COMPARATIVE ANALYSIS OF HEPATOPROTECTIVE AND ANTI-INFLAMMATORY POTENTIAL OF A POLYHERBAL FORMULATION PREPARED WITH PLANTS FROM KANDHAMAL DIST., ODISHA: AN *IN VITRO* STUDY

Avilas Bhatta[1]*, Sambit Kumar Parida[2]

Abstract:
In recent years, there has been a growing interest in natural medicine, with a shift towards utilizing herbal remedies for various health conditions. This study protocol was designed to investigate the hepatoprotective and anti-inflammatory potential formulations derived from selected indigenous medicinal plants, *Cayratia trifolia*, *Sesbania grandiflora*, *Cordia dichotoma*, and *Tephrosia purpurea*, collected from Kandhamal district, Odisha.

The hepatic safety potential of this formulation was evaluated on normal liver and kidney cell lines to assess its safety profile. Additionally, the anti-inflammatory activity of the formulation was evaluated through the inhibition of albumin denaturation, membrane stabilization, and proteinase activity. The hepatoprotective studies revealed that the polyherbal formulation did not induce any abnormalities or adverse effects in normal liver and kidney cells, indicating its safety for potential therapeutic use. The formulation exhibited significant inhibition of heat-induced albumin denaturation, with a maximum inhibition of 87.95% at a concentration of 800 µg/ml. The IC_{50} value was determined to be 287.05±2.79 µg/ml, demonstrating its effective anti-inflammatory activity. Moreover, the above polyherbal formulation demonstrated significant inhibition of heat-induced hemolysis, with a maximum inhibition of 78.79±0.54% at a concentration of 800 µg/ml. This suggests that the formulation stabilizes red blood cell membranes, potentially impeding the release of lysosomal content from neutrophils and reducing tissue inflammation and damage. The IC_{50} value for membrane stabilization was found to be 469.14±9.59 µg/ml. Furthermore, the polyherbal formulation exhibited substantial antiproteinase activity, with a maximum inhibition of 83.49±0.48% at a concentration of 800 µg/ml. This activity is significant in protecting against tissue damage during inflammatory reactions, highlighting the potential of the formulation as an anti-inflammatory agent. In conclusion, this study provides valuable insights into the efficacy and safety of polyherbal formulation, indicating its potential as a hepatoprotective and anti-inflammatory agent.

Keywords: Polyherbal Formulation, Hepatoprotective Activity, Anti-inflammatory activity, *In Vitro* Study, Cell Line Models.

[1]*Department of Pharmacy, Biju Patnaik University of Technology, Rourkela, Odisha, India.
[2]Seth Vishambhar Nath Institute of Pharmacy, Uttar Pradesh, India

***Corresponding Author:** Avilas Bhatta
*Research Scholar, Department of Pharmacy, Biju Patnaik University of Technology, Rourkela, Odisha, India.
Email: avilas.bhatta@gmail.com

DOI: 10.48047/ecb/2023.12.si10.00108

INTRODUCTION

In recent years, there has been a global shift from synthetic to natural medicine, with an increasing focus on utilizing medicinal herbs for treating various diseases. Traditional Indian medical systems, including Ayurveda, Siddha, Unani, Homeopathy, Yoga, and Naturopathy, have gained widespread acceptance, and are practiced for maintaining good health. As a result, extensive research is being conducted on phytomedicine, with numerous botanical preparations being evaluated for their therapeutic efficacy. Particularly, the development of botanical drugs with hepatoprotective and anti-inflammatory properties has gained significant attention. [1,2]

The incidence of hepatic damage and inflammation is rising due to various factors such as environmental pollution, dietary changes, and lifestyle modifications. The *in vitro* evaluation of the hepatoprotective and Anti-inflammatory activityof the polyherbal formulation derived from plants *Cayratia trifolia*, *Sesbania grandiflora*, *Cordia dichotoma*, and *Tephrosia purpurea* collected from Kandhamal District, Odisha, demonstrates its potential as a valuable candidate for further preclinical and clinical studies.[2,3,4]

In this study, a specific polyherbal formulation composed of hydroalcoholic extracts from different indigenous medicinal plants, prepared in specific ratios, was selected for investigation. The primary objective of this research is to assess the hepatoprotective potential and Anti-inflammatory activity of this polyherbal ayurvedic formulation using a cell line model and through the inhibition of albumin denaturation, membrane stabilization, and proteinase activity respectively.

METHODS: [4-9]

Chemicals and Cell Lines:

All chemicals and reagents used in this study were of analytical grade and were obtained from Loba Chemie Laboratory Reagents and Hi Media. The cell lines utilized in the experiments were obtained from the National Centre for Cell Sciences in Pune, India.

Collection and authentication of plant materials: The selected four plants were identified and authenticated by taxonomist Dr. S. K. Dash- H.O.D of Bioscience; College of Pharm. Science-Mohuda. The voucher herbarium specimens (no.- CPS/HS-0032,0033,0034 & 0035) were deposited in the herbarium of P.G. Dept. Of Phytochemistry- College of pharm. Sciences- Mohuda for future reference. After authentication, fresh leaves, barks, and flowers were collected separately (during its flowering time in Mar-April-2007) in bulk from young, matured plants from the rural hill area of Kandhamal forest area- Odisha.

Development of formulation: The drugs of plant origin were dried and made into fine powders, separately. The fine powder of drugs were taken in equal proportion and polyherbal formulation is prepared.

Table 1: Composition of Formulation

Sl no	Plant Name	Plant Part Used	Proportion
1	*Cayratia trifolia*	Roots	1 part
2	*Sesbania grandiflora*	Flower	1 part
3	*Cordia dichotoma*	Bark	1 part
4	*Tephrosia purpurea*	Seed	1 part

Preparation of Extract:

The polyherbal formulation consisted of 4 herbal ingredients in varying ratios. The extraction of phytochemicals was performed using the cold maceration method. Double-distilled water and ethanol in a 7:3 ratio was used for the extraction process. The mixture was allowed to stand for 48 hours with intermittent shaking. The resulting mixture was filtered through double-lined muslin cloth and then subjected to evaporation under reduced pressure using a vacuum evaporator. The final product obtained was a greenish-black material which was stored in an airtight container at 4°C. [10]

Determination of *In Vitro* hepatoprotective activity:

The *in vitro* hepatoprotective activity of the polyherbal formulation were assessed using the 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Two separate experiments were conducted following the same procedure to evaluate this efficacy of the test drug. The percentage of cell viability and mortality was calculated using a standard formula. [11-15]

Assessment of *in vitro* anti-inflammatory activity

Anti-inflammatory activity of this polyherbal ayurvedic formulation was conducted through the

inhibition of albumin denaturation, membrane stabilization, and proteinase activity respectively.

Albumin denaturation method

The assessment of *in vitro* anti-inflammatory activity was conducted using the inhibition of albumin denaturation method. The reaction mixture consisted of varying concentrations of the test formulation and a 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted to 6.8 using a small amount of 1N HCl. The samples were then incubated at 37°C for 20 minutes and subsequently heated at 57°C for 20 minutes. After cooling, the turbidity of the samples was measured spectrophotometrically at 660 nm. The experiment wasperformed in triplicate and average was taken. The percentage inhibition of protein denaturation was calculated as follows:

% Inhibition of denaturation =

$$\frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control} \times 100$$

Membrane Stabilization Test

Preparation of Red Blood Cell (RBC) Suspension

A total of 10 ml of fresh whole human blood was collected (who has not taken any NSAIDS for 2 weeks) and transferred to heparinized centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 10 minutes, and the resulting pellet was washed three times with an equal volume of normal saline. The volume of the blood was measured and reconstituted to a 10% v/v suspension using normal saline.

The reaction mixture (2 ml) consisted of 1 ml of the test drug solution and 1 ml of a 10% RBCs suspension. For the control test tube, saline was added instead of the drug. Aspirin was used as the standard drug. All the centrifuge tubes containing the reaction mixture were incubated in awater bath at 56°C for 30 minutes. After incubation, the tubes were cooled under running tap water. The reaction mixture was then centrifuged at 2500 rpm for 5 minutes, and the absorbance of the supernatants was measured at 560 nm. The experiment was conducted in triplicate, and the percentage of membrane stabilization activity was calculated using the formula mentionedabove.

Proteinase Inhibitory Action

The reaction mixture (2 ml) contained 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH 7.4), and 1 ml of the test sample at different concentrations. The mixture was incubated at 37°C for 5 minutes, and then 1 ml of 0.8% (w/v) casein was added. The mixture was further incubated for 20 minutes. To terminate the reaction, 2 ml of 70% perchloric acid was added. The resulting cloudy suspension was centrifuged, and the absorbance of the supernatant was measured at 210 nm against a blank buffer. The experiment was performed in triplicate, and the percentage inhibition of proteinase inhibitory activity was calculated as per previously mentioned formula.

RESULTS AND DISCUSSION:

In Vitro hepatoprotective Studies: This study play a crucial role in evaluating the potential benefits and limitations of a model for screening and analyzing toxic compounds. The current investigation provide valuable insights into the cellular and molecular effects of potentially harmful substances through the utilization of various morphological and biochemical markers. Among the available methods, *in vitro* cell line models offer convenience and feasibility in laboratory settings.[16-18] In this study, the cytotoxicity of different concentrations of hepatoprotective polyherbal formulation was assessed against normal liver and kidney cell lines.

The results of the cytotoxicity studies revealed the impact of polyherbal formulation on the viability of the tested cell lines. The various concentrations of hepatoprotective formulation were analyzed to determine their effects on cell viability and assess the potential toxicity. The obtained data provides insights into the cytotoxic properties of hepatoprotective polyherbal formulation and enables a better understanding of itssafety profile. These findings are valuable for further investigations and contribute to the characterization of our polyherbal formulation as a potential therapeutic agent.[20,21,22]

Moreover, the use of *in vitro* cell line models offers several advantages, such as ease of handling, controlled experimental conditions, and the ability to study specific cell types. This enables researchers to gain valuable preliminary information regarding the effects of hepatoprotective polyherbal formulation on normal liver and kidney cells, laying the foundation for further investigations on its potential as a hepatoprotective agent. However, it is important to acknowledge the limitations of in vitro models, as they may not fully represent the complexity and dynamics of in vivo systems. Thus, it is crucial to validate these findings through further studies, including in vivo experiments and clinical trials.[23,24]

The *in vitro* cytotoxicity studies provided valuable insights into the effects of our selected polyherbal formulation on normal liver and kidney cell lines. The results highlight its potential cytotoxic properties and contribute to the understanding of its safety profile. These findings pave the way for future research, allowing for a better understanding of polyherbal formulation's therapeutic potential and its applicability as a hepatoprotective agent. Further investigations using in vivo models and clinical trials are warranted to validate and expand upon these preliminary findings.[25]

Table 2: Cytotoxic effect of different concentrations of Hepatoprotective polyherbalformulation on normal cell lines (Chang liver cells)

S. No	Concentration (µg/ml)	Cell mortality (%)	Cell viability (%)
1	500	31.92	68.07
2	250	25.38	74.61
3	100	23.67	76.32
4	68.5	18.38	81.61
5	35.25	7.58	92.41
6	13.63	3.72	96.27
7	7.80	1.47	98.52
9	Cell control	0	100

Table 3: Cytotoxic effect of different concentrations of Hepatoprotective polyherbalformulation on kidney (Vero) cell lines

S. No	Concentration (µg/ml)	Cell mortality (%)	Cell viability (%)
1	500	33.08	66.91
2	250	16.18	83.81
3	100	16.08	83.91
4	68.5	10.59	89.40
5	35.25	8.28	91.71
6	13.63	5.35	94.64
7	7.80	2.83	97.16
9	Cell control	0	100

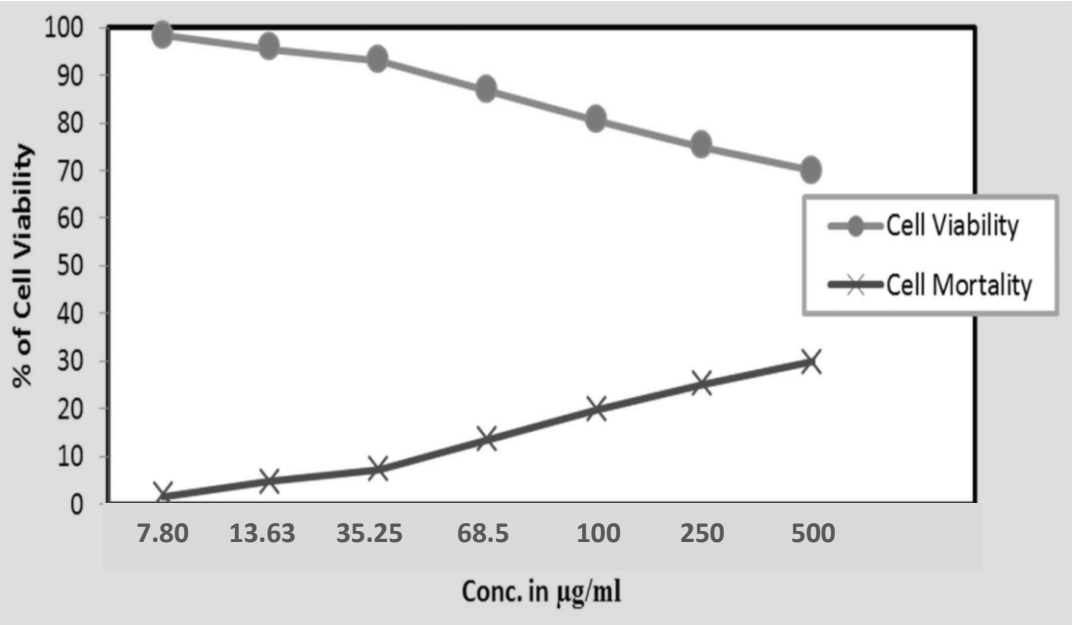

Fig. 1: Cytotoxic effect of Hepatoprotective polyherbal formulation on normal liver celllines percentage of viability and mortality

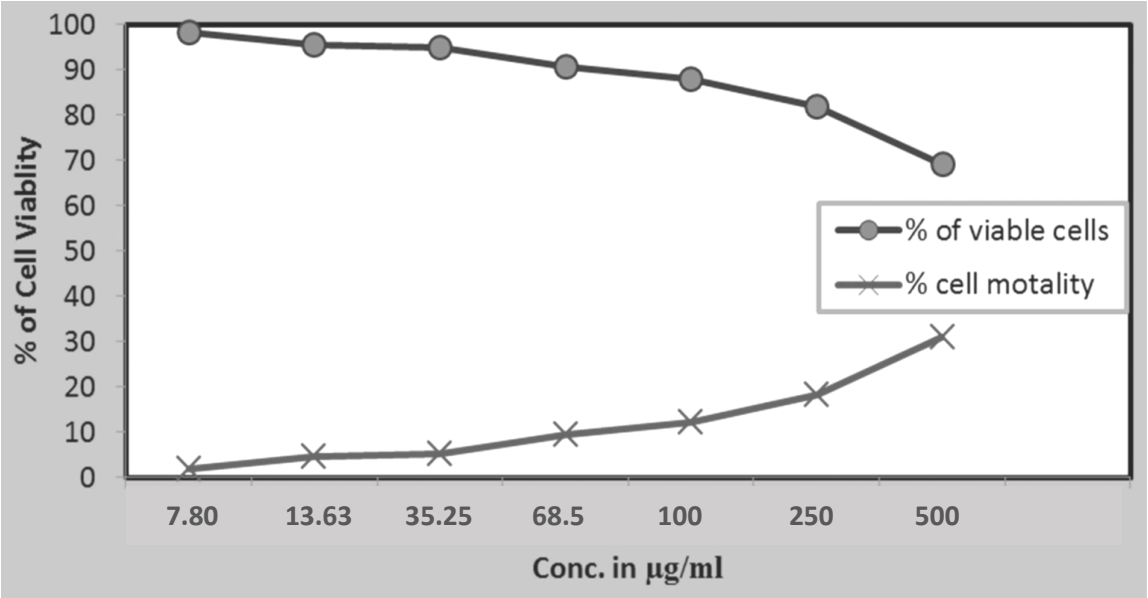

Fig. 2: Cytotoxic effect of Hepatoprotective polyherbal formulation on normal kidney(Vero) cell lines percentage of viability and mortality

In vitro cytotoxicity data play a crucial role in assessing the potential toxicity of a specific substance and can also provide insights into the need for additional toxicity tests. When a drug is administered, it undergoes metabolism primarily in the liver, which is the organ most exposed to drugs and toxic substances. After the metabolism process is completed, the drug is eliminated through the kidney. However, during the elimination process, there is a possibility of causing damage to the kidney cells.

The results obtained from the cytotoxicity studies of the polyherbal formulation indicate that it does not induce any abnormalities or adverse effects in normal liver and kidney cells. This suggests that the formulation is safe and does not pose a risk to the cellular integrity and function of these vital organs. This information is valuable in assessing the safety profile of the polyherbal formulation and provides confidence in its potential as a therapeutic agent. Further studies and tests can be conducted to explore its efficacy and safety in more detail. [25,26]

In vitro Assessment of Anti-inflammatory Activity

Inhibition of Albumin Denaturation

Protein denaturation is a well-known cause of inflammation, and certain anti-inflammatory drugs like phenylbutazone, salicylic acid, and flufenamic acid have demonstrated a dose- dependent ability to inhibit thermally induced protein denaturation [27,28]. As part of the investigation into the mechanism of anti-inflammatory activity, the ability of extract to inhibit protein denaturation was studied. The results, presented in Table 4, showed that the extract effectively inhibited heat-induced albumin denaturation at various concentrations. The maximum inhibition observed was 87.95±1.46 at a concentration of 800 µg/ml. The IC_{50} value was determined to be 287.05±2.79 µg/ml, with a correlation coefficient (r) of 0.948. Aspirin, a standard anti-inflammatory drug, exhibited the highest inhibition of 76.88±0.55 % at a concentration of 200 µg/ml.

Membrane Stabilization Test

To gain further insights into the anti-inflammatory action of Poly herbal formulation, the stabilization of red blood cell (RBC) membranes was examined. The extract exhibited significant inhibition of heat-induced hemolysis at various concentrations, suggesting that membrane stabilization contributes to its anti-inflammatory effect. This finding suggests that the extract may impede the release of lysosomal content from neutrophils at the site of inflammation. Neutrophil lysosomes contain bactericidal enzymes and proteases, which, when released extracellularly, can cause additional tissue inflammation and damage (29).

Table 4: Effect of Polyherbal formulation on Albumin Denaturation, Membrane Stabilization, and Proteinase Inhibitory Activity

Test Sample Conc.(µg/ml)	% Inhibition of AlbuminDenaturation	% Membrane Stabilization	% Proteinase Inhibition
Polyherbalformulation			
50	24.70±1.45	18.84±0.62	22.29±1.36
100	30.82±1.18	22.50±0.58	23.79±0.68
200	46.98±0.64	27.47±0.93	29.34±1.53
400	63.42±1.16	48.69±1.36	41.70±0.80
600	72.64±0.89	55.64±1.12	68.72±1.39
800	87.95±1.46	78.79±0.54	83.49±0.48
Correlation coefficient value (r)	0.948	0.998	0.998
IC50 value	287.05±2.79	469.16±9.59	438.28±5.92
Aspirin			
100	66.45±0.65	72.56±0.58	56.45±0.45
200	76.88±0.55	85.92±0.75	92.87±0.76

Values represent the mean ± SD of three replicates; linear regression analysis was used to calculatethe IC_{50} value.

The test extract, at concentrations ranging from 200 µg/ml to 800 µg/ml, demonstrated varying degrees of inhibition of heat-induced hemolysis, as presented in Table 4. The maximum inhibition of 78.79±0.54% was observed at 800 µg/ml. The IC_{50} value, indicative of the concentration required for 50% inhibition, was determined to be 469.16±9.59 µg/ml, with a high correlation coefficient (r) of 0.998. Aspirin, the standard drug, exhibited the highest inhibition of 85.92±0.75% at a concentration of 200 µg/ml.

Proteinase Inhibitory Activity

Proteinases have been implicated in arthritic reactions, and neutrophils are recognized as an abundant source of proteinases, housing numerous serine proteinases within their lysosomal granules. Previous reports have highlighted the crucial role of leukocyte proteinases in the development of tissue damage during inflammatory reactions. Significant protection against such damage has been observed with the use of proteinase inhibitors [30-33]

The Poly herbal formulation demonstrated substantial antiproteinase activity at different concentrations, as presented in Table 4. The maximum inhibition of 83.49±0.48% was observed at 800 µg/ml. The IC_{50} value, representing the concentration required for 50% inhibition, was determined to be 438.28±5.92 µg/ml, with a correlation coefficient (r) of 0.998. Aspirin exhibited the highest inhibition of 92.87±0.76% at a concentration of 200 µg/ml.

CONCLUSION

The findings of this in vitro study provide compelling evidence of the hepatoprotective and anti-inflammatory potential of the polyherbal formulation derived from plants in Kandhamal Dist., Odisha. The formulation exhibited no cytotoxic effects on normal liver and kidney cells, indicating its safety for potential therapeutic use. Furthermore, the formulation demonstrated significant anti-inflammatory activity by inhibiting albumin denaturation, stabilizing red blood cell membranes, and inhibiting proteinase activity. These results suggest that the formulation could protect against tissue damage during inflammatory reactions. The obtained data supports the notion that the polyherbal formulation holds promise as a therapeutic agent for hepatoprotection and anti-inflammatory treatment.

Overall, the results of this study provide a solid foundation for future research and development of polyherbal formulation as a potential therapeutic intervention for liver protection and inflammation management.

CONFLICTS OF INTEREST

Conflicts of interest were carefully addressed, and all authors have declared no conflicts.

REFERENCES

1. Aggarwal BB, Sethi G, Ahn KS, Sandur SK, Pandey MK, Kunnumakkara AB, et al. Targeting signal-transducer-and-activator-of-transcription-3 for prevention and therapy of cancer: Modern target but ancient solution. Ann N Y Acad Sci 2006;1091:151-69.
2. Aziz MY, Omar N. Anti-cancer activity of Bauhinia variegata (L.) whole plant extracts against MCF-7 breast cancer cell line. J Appl Pharm Sci 2012;2:59-62.
3. Boyd MR, Paull KD. Some practical considerations and applications of the national cancer institute in vitro hepatoprotective drug discovery screen. Drug Dev Res 1995;34:91-9.
4. Elango V, Thiruvengadam M, Subramanian S.

Evaluation of antioxidant and Anti-inflammatory activity of leaves of Alangium salvifolium Wang against Dalton's lymphoma ascites-induced Swiss albino mice. Int J Appl Pharm 2013;5:61-7.
5. Fotakis G, Timbrell JA. In vitro cytotoxicity assays: Comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicol Lett2006;160:171-7.
6. González LT, Minsky NW, Espinosa LE, Aranda RS, Meseguer JP, Pérez PC, et al. In vitro assessment of hepatoprotective agents against damage induced by acetaminophen and CCl4. BMC Complement Altern Med 2017;17:39.
7. Houghton PJ, Howes MJ, Lee CC, Steventon G. Uses and abuses of in vitro tests in ethnopharmacology: Visualizing an elephant. J Ethnopharmacol 2007;110:391-400.
8. Ilyas U, Katare DP, Aeri V, Naseef PP. A review on hepatoprotective and immunomodulatory herbal plants. Pharmacogn Rev 2016;10:66-70.
9. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
10. Jiménez-Medina E, Garcia-Lora A, Paco L, Algarra I, Collado A, Garrido F, et al. A new extract of the plant Calendula officinalis produces a dual in vitro effect: Cytotoxic anti-tumor activity and lymphocyte activation. BMC Cancer 2006;6:119.
11. Kao YH, Hiipakka RA, Liao S. Modulation of endocrine systems and food intake by greentea epigallocatechin gallate. Endocrinology 2000;141:980-7.
12. Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet RA, Doody LA, et al. Chemo preventive drug development: Perspectives and progress. Cancer Epidemiol Biomarkers Prev 1994;3:85-98.
13. Lu H, Meng X, Yang CS. Enzymology of methylation of tea catechins and inhibition of catechol-O-methyltransferase by (-)-epigallocatechin gallate. Drug Metab Dispos 2003;31:572-9.
14. Manosroi A, Akazawa H, Akihisa T, Jantrawut P, Kitdamrongtham W, Manosroi W, et al. In vitro anti-proliferative activity on colon cancer cell line (HT-29) of Thai medicinal plants selected from Thai/Lanna medicinal plant recipe database "MANOSROI III". J Ethnopharmacol 2015;161:11-7.
15. Moon DO, Kim MO, Choi YH, Park YM, Kim GY. Beta-lapachone (LAPA) decreases cell viability and telomerase activity in NCI-H460 human lung cancer cells via apoptosis induction and hTERT downregulation. Phytother Res 2009;23:269-74.
16. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
17. Nayak BS, Kanhai J, Milne DM, Pereira LP, Swanston WH. The use of plants in the traditional management of diabetes in St. Vincent and the Grenadines. Pharm Biol 2009;47:566-71.
18. Ortega A, Ferrer P, Cascante M. Use of metabolomics and in vitro systems biology in cancer research. Curr Drug Metab 2009;10:881-94.
19. Pandey MK, Sung B, Kunnumakkara AB, Sethi G, Chaturvedi MM, Aggarwal BB. Berberine modifies cysteine 179 of Ikappa Balpha kinase, suppresses nuclear factor-kappaB- regulated antiapoptotic gene products , and potentiates apoptosis. Cancer Res 2008; 68:5370-9.
20. Patel S, Gheewala N, Suthar A, Shah A. In-vitro cytotoxicity activity of Solanum nigrum extract against Hela cell line and Vero cell line. Int J Pharm Pharm Sci 2009;1 Suppl 1:38-46.
21. Paulmurugan R, Malhotra M, Wu L, Mc Macken J, Contag CH, Gambhir SS. A novel far- red bimolecular fluorescence complementation system that allows for efficient visualization of protein interactions under physiological conditions. Biosens Bioelectron 2006;21:2015-22.
22. Perumal P, Nair RR, Nair MG. Chemical composition, antioxidant, and hepatoprotec tive activities of the essential oil of Wedelia chinensis (Osbeck) Merr. with reference to chemical profiling of volatile oils of the genus Wedelia. Chem Biodivers 2010;7:2040-7.
23. Präbst K, Engelhardt H, Ringgeler S, Hübner H. Basic colorimetric proliferation assays: MTT, WST, and resazurin. Methods Mol Biol 2017;1601:1-7.
24. Prashant A, Rangaswamy C, Yadav AK, Reddy V, Sowmya MN, Madhunapantula S, et al. In vitro Anti-inflammatory activity of ethanolic extracts of Piper nigrum against colorectal carcinoma cell lines. Int J Appl Basic Med Res 2017;7:67-72.
25. Ray S, Ray A, Bhattacharya S, Chattopadhyay S, Chakraborty R. Evaluation of anti-proliferative activity of leaf extract of Anthocephalus cadamba on different cancer

cell lines. Asian J Pharm Clin Res 2014;7:46-9.
26. Banskota AH, Tezuka Y, Kadota S. Recent progress in pharmacological research of medicinal plants and natural products. Phytotherapy Research. 2001;15(8):677-90.
27. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. The Scientific World Journal. 2013; 2013:162750.
28. de Sousa Araújo TA, de Melo MG, de Carvalho MG, de Sousa DP. Mechanisms of action underlying the anti-inflammatory and immunomodulatory effects of propolis: a brief review. Revista Brasileira de Farmacognosia. 2015;25(6):e-1-e-5.
29. Jantan I, Ahmad W, Bukhari SN. Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. Frontiers in Plant Science. 2015;6:655.
30. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: a review. European Journal of Medicinal Chemistry. 2015;97:55-74.
31. Tabassum N, Ahmad F. Role of natural herbs in the treatment of hypertension. Pharmacognosy Reviews. 2011;5(9):30-40.
32. Wojcikowski K, Wohlmuth H, Johnson DW, Gobe G. Dioscorea villosa (wild yam) induces chronic kidney injury via pro-fibrotic pathways. Food and Chemical Toxicology. 2008; 46(10):3122-31.
33. Gupta M, Mazumder UK, Gomathi P, Selvan VT. Anti-inflammatory evaluation of leaves of Plumeria acuminata. BMC Complementary and Alternative Medicine. 2006;6:36.