



Pharmacokinetic and Phytochemical Appraisal of *Trigonella foenum* and *Andrographis lineata* in Dimethylnitrosamine Induced Injury in Albino Rats

**Sara Zahid^{1*}, Asma Ahmed¹, Hasan Akbar Khan¹, Fatima Zahid²,
Zeeshan Akbar³, Hafsa Waheed¹ and Ambreen Abbas¹**

¹Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan.

²Department of Pharmacy, The University of Lahore, Islamabad, Pakistan.

³Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i46B32917

Editor(s):

(1) Dr. Juan Carlos Troiano, University of Buenos Aires, Argentina.

Reviewers:

(1) Prashant V. Ajmire, Dr. Rajendra Gode IOP, India.

(2) Ioana Stanciu, University of Bucharest, Romania.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/75341>

Received 02 August 2021

Accepted 09 October 2021

Published 18 October 2021

Original Research Article

ABSTRACT

Trigonelline is a plant alkaloid and Andrographolide is a diterpene lactone, both exhibiting anti-inflammatory, antioxidant and neuroprotective activities. The present study was designed to evaluate the antioxidative and anti-inflammatory activity of the above said compounds in Dimethylnitrosamine induced toxicity in albino rats. Extraction of *Trigonella foenum* and *Andrographis lineata* was carried out by using methanol, petroleum ether, ethyl acetate and ethanol assisted with suitable temperature, followed by DPPH scavenging activity (IC₅₀) of these extracts. C_{max}, T_{max}, t_{1/2}, CL, V_d and AUC were evaluated as pharmacokinetic parameters by using calibration curves of Andrographolide and Trigonelline. Extracts of *T. foenum-graecum* and *A. lineata* have antioxidant activity by inhibiting DPPH (IC₅₀ value was 69.04±3.65% and 71.76±6.99%, respectively) comparable with ascorbic acid (53.99±4.88%). Phytochemical analysis of *T. foenum-graecum* and *A. lineata* was found with maximum number of phenols in them, with least recovery in the ethyl acetate extract while maximum phenols were found in

*Corresponding author: E-mail: sarazahid61@yahoo.com, sara.zahid@imbb.uol.edu.pk;

T. foenum graecum. All the pharmacokinetic parameters of trigonelline and andrographolide administered in low dose (200 mg/kg and 50 mg/kg respectively) confirmed the better antioxidative activity than that of ascorbic acid used as a potent antioxidant.

Keywords: Antioxidant; bioavailability; secondary metabolites; therapeutic activity; scavenging potential; ascorbic acid.

1. INTRODUCTION

The genomic technology has ability to give insight into the investigations in altering the gene expression caused by acute effect with Dimethylnitrosamine. Its acute administration in rats exhibits overall significant changes in the profile of gene expression [1]. The most considerable changes were examined and were related to genes involving DNA damage, stress, cell proliferation, and alteration in metabolic enzymes [2]. The gene expression profiles have catalogued the molecular responses to acute Dimethylnitrosamine toxicity and revealed the genetic basis of hepatic toxicity [3]. Nature has always been explored by mankind since ancient times and plants have always been explored in search of new drugs [4]. Plants secondary metabolites have medicinal values and show therapeutic potential like anti-viral, anti-inflammatory and immune-modulatory effects on hepatocytes proved to be crucial in chronic hepatitis [5]. *Trigonella Foenum-Graecum* (Trigonelline) has antioxidative, anti-lipidemic, anti-fibrotic, anti-inflammatory, membrane stabilizing, immunomodulatory and liver regenerating properties [6]. Trigonelline, which is the bioactive secondary metabolite of *Trigonella Foenum* protected completely against significant increase in the membrane ratios of cholesterol [7], phospholipids and sphingomyelin, phosphatidylcholine in rats with carbon tetrachloride induced cirrhosis [8]. Andrographolide, the major component of *Andrographis Lineata* has been reported to possess the strong anti-inflammatory activity [9], and is a lactone diterpene that gives the plant a bitter taste [10]. Enormous literature has focused on the anti-viral, immunomodulatory, hepatoprotective and anticancer potential of andrographolide [11]. The purpose of this study was first to analyze the anti-inflammatory potential of *Andrographis lineata* and *Trigonella Foenum* along with their bioactive secondary metabolites Andrographolide and Trigonelline through its pharmacokinetic parameters [12].

2. MATERIALS AND METHODS

All chemicals and reagents used were of analytical grades and were purchased from

Sigma Chemical Co. (St Louis, MO, USA). Standardized 90% ethanolic extracts of *Trigonella Foenum* and *Andrographis Lineata*, 80% ethanolic extracts of both Trigonelline and Andrographolide were purchased from Sigma Chemical Co. A *p*-value of <0.05 was considered as statistically significant.

2.1 Experimental Design

Chart 1. All chemical treatments

Groups	Treatments
Normal	Control
Positive control	Dimethylnitrosamine
C	Dimethylnitrosamine +Andrographolide
D	Dimethylnitrosamine +Trigonelline
E	Dimethylnitrosamine +Andrographolide+Trigonelline

2.2 Administration of Drugs

Twenty male Wister rats (100-150 gms) were used in the present project each group containing five rats. Dimethylnitrosamine was injected intraperitoneally (0.5 ml/kg body weight) for one week. Dose of trigonelline and andrographolide (200 mg/Kg/ml b.w and 50 mg/Kg/ml b.w respectively) was given by intragastric administration. 0.5 ml of blood sample was collected by venipuncture at different time intervals (0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 30, 35 and 60hrs) for one week. Heparinized eppendorf tubes were used for blood sample and centrifuged at 12000rpm for 3-5 minutes. Plasma was stored at -20°C (Rent et al., 2010).

2.3 Calibration Curves

10 µl of working solution of Andrographolide and Trigonelline was added to 90µl of drug free rats' plasma. After centrifugation, calibration curves were generated through different samples of plasma (1 ml) with 20µl from each working solution of Andrographolide and Trigonelline. The range of 0.05 - 10µg/ml was helpful in getting good linearity at regression equation of $0.191x + 0.49$ with $r^2=0.968$.

2.4 Antioxidative and Phytochemical Analysis of Plant Extracts

Antioxidative and scavenging activity of *Andrographis Lineata* and *Trigonella Foenum* was analyzed by DPHH assay [13]. Various phytochemicals tannins, alkaloids, phenols, flavonoids and carotenoids were also analyzed [14].

2.5 Pharmacokinetic Analysis for Trigonelline and Andrographolide

Kinetic version 5.5 was used to study the various pharmacokinetic aspects like peak serum concentration (C_{max}), time to reach peak concentration (T_{max}), elimination half life ($t_{1/2}$), total body clearance (CL), volume of distribution (V_d) and area under curve (AUC) were evaluated for Trigonelline and andrographolide. Various pharmacokinetic parameters including peak serum concentration (C_{max}), time to reach peak concentration (T_{max}) were evaluated from concentration-time data. Moreover, elimination half life ($t_{1/2}$), total body clearance (CL), volume of distribution (V_d) and area under curve (AUC) were also evaluated by non-compartmental method by pharmacokinetic software Kinetic- Version 5.0 and calculated using the trapezoidal rule.

2.6 Statistical Analysis

Results has been expressed as MEAN \pm SD while P- values less than 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

3.1 Pharmacokinetic Studies in Rats after Oral / Intra gastric Administration of Trigonelline And andrographolide

The oral administration of trigonelline and andrographolide attains a therapeutic use as an antioxidative agent. The pharmacokinetic parameters of these two respective drugs are compartmentalized and mostly dose dependent. The absorption of trigonelline administered by oral route and observed after 24 hours from rat bile is decreased to 2-3%. The Table 1 shows that half-life of trigonelline is 4.14 hours which indicates it as a short acting drug. Other parameters calculated are C_{max} , t_{max} , AUC, K_e , V_d and Cl . Results of all these parameters link together to show that trigonelline is rich in

antioxidative phytochemicals i.e phenols and their scavenging activity against hepatic injury induced by dimethylnitrosamine is higher as compared to that of ascorbic acid. Less half-life of trigonelline metabolite results in a mean residence time of 9 hours and an elimination rate constant of 0.43 hr^{-1} indicating a short duration of time in blood plasma. The less volume of distribution of trigonelline (1.63 L) in albino rats results in a C_{max} of 3.7 to reach the optimum therapeutic response with a good bioavailability relative to that of standard ascorbic acid and the graph shows time approaching the respective C_{max} to be 5 hours. The area under curve evaluated by using trapezoidal rule is calculated as 217.25 with a clearance rate of 155.12. The results of all the pharmacokinetic parameters of trigonelline administered in low dose (200 mg/kg) confirmed the better antioxidative activity than that of ascorbic acid used as a potent antioxidant (Table 1). The increased rate of absorption in case of andrographolide caused a valuable and abrupt change in the results obtained by statistical approach. The pharmacokinetic variability of andrographolide and trigonelline was found valuable in diseased state of hepatic injured rats. The dose adjustment at a t_{max} of 5 hours (similar to that of trigonelline) to meet the effective C_{max} is 1.9 which is approximately half the optimum dose required for trigonelline. Due to very less plasma peak concentration of andrographolide, there is a prominent change in pharmacokinetic parameters as compared to optimum dose of ascorbic acid required to reach the effective therapeutic response. As the results indicate that half-life of andrographolide is 12.54 hours therefore less dose is required to keep a constant and steady state plasma drug concentration. The results show the values of AUC and V_d as 525.001 and 2.3 respectively. Higher value of AUC in case of andrographolide indicates its longer stay in plasma which results in higher scavenging activity despite of very less C_{max} . An increased value of MRT (19.58 hours) and decreased value of K_e (0.41) with a clearance rate of 205.66 provides an easy compliance for administration of a prolonged and steady state concentration of under discussed drug molecules. *T. foenum-graecum* absorption is very low through oral route about 2-3%, observed after 24hrs from rat bile. The excreted form of *T. foenum-graecum* through bile is in the form of glucuronidase conjugates and sulphates in case of human beings. Peak plasma concentration could be seen in 6 to 8hrs and elimination half-life was found to be 6hrs. Pharmacokinetic analysis of *T. foenum-graecum*

has revealed that it is eliminated through bile as metabolites because it is taken through oral route and distributed in various organs and seems to be circulated in enterohepatic system. Moreover, the complex of *T. foenum-graecum* and phosphatidylcholine has shown an increased bioavailability by oral route in healthy volunteers may be due to the facilitating ole of drug complex through the alimentary canal (Ghosh et al., 2010).The data regarding plasma concentration of *T. foenum-graecum* versus time profile in rats is presented (Fig. 1). The results of the present pharmacokinetic studies (Table 1) shows that C_{max} , T_{max} , $t_{1/2}$, AUC, MRT, Vd, C1T and Kcof *T. foenum-graecum* after a single dose administration in rats was $3.3 \mu\text{g ml}^{-1}$, 1.5h, 3.5h, 13.42, 5.1hr, 1.63 L/Kg, 281.79ml/h/Kg and 0.43h respectively. Complex formation of chemical constituents in herbal medicines shows

difficult evaluation inside the body and always have recognized list of active chemical components, with a great demand for analytical methods (Ernst, 2000; Ahmad et al., 2006).

3.2 Secondary Metabolites and Antioxidative Characterization of *T. foenum-graecum* and *A. lineata*

In the first phase of the experiment the crude extract of *T. foenum-graecum* and *A. lineata* was extracted in different solvent systems e.g., methanol, ethanol, chloroform, ethyl acetate, petroleum ether. The results (Fig. 2) shows that the maximum extraction was observed in petroleum ether (11.67%) followed by chloroform, ethanol, ethyl acetate and methanol (5.78%, 5.76%, 4.87% and 5.78% respectively).

Table 1. Pharmacokinetic parameters of trigonelline(@ 200 mg/kg BW) and andrographolide (@of 50 mg/kg BW) in rats after oral (intragastric) administration

Parameters	Trigonelline	Andrographolide
$C_{max}(\mu\text{g ml}^{-1})$	3.7	1.9
T_{max} (h)	5.0	5.0
$t_{1/2}$ (h)	4.14	12.54
AUC ($\mu\text{g h ml}^{-1}$)	217.25	525.001
MRT (h)	8.82	19.58
$-1K_e$ (h^{-1})	0.43	0.41
V_d (L/Kg)	1.63	2.3
Cl(ml/h/Kg)	155.12	205.66

Results has been expressed as MEAN±SD

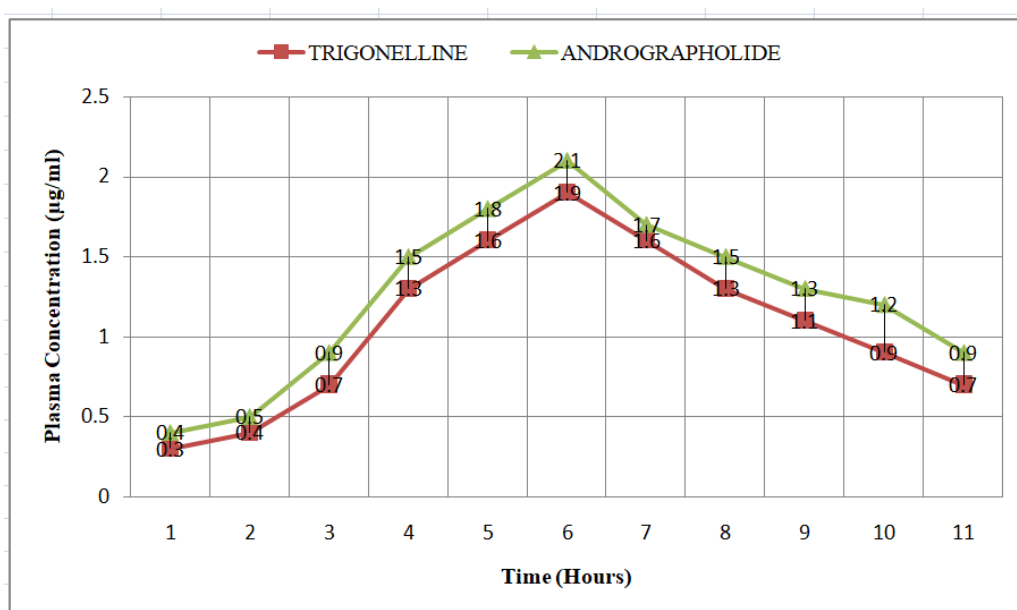


Fig. 1. Mean plasma concentration-time curve of trigonelline (at the rate of 200 mg/kg b.w) and Andrographolide (at the rate of 50 mg/kg b.w) in rats after oral (intragastric) administration

The lowest recovery was recorded by ethyl acetate. In second phase petroleum ether extract was evaluated to estimate total phenolic, tannins, alkaloid, flavonoid and carotenoids contents. The maximum phenols were recorded in *T. foenum-graecum* (13.65±1.45mg of GAE/g of extract) followed by *A. lineate* (9.45±2.87 mg of GAE/g of extract). Maximum tannins were recorded in *A. lineate* (48.87±6.76 mg of GAE/g of extract) followed by *T. foenum-graecum* (33.67±4.87 mg of GAE/g of extract). Maximum alkaloids were recorded in *T. foenum-graecum* (54.56±6.89 mg of GAE/g of extract) followed by *A. lineate* (33.87±3.67 mg of GAE/g of extract). Maximum flavonoids were recorded in *T. foenum-graecum* (70.67±9.56 mg of QE/g of extract) followed by *A. lineate* (39.09±4.98 mg of QE/g of extract) respectively. Maximum carotenoids were recorded in *T. foenum-graecum* (34.76±4.98 mg of GAE/g of extract) followed by *A. lineate* (19.76±2.76 mg of GAE/g of extract). In Fig. 4 petroleum ether extracts of *T. foenum-graecum*

and *A. lineata* has antiradical activity by inhibiting of DPPH radical (IC₅₀ value of 69.04±3.65% and 71.76±6.99% respectively), as compared to ascorbic acid standard (IC₅₀= 53.99±4.88%). IC₅₀ value is the effective concentration at which the antioxidant activity is 50%. This means these extracts were able to reduce the stable radical of DPPH to yellow colored diphenyl picrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, poly-hydroxy aromatic compounds (hydroquinone, pyrogallol, gallic acid, etc.) reduce and decolorize DPPH by their hydrogen donating ability. It appears that extracts of *T. foenum-graecum* and *A. lineata* possess hydrogen donating abilities to act as an antioxidant. Fig. 4 shows the highest DPPH radical scavenging effect in petroleum ether extracts of *A. lineate* (71.76±6.99%) and *T. foenum-graecum* (69.04±3.65%). The data were even superior to standard antioxidant ascorbic acid (53.99±4.88%).

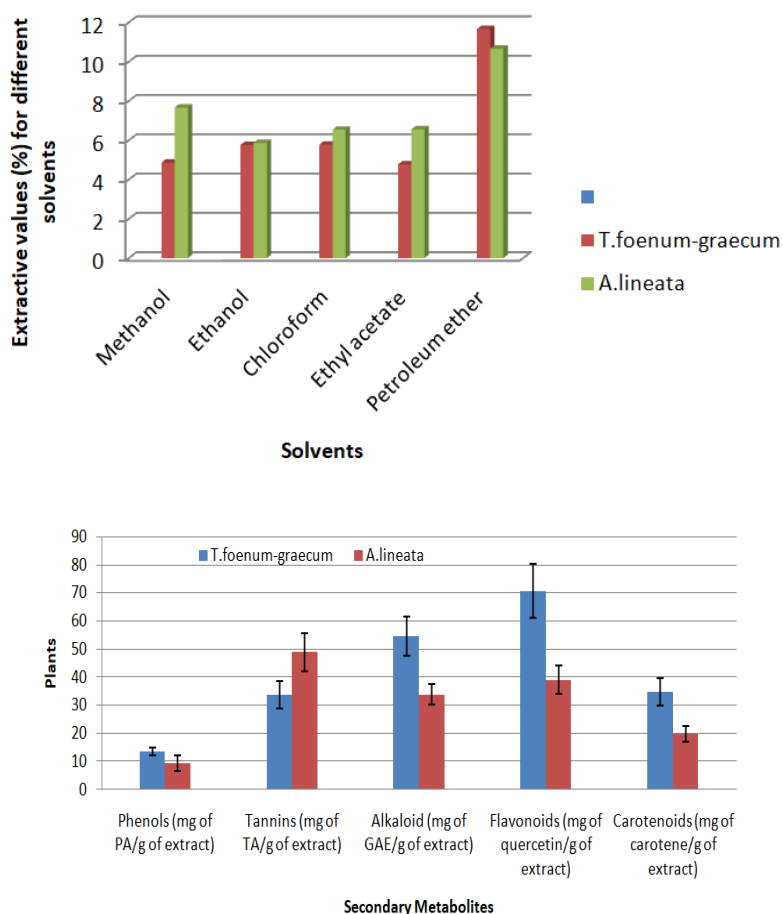


Fig. 2. % Yield in different solvents and Phytochemicals in *T. foenum-graecum* and *A. lineate*

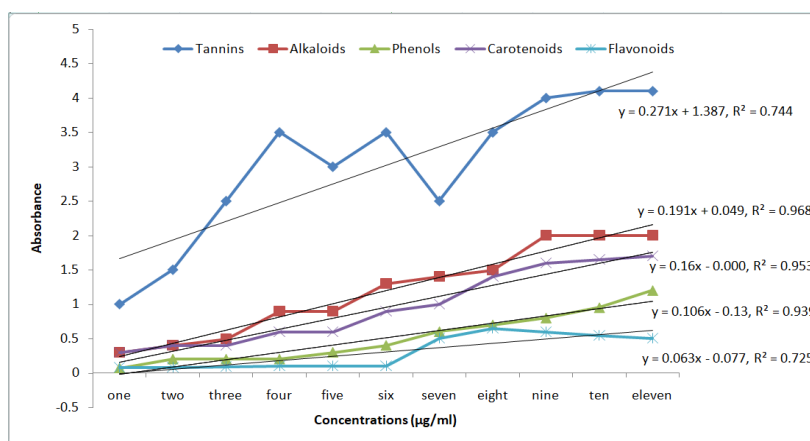


Fig. 3. Calibration curves for secondary metabolites

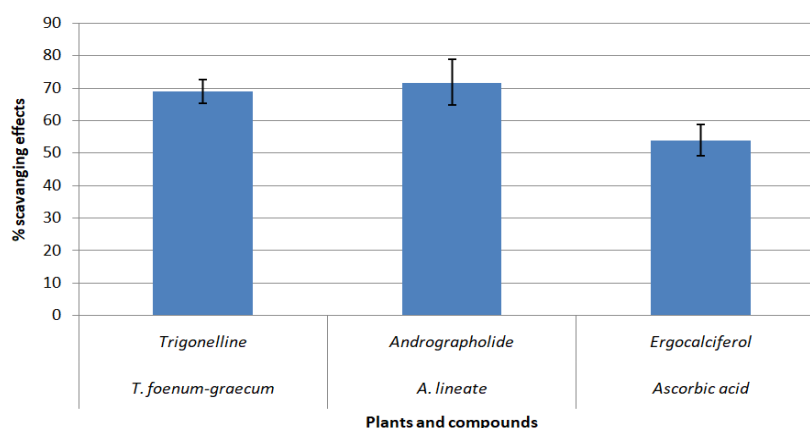


Fig. 4. Scavenging activity of DPPH (Ic-50 %)

4. CONCLUSION

The present study concluded that maximum recovery of the active compounds was obtained in petroleum ether, then in chloroform, ethanol and methanol. However, least recovery was observed with ethyl acetate. Both trigonelline and andrographolide possess therapeutic anti-inflammatory and antioxidative properties at their specific dose regimen.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the Institutional Review Board of The University of Lahore (Approval No: USM/Animal Ethics approval/2009/ [45] [140]) and all animal experiments were performed in accordance with

the Institutional Guidelines for the Care and Use of Animals for Scientific Purposes.

ACKNOWLEDGEMENTS

The authors are grateful for the valuable contribution of Prof. Dr. Muhammad Ashraf Director Institute of Molecular Biology and Biotechnology (IMBB)/Centre for Research in Molecular Medicine (CRIMM), University of Lahore-Pakistan for the financial support and for critically reviewing the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Alvari A, Ohadi Rafsanjani MS, Ahmad FJ, Abdin MZ. Current status and future

- prospects of hepatoprotective herbal medicines: Contemporary overview into the clinical trials. *Rev Recent Clin Trials*. 2012;7:214–23.
- Ferrucci L, Fabbri E. Inflammation ageing: Chronic Inflammation in Ageing, Cardiovascular Disease, and Frailty. *Nat. Rev. Cardiol*. 2018;15(9):505–522.
 - Sinha M, Gautam L, Shukla P, Kaur KP, Sharma S, Singh, TP. Current Perspectives in NSAID-Induced Gastropathy. *Mediators Inflammation*. 2013;25:82-09.
 - Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive Oxygen Species in Inflammation and Tissue Injury. *Antioxidants & Redox Signaling*. 2014; 20(7):1126–1167.
 - Kuhn H, Banthiya S, van Leyen K. Mammalian Lipoxygenases and Their Biological Relevance. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids*. 2015;1851(4): 308–330.
 - Hughes SD, Ketheesan N, Haleagrahara N. The Therapeutic Potential of Plant Flavonoids on Rheumatoid Arthritis. *Crit. Rev. Food Sci. Nutr*. 2017;57(17):3601–3613.
 - Xiao JB. Dietary Flavonoid Aglycones and Their Glycosides: Which Show Better Biological Significance?. *Crit. Rev. Food Sci. Nutr*. 2017;57(9):1874–1905.
 - Valadez-Carmona L, Plazola-Jacinto C, Hernandez-Ortega P, Hernandez-Navarro M, Villarreal MD, Necoechea-Mondragon F, OrtizMoreno H, Ceballos-Reyes AG. Effects of Microwaves, Hot Air and Freeze-drying on the Phenolic Compounds, Antioxidant Capacity, Enzyme Activity and Microstructure of Cacao Pod Husks (*Theobroma Cacao L.*). *Innovative Food Sci. Emerging Technol*. 2017;41:378–386.
 - Pekal A, Pyrzynska, K. Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. *Food Analytical Methods*. 2014;7(9):1776–1782.
 - Nema NK, Maity N, Sarkar BK, Mukherjee PK. Matrix Metalloproteinase, Hyaluronidase and Elastase Inhibitory Potential of Standardized Extract of *Centella Asiatica*. *Pharmaceutical Biology*. 2013;51(9):1182–1187.
 - Herald TJ, Gadgil P, Tilley A. High-throughput Micro Plate Assays for Screening Flavonoid Content and DPPH-scavenging Activity in Sorghum Bran and Flour. *J. Sci. Food Agric*. 2012;92(11): 2326–2331.
 - Perera H, Samarasekera J, Handunnetti S, Weerasena, MO. *In vitro* anti-inflammatory and anti-oxidant activities of Sri Lankan medicinal plants. *Ind. Crops Prod*. 2016; 94:610–620.
 - Wu DL, Yotnda P. Production and Detection of Reactive Oxygen Species (ROS) in Cancers. *J. Visualized Exp*. 2011; 57:33-57.
 - Yadav R, Kalia K, Kumar P, Jain RV. Antioxidant and Nutritional Activity Studies of Green Leafy Vegetables. *Intl. J. Agric. Food Sci. Technol*. 2013;4(7):707–712.

© 2021 Zahid et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:

<https://www.sdiarticle4.com/review-history/75341>