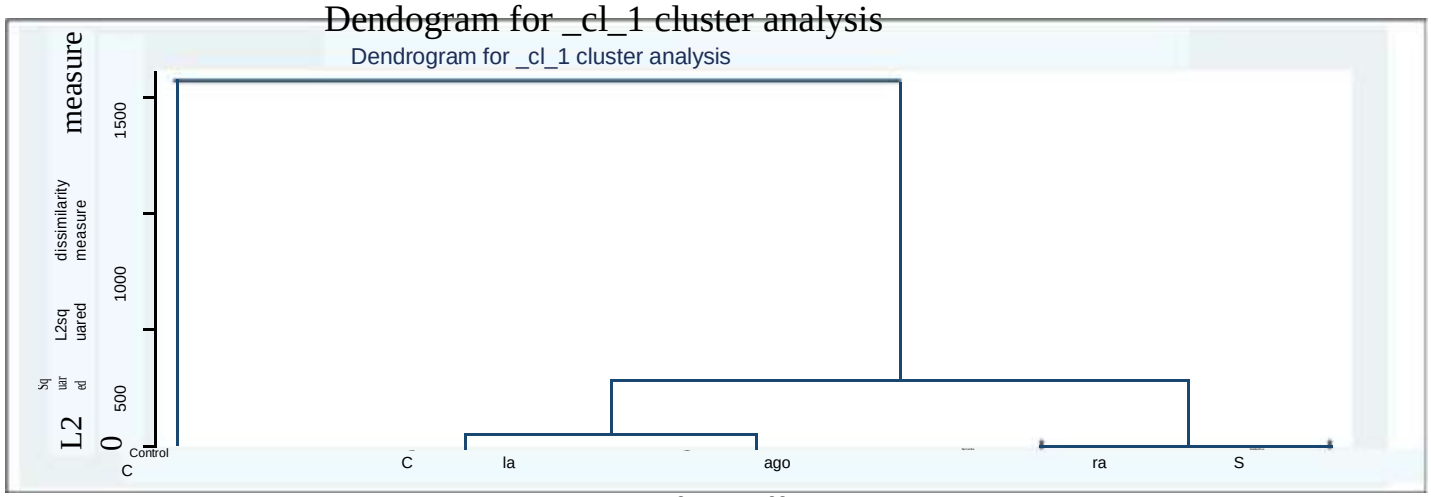
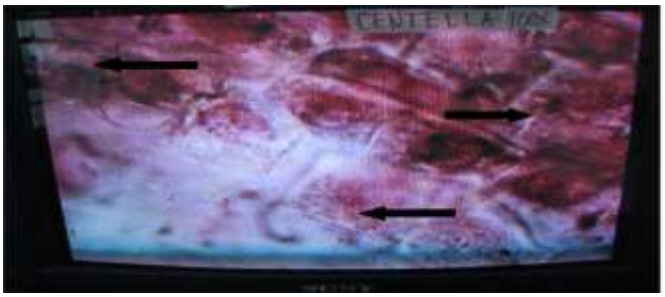
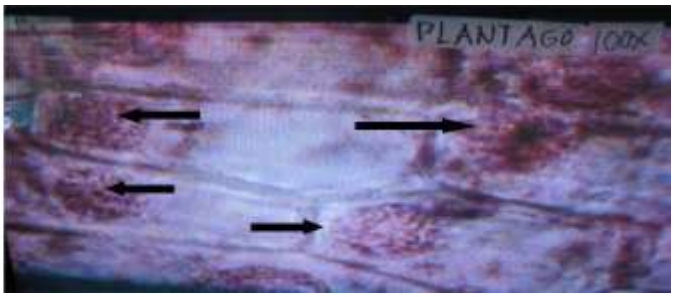


Figure 1. Cluster analysis on the cytotoxicity level of the different plant treatments

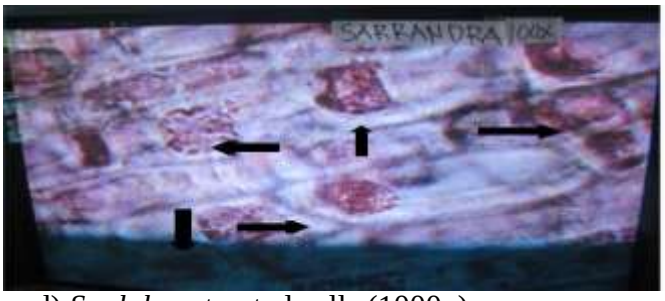
a) Distilled water–treated cells (1000X) showing normal stages in: a. interphase, b. prophase, c. metaphase, d. anaphase, e. telophase



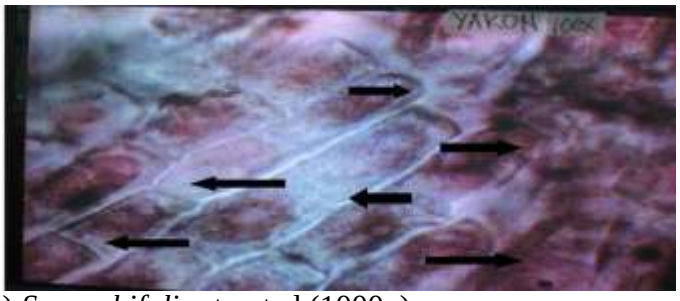
b) *C. asiatica*- treated cells (1000x)



c) *P. major* – treated cells (1000x)



d) *S. glabra*- treated cells (1000x)



e) *S. sonchifolia*- treated (1000x)

Figure 2. The medicinal plant extract-treated onion root cells under metaphase stage as shown by the arrows

Chromosomal Abnormalities Noted on the Mitotic Stages

The plant extract-treated onion cells as seen in Figure 3 exhibited mitotic abnormalities. Mitotic abnormalities such as anaphase chromosome bridges in C. asiatica- treated cells (a); c-metaphase or c-mitosis noted in *P. major, S. glabra* and *S. sonchifolia* extracts-treated onion cells (b). The microtubule cytoskeleton plays a crucial role in the cell cycle and in mitosis. According to Aranez and Rubio (1993), insecticides such as Folidol and Malathion, cause chromosomal abnormalities in *Allium cepa* roots because of the effects of the treatments on DNA or on the microtubules of the spindle fiber.

C-mitosis or C-metaphase is the common abnormality observed in *S. glabra* and *P. major*-treated cells which may have been due to disturbed microtubule by the plant extract contents wherein the cell had a failure to form the achromatic spindle; plant compounds like tannins for example are good enzyme inhibitors and act as antidotes for certain types of alkaloids. Flavonoids, on the other hand are responsible for the antimutagenic of cells, and probably anticarcinogenic (Bu-Abbas *et al.*, 1996); anaphase chromosome bridges happen during the translocation of the unequal chromatid exchange or due to dicentric chromosome presence. In *Allium* test, Pb at a lower and higher concentration exhibit mitodepression and exerted inhibition of prophase and finally resulted in the disappearance of mitosis which is a form of lethal toxicity.

Effects of the Four Plant Extracts on Root Growth and Number of Roots on the 9th Day

Among the macroscopic parameters, the root length (Figure 4) is the most important, but root forms such as presence of twists (crochet hook) or swellings (c-tumors; *C. asiatica*-treated root), broken root tips; color and others, may be useful signs, as well as the length of the green shoots (control). It was observed in *Smallanthus sonchifolia* that some of the roots rotted within the 9- day of observation. Furthermore, it was observed that in *Centella asiatica* and *Sarcandra glabra*, there was a thickening and thinning of roots, respectively, as compared to the size of the normal roots. Root color also was observed where *C. asiatica* treated-roots are white with brown spots, *P. major*-treated roots were dirty white, *S. glabra* was dark brown and *S. sonchifolia* was brownish, while the distilled water (control) showed normal white roots.

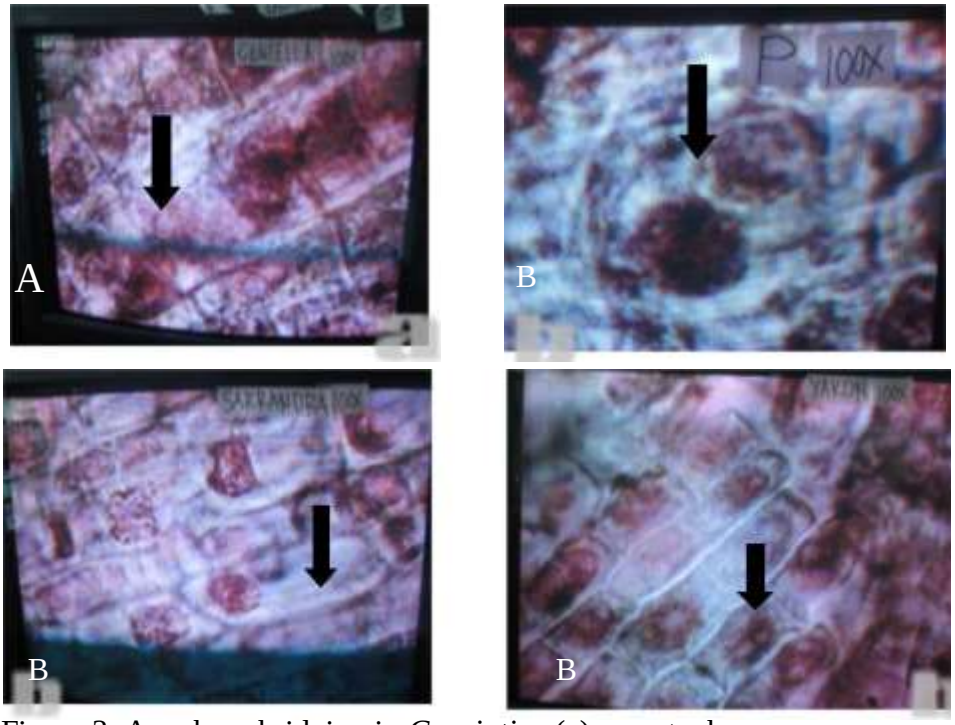
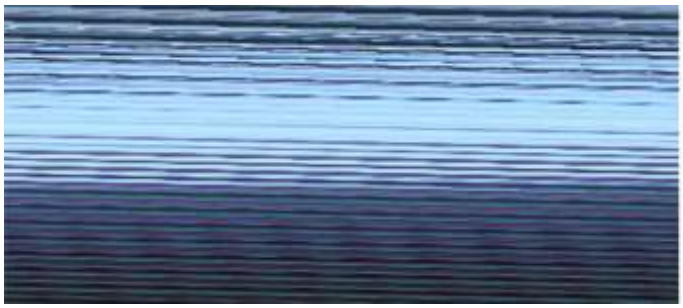
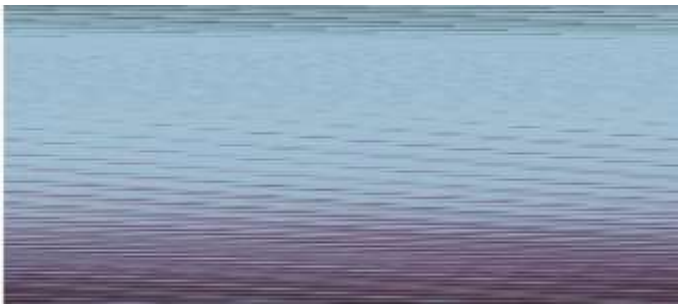


Figure 3. Anaphase bridging in *C. asiatica* (a), c metaphase or c mitosis observed in *P. major, S. glabra* and *S. sonchifolia* (b).

Normal root growth in distilled water (Control)





Centella asiatica- treated roots
Thickened Roots

Sarcandra glabra- treated roots
Thinned Roots

Plantago major- treated roots
Change of Color

Smallanthus sonchifolia-treated roots
Change of Color

Figure 4. Root bulbs of the different treatments taken on the 9th day

Effect on Root Length

Mean root length on the 3rd, 6th and 9th day shows that the control differs significantly from the length measured in all plant-extract treated onion bulbs (Figure 5). Expectedly, root growth was fast in distilled water (control) where it shows the continuous rise and constant increase of the root length with time. The control increased from 1.72 to 4.06 cm; in. *C. asiatica* from 0.26 to 0.6, in *P. majo*r from 0.49 to 0.62, in *S. glabra* from 0.20 to 0.28 and in *S. sonchifolia* from 0.14 to 0.34. Table 2 revealed higher degree of inhibition in root length in the *S. glabr*a and *S. sonchifolia* treated extracts (95.21% and 94.19% inhibition) as compared to 89.74 and 89.40% inhibition in *C. asiatica* and *P. major*, respectively.

The plant extracts evidently inhibited root growth. Coutts et al. (2003) proved that alfalfa extracts reduced root elongation of *Allium cepa* at rate by up to 90% due to the toxicity factors. Inhibition was slowed mainly by osmotic factors whereas reduced root elongation was due mainly to toxic factors of the leaf extract. This is strongly supported by root decoction of *Mimosa pudica* proven to be effective in large doses towards breast cancer cell (Bojo *et al.*, 2005).

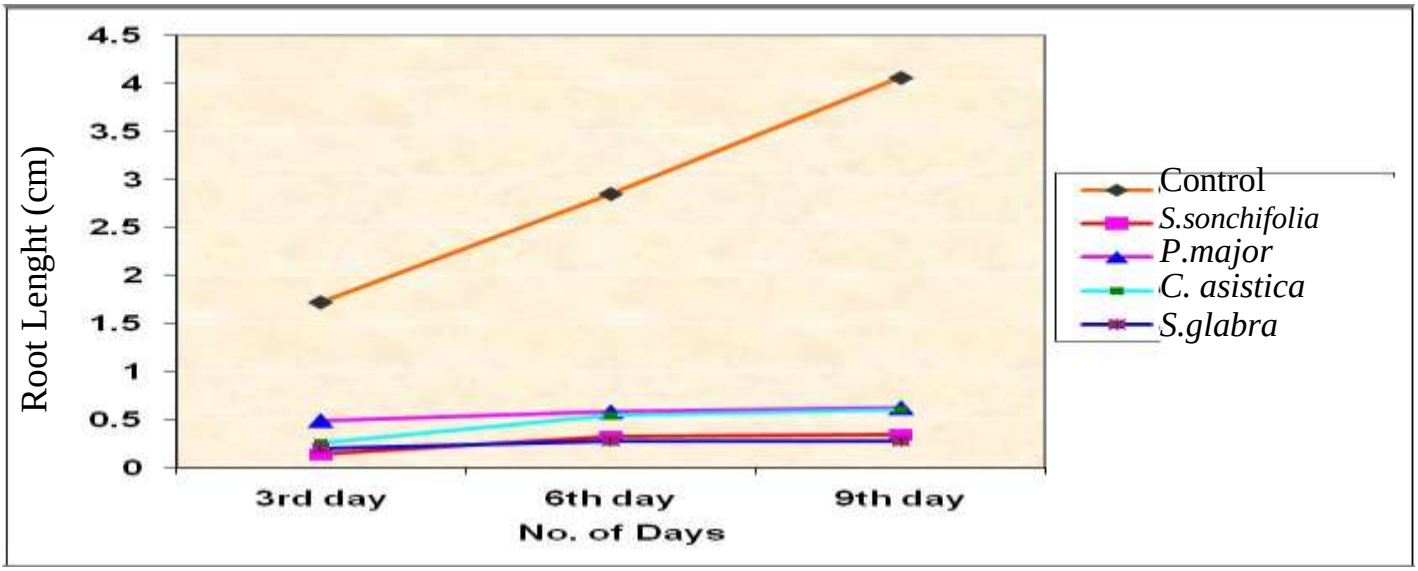


Figure 5. Mean root length of *A. cepa* within the 9-days observation period in the plant extract-treated and in distilled-water treated bulbs

Table 2. Mean length of roots (cm) on the 9th day

TREATMENTEATMENTS	MEAN ROOT LENGTH (9^{TH} DAY)	COMPUTED PERCENT INHIBITION*
Distilled water	5.85^{a}	-
Centella asiatica	0.60^{b}	89.74^{b}
Plantago major	0.62^{b}	89.40^{b}
Sarcandra glabra	0.28^{b}	95.21^{b}
Smallanthus sonchifolia	0.34^{b}	94.19^{b}

Means with the same letters within the column are not significantly different by DMRT (P = 0.05).

* $\frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$ % Inhibition

Effect on the Number of Roots

Computed percent inhibition (Table 3) on the effect on the number of roots showed that the four treatments allowed roots to emerge but slowed growth when exposed in a longer duration of time. The observation is explained by the study of Chon (2002). He mentioned that in the extract medium, longitudinal growth of the embryonic root cells had been exhausted, cell division was not fully activated, and the cell supply was limiting growth, likely due to disruption of mitosis by the components of the plants that stops elongation of the root. This response would allow the bulb to survive for a few days or weeks in a weakened condition and then either die or form root branches behind the tip to exist as a less thrifty autoconditioned plant (Jennings and Nelson, 2002). The roots in the four extract treatments showed poor development as compared to the control. However, among the four plants, growth was relatively faster in *C. asiatica* and in *P. major* extracts since there was no inhibition in root number, -24.93% and -24.93%, respectively as shown in Table 3. This observation conforms to the lower degree of cytotoxicity noted in these plant extracts, as mentioned earlier. This would imply safer use of these medicinal tea plants.

The biological activity of plant extracts is due to the various phytotoxic compounds present in the extracts. These compounds may independently or jointly contribute to cause plant growth regulatory effect and inhibit germination.

Decoction of the plant leaves caused a decrease in cellular division in root- tip cells compared with non-treated control. This significant inhibitory cytotoxic effect was seen and observed macroscopically and microscopically. This cytotoxicy was likewise observed by Camparoto *et al.,* (2002) in their study, "Effects of *Maytenus ilicifolia* Mart. and *Bauhinia candicans* Benth infusions on onion root-tip and rat bone marrow cells". He reported however, that this process could be reversible because slight recovery in cell division was observed after 24 hours in water. It is possible that a high concentration of any chemical will have an effect (either inhibitory or stimulatory) on the cell cycle, which is probably also true in this study. They found that the principal components of the leaf extracts are catechins, which seem to be responsible for antimutagenic activity, which varied from 4.3% in black tea to 26.7% for green tea.

Green tea is produced from non-fermented *Thea sinensis* leaves (the most popular beverage in the orient) used in Japan as an antipyretic, diuretic and antioxidant, showed to have antimutagenic and antitumor effects *in vitro* and *in vivo* (Wang *et al.,* 1989).

It has also been reported that the mortality rate due to human cancers in areas of tea cultivation is significantly lower than in areas where tea is not grown (Sasaki,1993), and that the catechin present in green tea suppresses the action of many environmental mutagens (Nakamura *et al.,* 1997).

Table 3. Mean number of roots counted on the 9th day of observation

TREATMENTTMENTS	MEAN NUMBER OF ROOTS	COMPUTED PERCENT INHIBITION*
Distilled water	10.67^{ab}	-
Centella asiatica	13.33^{a}	-24.93^{a}
Plantago major	13.33^{a}	-24.93^{a}
Sarcandra glabra	8.33^{ab}	21.93^{ab}
Smallanthus sonchifolia	4.33^{b}	59.42^{ab}

Means with the same letters within the column are not significantly different by DMRT (P = 0.05).

* % Inhibition = $\frac{\text{Control – Treated x 100}}{\text{Control}}$

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This study provided insights on the safe use of the selected commonly-used medicinal plants extracts from *C. asiatica*, *P. major*, *S. glabra* and *S. sonchifolia* on the cells of the *Allium cepa* L. root tips on the following: micro parameters particularly mitotic index, possible cytotoxic effects on the occurrence of chromosome aberrations, and on the macro parameters such as number of onion roots per bulb, root length, changes in root color and occurrence of abnormal roots like presence of swellings (c-tumors), twists (crochet hook) and broken root tips.

1. The significantly lower mitotic indices in inter-phase, prophase and telophase in the treatments using extracts from *C. asiatica, P. major, S. glabra* and *S. sonchifolia* as compared with the control (distilled water) indicated slowed cell division.

2. Several chromosome abnormalities were observed such as C-mitosis or c metaphase in *S. glabra, S. sonchifolia* and *P. major* and anaphase chromosome bridges in *C. asiatica*. Expectedly, onion roots in the control showed normal cell development.

3. While the control exhibited the fastest root growth as indicated by the continuous rise in the root length, the four extract treatments showed poor and much slowed development. Rotting of some roots on the 9_{th} day observation was seen in *S. sonchifolia*.

Conclusions

1. The four plant extract treatments inhibit cell division apparently due to the disturbance of the normal cell cycle process which could be assumed in preventing biosynthesis of DNA and/ or microtubule formation which may support the function of medicinal plant extract specifically as an alternative anticancer agent.

2. Four plant extract treatments allow root emergence but root length increase shows slow development.

   This is a proof that the four medicinal plants are effective in inhibiting normal cell division and slows down cell development or either stops it.

3. The onion root tips exhibited mitotic abnormalities when soaked in the plant extracts: C -metaphase or C - mitosis was observed in *P. major*, *S. glabra* and *S. sonchifolia* and anaphase bridging was observed in *C. asiatica*. These are the stages that were affected proving slow cell division. *S. glabra* and *S. sonchifolia* show that both have higher cytotoxicity on the A. cepa roots as compared to *P. major* and *C. asiatica.*

Recommendations

1. A further study using different concentrations of the different plant extracts and prolonged day of observation should be conducted.

2. Related study on other herbal medicines that are commonly-used in the Philippines especially here in the Cordillera should be done.

3. There is a need to study the different commercially prepared teas sold in the supermarkets.

4. In addition, plant extracts must be tested in vivo also on experimental animals for verification.

5. Another method of determining toxicity of the plant extracts may be conducted, like the brine shrimp assay.

Finally, it is recommended that, while tea preparations are taken in the simple manner of plain decoctions, its prolonged use may not be good. Medicinal plants should not be used indiscriminately, as even ginger tea thought to be safe and already widely used, was likewise to be consumed at a certain dosage in a day.

LITERATURE CITED

ARANEZ, A. and R. RUBIO. 1993. Genotoxicity of Two Organophosphate Insecticides Based on Allium Test. Institute of Biology.

BADR, A. and A. IBRAHIM. 1987. Effect of Herbicide Glean on Mitosis, Chromosomes an Nucleic Acids in *Alium cepa* and *Vicia faba* Root Meristems. *Cytologia*.

BIANCHI, N., I. LAMPRONTI, M.T.H. KHAN, M. BORGATTI and R. GAMBARIL. 2007. Inhibitory Effects of Bangladeshi Medicinal Plant Extracts on Interactions Between Transcription Factors and Target DNA Sequences. eCAM 2008;5(3)303–312. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2529391/pdf/nem042.pdf.

BOJO, Z. et al. 2005. C ytotoxicity of *Mimosa pudica* Linn. 20th Philippine Chemistry Congress, Baguio Country Club, Baguio City. P.98.

BOUGHTON, B and R. FREY. 2005. "Naturopathic Medicine." Gale Encyclopedia of Alternative Medicine. 2005.Encyclopedia.com. Retrieved at <http://www.encyclopedia.com>.

BU-ABBAS, A., X. NUNEZ, M.N CLIFFORD, R. WALKER and C. IOANNIDES. 1996. A Comparison of the Antimutagenic Potential of Green, Black and Decaffeined Teas: Contribution of Flavanols to the Antimutagenic Effect. Mutagenesis, Oxford.

CALDECOTT, T. 2008. Plantain. Home Publication Western Herbs.

CAMPAROTO, M. et al. 2 0 0 2. E ffects of *Maytenus ilicifolia* Mart. and *Bauhinia candicans* [Benth] Infusios on Onion Root-Tip and Rat Bone-Marrow Cells.

CANTO-DOROW, T., M.F. KNOLL., A.C.F. DA SILVA and S.B. TEDESCO. 2006. Effects of *Pterocaulon polystachyum* DC. (Asteraceae) on Onion (*Allium cepa*) Root-Tip Cells. Genet. Mol. Biol. vol.29 no.3

CAPRIOTTI, T. 1999. Exploring the "herbal jungle" Med Surg Nursing.

CHEVALIER, A. 1997. Encyclopedia of medicinal plants: A Practical Guide over 550 key herbs and their medicinal uses. London Dorling Kindersley ltd.

CHON, S. 2002. Effects of Alfalfa Leaf Extracts and Phenolic Allelochemicals on Early Seedling Growth and Root Morphology of Alfalfa and Barnyard Grass.

COUTTS, J. CHON and C. NELSON. 2003. Osmotic and Autotoxic Effects of Leaf Extracts on Germination a n d Seedling Growth of Alfalfa Dep. of Agron., Univ. of Missouri, Columbia, MO 65211.

DA SILVA, R.F., A. MENDEZ-YUFFA, L. RIOS and N.X. DE ENRECH. 1999. Mitotic aberrations in coffee (*Coffea arabica* cv. 'Catimor') leaf explants and their derived embryonic calli.

ENNERVER, R. et al. 1988. Genotoxicity testing of quizalofor-p-ethyl herbicide using the *Allium cepa* anaphase-telophase chromosome aberration assay. Volume 61.

FISKEJO, G. 1985. *Allium* Test 1:A2-3 Day Plant Test for Toxicity Assessment by Measuring the Mean Root Growth of Onions (*Allium cepa*).

HORIKAWA, K., T. MOHRI, Y. TANAKA and H. TOKIWA. 1994. Moderate inhibition of mutagenicity and carcinogenicity of benzo[a]pyrene,1,6-dinitropyreneand3,9-dinitrofluoranthene by Chinese medicinal herbs. Mutagenesis. (6):523-6.

JAIN, A.K., K. SHIMOI, Y. NAKAMURA, T. KADA, Y. HARA and I. TOMOTA. 1989. C r u d e Tea Extracts Decrease the Mutagenic Activity of N-methyl-N'-nitro-N-nitrosoguanidine *in vitro* and in Intragastric Tract of Rats. Mutation Research. 210, 1-8.

JENNINGS, J. and C. NELSON . 2002. Zone of Autotoxic Influence Around Established Alfalfa Plants.

KAUFMAN, L. and P. ROUSSEEUW. 1990. Finding Groups in Data. New York:Wiley.

KNOWLEDGERUSH, 1999-2003.

LIRIO, L. 2000. Antibacterial activity of *Piper betle* Linn a n d identification of hydroxychavicol. Ph.D. Dissertation. Ghent Univ. Ghent, Belgium.

MARTINS, E.R., and R.H.S. SANTOS et al. 1995. *Plantas medicinais*: uma alternativa terapeutica de baixo custo. Vicosa:Imprensa Universitaria da Universidade Federal de Vicosa.

NAKAMURA, T., Y. NAKAZAWA, S. ONIZUKA, S. SATOH, A. CHIBA, K. SEKIHASHI, A. MIURA, N. YASUGAHIRA and Y.F. SASAKI. 1997. Antimutagenicity Of Tochu Tea (An Aqueous Extract of *Eucommia Ulmoides* Leaves): 1. The Clastogen-Suppressing Effects of Tochu Tea in Cho Cells and Mice. Mutat Res. 388(1):7-20.

OHTSUKA, M. et al. 1995. Effects of the nine active ingredients in chinese herbal medicine sho-saiko-to on 2-(2-furyl)-3-(5-nitro-2-fury) acrylamide mutagenicity. Japanese Journal of Cancer Research.

PICAR J., V. BALAYO and A. RIVAS. 2006. Efficacy of Gotu Kola as a Kidney Stone Solvent: Preliminary Study. Davao del Norte State College, New Visayas, Panabo City.

RAMLAN, M., V. BALASUBRAMANIAM, S.S. HASSAN, A.R. OMAR, M. MOHAMED, S.M. NOOR and I. OTHMAN. 2003. Cellular transcripts regulated during infections with Highly Pathogenic H5N1 Avian Influenza virus in 3 host systems.Bio Med Central.

RAINTREE NUTRITION, 1996. Method for Estimation of Tannin in Grain Sorghum.

SADIA, K. and A. VAHIDY. 1994. Cytotoxic Effect of Herbicide Ronstar on Meristamic Cells of *Allium cepa*, L. *Pak. J. Bot*.

SAMUELSEN, A. 2000. The Traditional Uses, Chemical Constituents, and Biological Activities of Plantago major. Volume 71. Pp1-21

SASAKI, Y. 1993. The Clastogen-Suppressing Effects of Green Tea, Po-lei Tea and Rooibos Tea in CHO Cells A Mice. Mutation Research.

SOTHEESWAAM, s. et al. 2008. Studies on phytochemical constituents of six Malaysian medicinal plants. Journal of Medicinal Plants Research Vol. 3(2), Pp. 067-072.

TEIXEIRA, R., M.L. CAMPAROTO, M.S. MATOVANI, V.E. VICENTINIL. 2003. Assessment of Two Medicinal Plants *Psidium guajava* L. and *Achillea millefolium* L., in *in vitro* and *in vivo* Assays. Genet. Mol. Biol. vol.26 no.4.

TEIXEIRA, R. et al. 2003. Hepatocytes Detoxify *Atunaracemosa* Extract. Society for Experimental Biology and Medicine.

WALITANG, D. and L. LIRIO. 2005. Physiological Effects of *Taraxacum officianale* Hot Water Crude Extract Using *Allium* Test. BSU Research Journal No. 47. P. 90.

WANG, Z-Y. et al. 1989. Protection Against Polycyclic Aromatic Hydrocarbon-Induced Skin Tumor Initiation in Mice by Green Tea Polyphenols.

YEN, G-C and H-Y. CHEN 1994. Comparison of Antimutagenic Effect of Various Tea Extracts (Green, Oolong, Poucho ng and Black Tea). Journal of Food Protection.