IJBPAS, May, 2025, 14(5): 2475-2485

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF THE CYTOTOXIC POTENTIAL OF *O. RECURVIPETALA* AGAINST ORAL SQUAMOUS CARCINOMA CELL LINES BY LC-MS PROFILING AND MTT ASSAY

VISHNUPRIYA S[1*], ANIL KUMAR R[1], REDDY AN[1], ANUSHREE R[1], ARSHA MOHAN K[1], KURABET A[1] AND G VENKATA KARTHIK REDDY[2]

1: Department of Pharmaceutical chemistry, The Oxford College of Pharmacy, Bangalore, Karnataka - 562107

2: Department of Pharmacology, The Oxford College of Pharmacy, Bangalore, Karnataka-562107

***Corresponding Author: Ms. Vishnupriya S: E Mail: vishnupriya423@gmail.com**

Received 15th Dec. 2023; Revised 19th Jan. 2024; Accepted 27th June 2024; Available online 1st May 2025

https://doi.org/10.31032/IJBPAS/2025/14.5.8945

ABSTRACT

Rubiaceae plants, specifically ophiorrhiza plants, are used for healing in traditional and modern medicine. Recently, *Ophiorrhiza recurvipetala* was discovered in Assam, India. The scientific data supporting its potential to aid with health issues is scant. The LC-MS profile and anticancer activity of *Ophiorrhiza recurvipetala* aversion on oral squamous carcinoma cell lines (OSCC) were investigated using MTT assay. Fifteen compounds were found in LC-MS analysis, including quercetin, beta-sitosterol, camptothecin, and betulinic acid and etc., The IC_{50} of 264µg/ml demonstrated a considerable anti-cancer impact of the extracts on oral SCC. *Ophiorrhiza recurvipetala* extract bioactive ingredients analysed in LC-MS prevented oral SCC, as demonstrated by MTT. Based on these findings, *Ophiorrhiza recurvipetala* warrants further investigation for potential medicinal applications as a potential source of naturally occurring chemicals that combat cancer.

Keywords: *Ophiorrhiza recurvipetala*, OSCC, LC-MS, MTT, IC_{50}

INTRODUCTION

In the hunt for novel and secure anti-cancer therapies, natural products are a useful resource. The multitude of phytoconstituents, including polyphenols, flavonoids, and alkaloids, that are present in plant extracts gives rise to a broad range of bioactive chemicals. In the search and development of new medications, they are essential **[1]**. Additionally, plants have a major impact on human health and are the primary source of many medicinal compounds. Many plants have long been recognised for their medicinal and restorative qualities **[2]**. Furthermore, there is a notable increase in the popularity of herbal therapies as more people are turning away from conventional drugs and embracing alternative medicine, also referred to as parallel herbal systems **[3]**. This study describes and illustrates *Ophiorrhiza recurvipetala*, a newly discovered species from Assam, India, classified within the Rubiaceae family. While the species is distinguished by its attenuated leaf base, 3 to 4 cm inflorescence, pearly white flowers, 5 to 7 cm peduncle, and hairy capsule, these characteristics unite it with *Ophiorrhiza ochroleuca* Hook. f. The genus Ophiorrhiza, belonging to the Rubiaceae family, is widely distributed across tropical and subtropical regions of Asia, Australia, New Guinea, and the moist forests of the Pacific Islands. It is an Indo-Malaysian genus. Periatric herbs comprise most of this genus, exhibiting a typical growth span of approximately 10 centimeters to 1 meter. Typically, succulent stems, numerous capsular sords with small rhomboid morphologies, five-petaled flowers featuring opposing leaves that are somewhat irregular, and laterally compressed fruits are characteristic of the Ophiorrhiza genus **[4]**. Our subspecies comprise twenty-one species and five variations of the Ophiorrhiza genus. In particular, the eastern states and western Ghats of India are inhabited by 46 species and five variants, while Kerala is home to 16 species and three varieties. The Ophiorrhiza genus of plants has been associated with assertions of diverse therapeutic advantages and widespread applications in traditional and modern medicine. Throughout history, botanicals have been employed to treat cancer, inflammation, pain, and bacterial and viral infections. Furthermore, Ophiorrhiza species possess analgesic, antitussive, and stomatitis properties; they are also effective in treating gastropathy, leprosy, amenorrhea, lesions, and snake bites. In contemporary medicine, camptothecin, a constituent of Ophiorrhiza plants, is widely recognized for its anti-cancer properties. Because camptothecin inhibits DNA topoisomerase-1, this is the case. However, diverse cultures may

uniquely employ them to remedy specific ailments. For example, the Mama tribe uses the leaves to prepare tea to alleviate body pains and the juice for treating diarrhoea. Additionally, the Nanchang people of Bangladesh utilize a paste composed of O. rugosa var. prostrate (D. Don) to treat boils and earaches, respectively (D. Don & Mondal). The Chakma tribe treats a painful area with sun-dried, pulverized leaves. Ophiorrhiza species are abundant in bioactive compounds that exhibit remarkable pharmacological effects and can cure a broad spectrum of conditions, from mild to chronic **[5]**. The present investigation utilized oral squamous cell carcinoma as a model organism and assessed the antineoplastic potential of the compounds detected via LC-MS analysis.

MATERIALS AND METHODS

PLANT MATERIAL

Ophiorrhiza recurvipetala was collected in Dima Hasao, Assam. It was examined and authenticated by Sri Venkateshwara University botanist Madhav Shetty in Tirupati, Andhra Pradesh.

PLANT EXTRACTION

The plant leaves were rinsed and dried in the shade with faucet water. That was followed by Soxhlet extraction in ethanol. For 4 hours at 40°C, Soxhlet extracted 50 grams of drug powder. A rotary evaporator at 50°C thickened the extract after filtering it with No. 42 Whatman filter paper.

LCMS ANALYSIS

The HPLC separation technique used a gradient of A_1 and A_2 solvents. A_1 and A_2 contained 1/10 percent formic acid (v/v) in acetonitrile and water. A 1.0 mL/min flow rate produced a gradient of 10-30% A_2 in 20 minutes and 30% A_2 in 20-30 minutes. The UV detector scanned 254, 280, and 370 nm at a 10-milliliter injection volume. ESI-MS observed eluent from m/z 100 to 800 in positive ion mode. 3.5 kV of voltage and 60% of impact energy were used for ESI. Dry gas was 99.999 percent pure nitrogen at 350 °C and 12 L/min. They used 40 psi nebulizer nitrogen **[6]**.

MTT ASSAY

We employed the usual MTT test procedure to evaluate cell viability. To achieve serial dilutions varied from 62.5 to 1000 µg/mL, plant extracts were dissolved in Dimethyl sulfoxide and diluted using a cell culture medium. The vehicle control (DMSO) concentration was maintained between 0.1 and 1 percent or lower. Triton-x served as the positive control, while control cells were untreated cells with the same medium quantity. 4 × 104 adhering cells per well were grown in 96-well plates, and the plates were incubated at 37 °C for 24, 48, and 72 hrs. Next, a 20 µL solution of MTT (5 mg/mL) was introduced to each well and incubated for 4 hrs. In every well, 150 µL of DMSO was used to dissolve the formazan

crystals. At 490 nm, the absorption data was recorded using a microplate reader.

Oral SCC cell lines were seeded in 96-well plates at a density of 4 × 104 cells/mL, and the medium extracts were applied in dilution series. For 5 minutes at 1000 × g the 96 well plates were spun, 4°C in a centrifuge compatible with microplates after adequately cultivated for 24 and 48 hrs. After that, the medium was drawn and treated with the MTT solution, as previously raised. The absorbance of treated and untreated cells was compared to evaluate the vitality of the cells. Based on the concentrations of the plant extract, the IC_{50} values were computed, which led to a 50% suppression of cell growth **[7, 8]**.

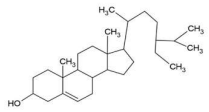
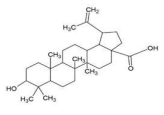
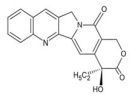
Cell viability was computed using the formula:

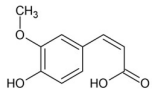
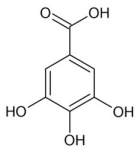
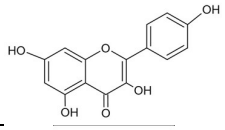
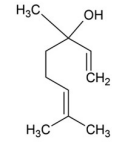
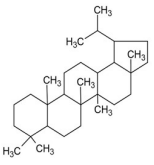
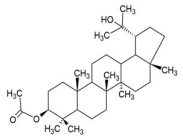
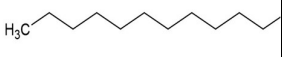
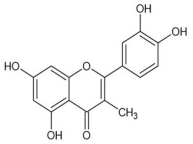
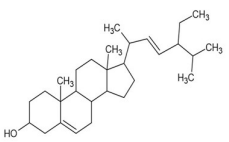
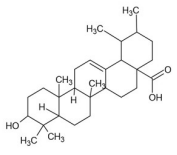
% Cell viability can be computed as Sample absorbance / Control absorbance × 100.

RESULTS

The identities of more than ten compounds, as well as their retention times, derived masses, theoretical masses, and mass fragment ions, were determined. The analysis of LC-MS data led to the identification of a total of fifteen compounds tabulated in (**Table 1)**. The spectra of MS of these compounds can be seen in (**Figure 1)**: (C_1) betasitosterol, (C_2) betulinic acid, (C_3) camptothecin, (C_4) doctriacontanoic acid, (C_5) ferulic acid, (C_6) gallic acid, (C_7) kaempferol, (C_8) linalool, (C_9) lupan, (C_{10}) lupan-20-ol-3(β)-yl acetate, (C_{11}) nonadecanoic acid, (C_{12}) hexadecanoic acid, (C_{13}) quercetin, (C_{14}) stigmasterol, (C_{15}) ursolic acid respectively.

Table 1: Phytocompounds identified through LC-MS in ethanolic extract of *Ophiorrhiza recurvipetala*

Compounds	t_R (min)	Mass (obtained)	Exact mass	Mass ion	Structures
Beta sitosterol	**17.537**	**415.65**	**414.71**	**$M+H^+$**	
Betulinic acid	**14.483**	**457.61**	**456.7**	**$M+H^+$**	
Camptothecin	**7.613**	**349.27**	**348.35**	**$M+H^+$**	
Doctriacontanoic acid	**10.121**	**453.73**	**452.82**	**$M+H^+$**	

Ferulic acid	**9.183**	**195.12**	**194.18**	**M+H$^+$**	
Gallic acid	**3.252**	**171.36**	**171.36**	**M+H$^+$**	
Kaempferol	**6.224**	**287.37**	**287.37**	**M+H$^+$**	
Linalool	**19.245**	**155.21**	**155.21**	**M+H$^+$**	
Lupan	**9.673**	**427.11**	**427.11**	**M+H$^+$**	
Lupan-20-ol-3(β)-yl acetate	**5.418**	**469.35**	**469.35**	**M+H$^+$**	
Nonadecanoic acid	**22.263**	**299.35**	**299.35**	**M+H$^+$**	
Hexadecanoic acid	**24.267**	**257.39**	**257.39**	**M+H$^+$**	
Quercetin	**8.611**	**303.17**	**303.17**	**M+H$^+$**	
Stigmasterol	**16.653**	**413.66**	**413.66**	**M+H$^+$**	
Ursolic acid	**15.659**	**457.71**	**457.71**	**M+H$^+$**	

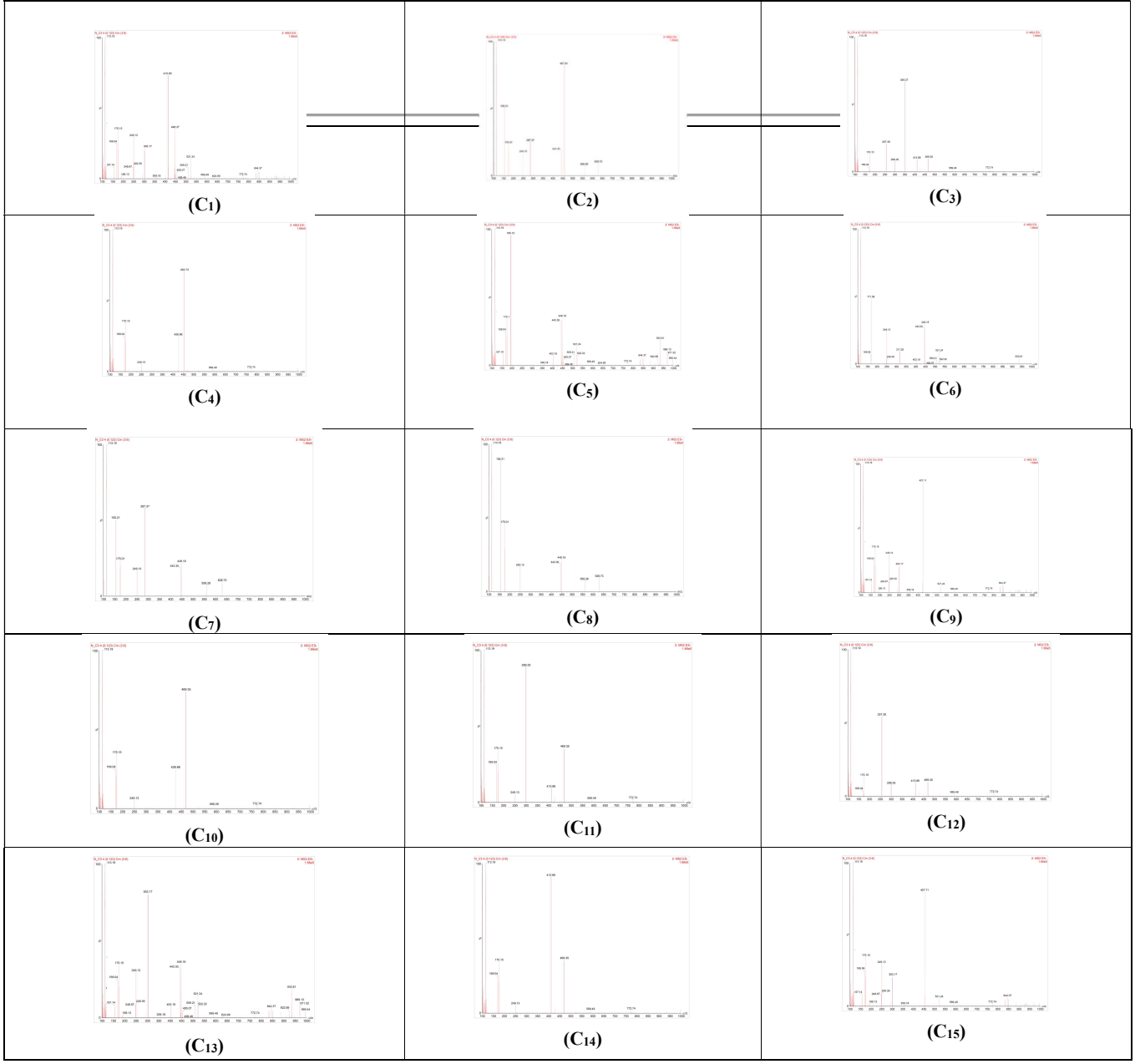


Figure 1: MS spectra of 15 phytoconstituents detected in *ophiorrhiza recurvipetala.* identities are listed in (table 1).

To evaluate the antiproliferative effect of an ethanolic extract of *Ophiorrhiza recurvipetala* on cell growth inhibition, dilutions from 62.5 to 1000 µg/ml was applied to the OSCC cell line for a duration of 24 hrs. MTT assay was employed to measure the viability of cells. The ethanolic extract of *Ophiorrhiza recurvipetala* slackened the cell viability in OSCC cell lines on its own **(Figure 1)**. *Ophiorrhiza recurvipetala* extract reduced OSCC cell proliferation. At high concentrations of 1000 µg/ml, extract of *Ophiorrhiza recurvipetala* slackened cell vitality by over 66.84% ($p < 0.01$), while at moderate doses (62.5-500 µg/ml), it slackened cell division by 23.86-51.87% ($p<0.05$). The ethanolic extract of *Ophiorrhiza recurvipetala* was classified as mildly active according to the NCI system, with an IC_{50} value of 264 µg/ml.

DISCUSSION

Ophiorrhiza plants possess medicinal properties but are not classified as drugs. These compounds have promising promise as lead molecules, particularly in the development of novel anti-cancer therapeutics. This could facilitate future researchers in gaining a more profound comprehension of diseases, providing more efficient treatments with a unique mechanism of action, enhancing patient adherence, reducing the adverse effects caused by synthetic anti-cancer drugs, and fostering the development of novel and innovative anti-cancer medications. Despite the great diversity of Ophiorrhiza species globally, there have been few investigations conducted.

We used LC/MS to look at the ethanolic extract of *Ophiorrhiza recurvipetala* and were able to identify 15 parts from different chemical families. *Ophiorrhiza recurvipetala* contains a variety of phytochemical compounds, including alkaloids (like camptothecin), polyphenols (viz., gallic acid, and ferulic acid), terpenoids (viz., betulinic acid, linalool, lupan, lupan-20-ol-3(β)-yl acetate, and ursolic acid), phytosterols (such as beta-sitosterol and stigmasterol), flavonoids (viz., kaempferol and quercetin), and long-chain fatty acids (viz., hexadecanoic acid, doctriacontanoic acid, and nonadecanoic acid). These compounds exhibit distinct activities biotically.

Camptothecin, is a frequently occurring modified monoterpene indole alkaloid in *Ophiorrhiza recurvipetala*. It has potent anti-cancer effects. There is a strong chemical called camptothecin in Ophiorrhiza plants that is very good at fighting cancer and stopping DNA topoisomerase I from working. By trapping the reaction intermediate, which is also known as the cleavable complex, while it breaks apart and puts itself back together, this part can selectively stop DNA topoisomerase I from working. So, it stops cancer cells from dividing and controls the production of microtubules, which makes the DNA topoisomerase even less effective **[9]**.

The extract of *Ophiorrhiza recurvipetala* contains various terpenoid components, including betulinic acid. Betulinic acid is a naturally occurring pentacyclic lupine-type triterpenoid. A lot of different medicinal and biological properties have been found for it. For example, it can stop the human immunodeficiency virus (HIV) and also fights bacteria, malaria, inflammation, parasites, pain, herpes, HSV-1, and cancer **[10]**. The pharmacological properties of lupane derivatives were said to have possible antiviral (specifically anti-HIV) and anti-cancer effects on these substances **[11]**. Linalool is a very effective aroma

enhancer for cosmetics, augmenting the preservation properties of the formulations utilized in them or serving as an anti-inflammatory agent for minor skin injuries. Prior research has demonstrated the utmost significance of this substance owing to its wide range of biological functions, such as its ability to serve as an antioxidant, reduce inflammation, combat cancer, protect the heart, and fight against pathogenic agents **[12]**. In recent years, it has been shown that ursolic acid has anti-cancer properties **[13]**. Additionally, Lupan-20-ol-3(β)-yl acetate has been reported to demonstrate antibacterial, anti-yeast, and fungicidal action.

It was discovered that the ethanolic extract of *Ophiorrhiza recurvipetala* contained ferulic acid and gallic acid, which are polyphenolic acids. Ferulic acid has many biological effects, such as removing metals from cells, changing enzyme activity, activating transcription factors, controlling gene expression, improving sperm viability, lowering inflammation, killing microbes, protecting the liver, preventing cancer, and stopping blood clots from forming **[14]**. Various fruits and medicinal plants contain a diverse range of natural phenolic chemicals, such as gallic acid. It is said to provide several advantages for your well-being. Gallic acid has antibacterial solid properties by inhibiting the motility, adhesion, and biofilm formation of several bacterial infections at a molecular level. Additionally, gallic acid has neuroprotective, anti-inflammatory, and cardioprotective properties, among others **[15]**.

Flavonoids, a subclass of phenolic chemicals, are characterized by the one or more hydroxyl substituents attachment and at least one aromatic ring. Previous research has confirmed that certain chemicals, namely quercetin and kaempferol, possess fungicidal, free radical scavenging, and antibacterial characteristics. Moreover, plants that include quercetin and kaempferol exhibit significant therapeutic potential for human well-being **[16]**. Furthermore, doctriacontanoic acid, hexadecanoic acid, and non-adecanoic acid, which are long chain fatty acids were detected.

It was found that the ethanolic extract of *Ophiorrhiza recurvipetala* was very effective at killing OSCC cell lines, with an IC_{50} value of 264 µg/ml. According to the National Cancer Institute (NCI) categorization, based on the ranges of IC_{50} value of substances categorised as highly active (<20 µg/ml), moderately active (21-200 µg/ml), and weakly active (201-500 µg/ml). If the IC_{50} value exceeds 500 µg/ml then the substances are categorised as inactive **[17]**. By the National cancer institute system categorization of anti-cancer drugs, it falls into the weakly active category. Nevertheless, the potential for many interactions among distinct

components in plant extracts might lead to surprising and intricate effects in herbal remedies.

Although there is an excellent range of variation around the globe, only a few numbers of Ophiorrhiza species have been extensively researched. In modern times, thanks to the progress in apparatus and equipment technology, it is now feasible to rapidly detect newly discovered bioactive compounds that possess properties capable of inhibiting cancer cells. Hence, further comprehensive examinations are required to ascertain the other bioactive chemicals present in this species and their therapeutic advantages.

CONCLUSION:

The ethanolic extract of *Ophiorrhiza recurvipetala* was carried out for LC-MS analysis and results confirmed the presence fifteen phytocompounds. Also, the anticancer activity of the extract was analysed by MTT assay and the results confirmed that the potency of extract was weakly active according to NCI system and further in vivo research studies could be carried out for this species to justify the hypothesis.

ACKNOWLEDGEMENTS

The authors are extremely thankful to The Oxford College of Pharmacy for conducting research work.

REFERENCES:

[1] Huang M, Lu JJ, Ding J. Natural products in cancer therapy: Past, present and future. Natural products and bioprospecting. 2021 Feb;11:5-13

[2] Theodoridis S, Drakou EG, Hickler T, Thines M, Nogues-Bravo D. Evaluating natural medicinal resources and their exposure to global change. The Lancet Planetary Health. 2023 Feb 1;7(2):e155-63.

[3] Salm S, Rutz J, Van Den Akker M, Blaheta R, Bachmeier B. Current state of research on the clinical benefits of herbal medicines for non-life-threatening ailments. Frontiers in Pharmacology. 2023 Sep 28;14:1234701

[4] Bhuyan B, Baruah S, Mehmud S. *Ophiorrhiza recurvipetala* (Rubiaceae) sp. nov. from Assam, India. Nordic Journal of Botany. 2021 Mar;39(3).

[5] Taher M, Shaari SS, Susanti D, Arbain D, Zakaria ZA. Genus Ophiorrhiza: A review of its distribution, traditional uses, phytochemistry, biological activities and propagation. Molecule

[6] Coppin JP, Xu Y, Chen H, Pan MH, Ho CT, Juliani R, Simon JE, Wu Q. Determination of flavonoids by LC/MS and anti-inflammatory activity in Moringa oleifera. Journal

of Functional Foods. 2013 Oct 1;5(4):1892-9.

[7] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983 Dec 16;65(1-2):55-63

[8] Somaida A, Tariq I, Ambreen G, Abdelsalam AM, Ayoub AM, Wojcik M, Dzoyem JP, Bakowsky U. Potent cytotoxicity of four cameroonian plant extracts on different cancer cell lines. Pharmaceuticals. 2020 Oct 31;13(11):357.

[9] Li F, Jiang T, Li Q, Ling X. Camptothecin (CPT) and its derivatives are known to target topoisomerase I (Top1) as their mechanism of action: did we miss something in CPT analogue molecular targets for treating human disease such as cancer?. American journal of cancer research. 2017;7(12):2350.

[10] Ghaffari Moghaddam M, Ahmad BH, Samzadeh-Kermani A. Biological activity of betulinic acid: a review. Pharmacology & Pharmacy. 2012;3(02):119-23.

[11] Tolstikova TG, Sorokina IV, Tolstikov GA, Tolstikov AG, Flekhter OB. Biological activity and pharmacological prospects of lupane terpenoids: I. Natural lupane derivatives. Russian Journal of Bioorganic Chemistry. 2006 Jan;32:37-49.

[12] Peana AT, Moretti MD. Linalool in essential plant oils: pharmacological effects. InBotanical medicine in clinical practice 2008 (pp. 716-724). Wallingford UK: CAB International.

[13] Seo DY, Lee SR, Heo JW, No MH, Rhee BD, Ko KS, Kwak HB, Han J. Ursolic acid in health and disease. The Korean journal of physiology & pharmacology: official journal of the Korean Physiological Society and the Korean Society of Pharmacology. 2018 May;22(3):235.

[14] Kumar N, Pruthi V. Potential applications of ferulic acid from natural sources. Biotechnology Reports. 2014 Dec 1;4:86-93.

[15] Kahkeshani N, Farzaei F, Fotouhi M, Alavi SS, Bahramsoltani R, Naseri R, Momtaz S, Abbasabadi Z, Rahimi R, Farzaei MH, Bishayee A. Pharmacological effects of gallic acid in health and diseases: A mechanistic review. Iranian journal of basic medical sciences. 2019 Mar;22(3):225.

[16] Jan R, Khan M, Asaf S, Lubna, Asif S, Kim KM. Bioactivity and therapeutic potential of kaempferol and quercetin: New insights for plant and human health. Plants. 2022 Oct 5;11(19):2623.

[17] Anywar GU, Kakudidi E, Oryem-Origa H, Schubert A, Jassoy C. Cytotoxicity of medicinal plant species used by traditional healers in treating people suffering from HIV/AIDS in Uganda. Frontiers in Toxicology. 2022 May 2;4:832780.