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# Variability in the Distribution of Daidzein and Genistein in Legume Sprouts and Their Anticancer Activity with MCF-7 Breast Cancer Cells

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**Abstract:** The possible growth inhibitory properties of isoflavones extract of legume sprouts on anticancer activity was measured in human breast cancer cells (MCF-7) using MTT assay and compared with isoflavones of soy beans sprouts in vitro. HPLC analysis of legume sprouts revealed the presence of daidzein and genistein as the predominant isoflavones in *Cajanus cajan* (Pigeon pea), *Cicer arietinum* (Chick pea), *Glycine max* (Soybean), *Macrotyloma uniflorum* (Horse gram), *Lens culinaris* (Lentil) and *Pisum sativum* (Garden pea). The most abundant isoflavone in the legume sprouts was found as genistein followed by daidzein. MTT assay demonstrated the dose dependent inhibition activities of daidzein and genistein on MCF-7 breast cancer cells. Lower concentrations of the extract did not exert a significant effect on cell growth inhibition and cell proliferation inhibition was more at higher concentrations of the extracts. *G. max* has recorded significant $IC_{50}$ value against MCF-7 breast cancer cells followed by *M. uniflorum* and *C. arietinum*. The results depicted the use of other legumes especially *M. uniflorum* and *C. arietinum* at their sprouts stage for preventing breast cancer apart from the commonly used *G. max* products.

**Key words:** Daidzein · Genistein · Isoflavones · Breast Cancer · Legume Sprouts

## INTRODUCTION

Breast cancer is still an important public health problem though intensive treatment has improved disease free survival and overall of survival of patients [1]. Estrogens are recognized as the main hormones that stimulate the growth and development of breast cancers [2, 3]. Diet is a primary contributing factor in the prevalence of breast carcinoma and high consumption of phytoestrogens is associated with lower incidence of breast cancer [4-6]. Legumes play an important role in many regions of the world as traditional diets [7] and are a source of phytoesterogens especially isoflavones [8, 9].

Phytoestrogens are plant substances that can mimic or modulate the actions of endogenous estrogens [10]. Phytoesterogens are produced by a wide variety of plants and are having anticancer activities [11, 12]. Among these compounds, isoflavones with a similar chemical structure to estrogen which binds to both estrogen receptors alpha and beta that makes them important compounds in biological systems [13, 14].

Soy beans are the major source of isoflavones and numerous studies have been reported about their nutritional and health benefits. However, little work has been done on anticancer activity of other leguminous plants against breast cancer cell lines especially at their sprouting stage. To better elucidate the effect of isoflavones on breast cancer, this study was carried by determining the in vitro anticancer activities of daidzein and genistein from legume sprouts against MCF-7 breast cancer cells.

## MATERIALS AND METHODS

**Germination Method:** Legume sprouts used in the study were *Cajanus cajan* (Pigeon pea), *Cicer arietinum* (Chick pea), *Glycine max* (Soybean), *Lens culinaris* (Lentil), *Macrotyloma uniflorum* (Horse gram) and *Pisum sativum* (garden pea). Seeds were soaked in water for 4 hours and transferred to a plastic container with several perforations at bottom placed in a chamber at 20°C with 80% humidity. Water was sprayed about 1 L $min^{-1}$ for 5 mins every 4 hours using a pump connected to a nozzle above the container.

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**Extraction of Isoflavones:** Legume sprouts were ground to a fine paste and subjected to extraction. It was mixed with 100 ml of hexane (1:10; w/v) in a flask and shaken at room temperature for 1 hr. The solution was centrifuged at 5000 rpm for 10 min and the supernatant was removed. The residue was further extracted successively with hexane three times. The residue was collected and the solvent was evaporated in fume hood. The defatted residue was extracted with 80% methanol (1:5; w/v) by shaking for 2 hrs for 4 hours at 25°C. The mixture was centrifuged at 6000 rpm for 20 mins at 25°C and the supernatant was concentrated in a rotary vacuum evaporator.

**HPLC Analysis of Daidzein and Genistein:** HPLC analysis was performed using 1260 infinity series LC system installed with a G1311C pump, a G1329B auto sampler and a G13166 column compartment and G4212B DAD detector. Chromatographic separation was carried out on a Zorbax Eclipse Plus C18 Analytical column (4.6 × 150 mm, 5 µ; Agilent Technologies). The mobile phase used was 0.05 M citric acid: acetonitrile (70:30) with the flow rate of 0.6 ml/min in column at ambient temperature with infusion volume of 20 µl in each experiment. Injection volume was 20 µl and detection was by UV absorbance at 254 nm. Stock standard solutions were prepared by accurately adding 20 mg each of daidzein and genistein to methanol in 50 ml volumetric flask, sonicated and made up to final volume. Working standards were prepared by diluting 5 ml of stock to 50 ml of diluent (Methanol: water-50:50) and filtered through 0.22 µm filter. Determination of detection and quantitation limits.

Both the detection and quantitation limit of daidzein and genistein standards were determined through calibration curves obtained by plotting concentration against area. They were also calculated by the standard deviation and the slope of the calibration curve and the values were compared with those obtained by the response of the diluted. The optimized method was validated according to ICH guidelines for the validation of analytical methods [15].

**Statistical Analysis:** Experiments were repeated thrice and statistical analyses were conducted using ANOVA and t-test. Individual peak areas and the total areas were compared with each other using least significant diffierence (LSD) method to determine any significant diffierence ($p < 0.05$) among them.

**Cell Culture Conditions:** MCF-7 cells were (ATCC) cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), BME and MEM amino acids, L-glutamine, sodium pyruvate, penicillin-streptomycin and porcine insulin ($10^{-8}$M) (Sigma). The culture flasks were maintained in a cell incubator at a humidified atmosphere of 5% $CO_2$ at 37°C.

**MTT Assay:** The daidzein and genistein fractions from HPLC were tested for in vitro cytotoxicity using MCF-7 cells by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Briefly, different concentrations of the extracts were incubated in Dulbecco's modified eagle medium (DMEM) without fetal bovine serum (FBS) and antibiotics for 24 hrs. The cultured MCF-7 cells were harvested by trypsinization and plated into the different extracts at a density of 5 x $10^4$ cells/well followed by incubation at 37°C in a humidified 5% $CO_2$ incubator for 24 h. Medium was removed by gentle inversion of the microplate and the cells were washed with PBS prior to the incubation with 100 µL (5 µg/well) of MTT reagent for 3-4 hrs at 37°C. After incubation, the media with MTT reagent was removed from the wells and DMSO (100 µL) was added to rapidly solubilize the formazan. The absorbance for each well was measured at 590 nm in a microtitre plate reader (Tecan Infinite F500) and the percentage inhibition was calculated using the formula.

$$Inhibition\ (\%) = \frac{(ControlA_{590} - SampleA_{590})}{ControlA_{590}} x100$$

## RESULTS

Identification of isoflavones extracted from leguminous sprouts were analysed by HPLC and identified by comparing retention times and UV absorption spectra with the standards analysed by the same procedure. From the HPLC analysis, daidzein and genistein were found as the predominant isoflavones along with other minor peaks. The daidzein content of different leguminous sprout extracts were in range from 34 to 321 µg $g^{-1}$ of sprouts where as genistein content was from 40 to 340 µg $g^{-1}$ (Fig. 1). Variations in the isoflavone contents were observed with the sprout extracts and amongst the extracts, *G. max* has the highest genistein (340 µg $g^{-1}$) and daidzein (321 µg $g^{-1}$) content followed by *M. uniflorum* (276 µg $g^{-1}$ and

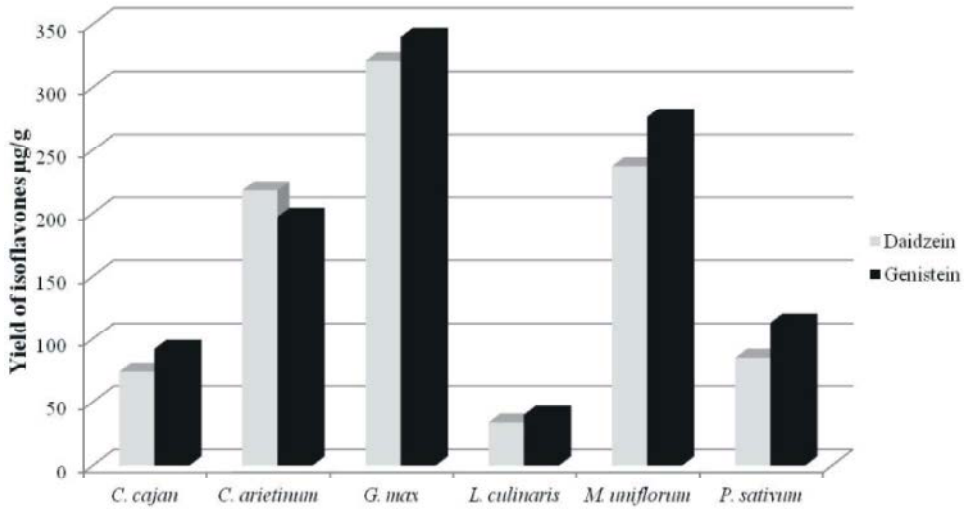


Fig. 1: Daidzein and genistein content of legume sprouts

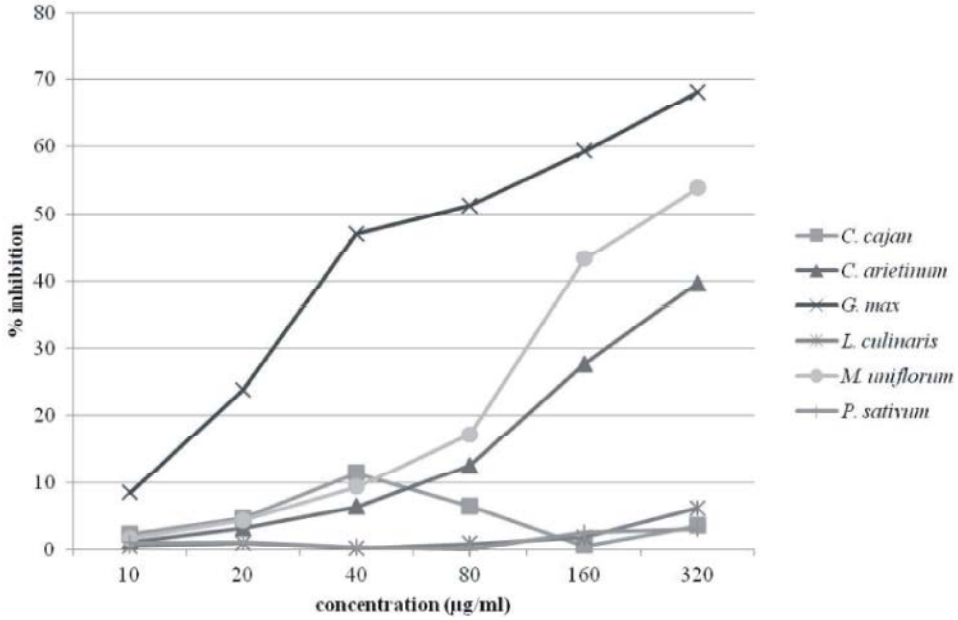


Fig. 2: MTT assay of isoflavone extracts from legume sprouts against MCF-7

237 µg $g^{-1}$), *C. arietinum* (197 µg $g^{-1}$ and 218 µg $g^{-1}$) and *P. sativum* (113 µg $g^{-1}$ and 85 µg $g^{-1}$). The other two sprouts namely *C. cajan* and *L. culinaris* have lesser amount of isoflavones when compared to other sprouts. In general, higher levels of daidzein were present in sprouts except in *C. arietinum* and *C. cajan* where there was a 9.63% and 19.6% increase over genistein level.

The effect of anticancer activity from isoflavones on MCF-7 was evaluated through micro-culture tetrazolium assay (MTT). The multiple concentrations of isoflavones extracts from leguminous sprouts were used and effective doses were calculated from dose-response curve. Results of the cytotoxicity evaluation against MCF-7 cell line of the legume sprout extracts are shown in Fig. 2. Various concentrations stating from 10, 20, 40, 80, 160 and 360 µg $ml^{-1}$ were used in cell inhibition assay and the treatment resulted in a dose dependent inhibition of cell growth. Lower concentrations of the extract did not exert a significant effect on cell growth inhibition and cell proliferation inhibition was more at higher concentrations of the extracts. *G. max* has recorded significant inhibitory activity against MCF-7 breast cancer cells with an $IC_{50}$ value of 150.9 µg $ml^{-1}$ followed by *M. uniflorum* (266.36 µg $ml^{-1}$) and *C. arietinum* (379.55 µg $ml^{-1}$).

However poor inhibition was seen with *P. sativum, C. cajan* and *L. culinaris* with a maximum percent inhibition of 6.1%, 3.55% and 3.01% at highest concentrations.

## DISCUSSION

Isoflavones in aglycone forms are essential for the absorption in the digestive tract and daidzein, glycitein and genistein are the aglycones. To maximize the yields of isoflavones and minimize the production cost, the extracts were analyzed by HPLC. Using HPLC to collect fractions, the active components in leguminous sprout extract were determined to be the isoflavones daidzein and genistein. It can be also noticed that the most abundant isoflavone in the legume sprouts was genistein followed by daidzein. Genistein is commonly known as phytoestrogen, which targets estrogen- and androgen-mediated signalling pathways in the processes of carcinogenesis [16].

Isoflavones could significantly inhibit the growth and induce the apoptosis of MCF-7 cells [17, 18]. The anti-proliferation action of isoflavones has been studied with a number of cancer cell lines and many mechanisms have been postulated such as topo-isomerase II inhibition [19], induction of differentiation [20], inhibition of angiogenesis [21] and inhibition of protein tyrosine kinase activity [22].

Daidzein and genistein are found in species of the Fabaceae family [23]. Generally, leguminous sprouts contain higher concentrations of isoflavones especially daidzein and genistein than the seeds [24] and this study focused on sprouts of legumes. Genistein has been reported as potent MCF-7 cells growth inhibitor [25] and daidzein exhibited a dose-dependent inhibition of MCF-7 cell growth [26, 27]. We evaluated the combined effect of daidzein and genistein in the MCF-7 breast cancer cells and genistein had higher cytotoxicity than daidzein in our study. Isoflavones are important components in chickpea seeds and sprouts [28] and the presence of genistein and daidzein in chick pea and horsegram were reported [29]. Previous studies reported the presence of more isoflavones in sprouts of chickpea [30]. Isoflavones from *Cicer arietinum* has inhibited cell proliferation of MCF-7 cell growth [31] but in our study, significant inhibition of MCF-7 breast cancer cells by *M. uniflorum* isoflavones was observed.

## CONCLUSION

These results contributed to the understanding of the relationships between isoflavones and anticancer activities of year-round leguminous sprouts for their effective utilization as functional food ingredients. Further, it depicts the use of other legumes especially *M. uniflorum* and *C. arietinum* at their sprouts stage for preventing breast cancer apart from the commonly used *G. max* products.

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