



## Original Research Article

# *In vitro* immuno-stimulatory and anticancer activities of *Oroxylum indicum* (L.) Kurz.: An evidence for substitution of aerial parts for conservation



Deeksha Rai <sup>a</sup>, H.N. Aswatha Ram <sup>b,\*</sup>, K. Neeraj Patel <sup>c</sup>, U.V. Babu <sup>c</sup>, L.M. Sharath Kumar <sup>c</sup>, R. Kannan <sup>c</sup>

<sup>a</sup> Department of Pharmacognosy, Yenepoya Pharmacy College & Research Centre, Yenepoya Deemed to be University, Mangalore, 575018, India

<sup>b</sup> Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, 576104, India

<sup>c</sup> Phytochemistry Division, R and D Center, The Himalaya Drug Company, Makali, Tumkur Road, Bangalore, 562162, India

## ARTICLE INFO

## Article history:

Received 8 January 2021

Received in revised form

1 September 2021

Accepted 2 September 2021

Available online 22 November 2021

## Keywords:

Leaves

*Oroxylum indicum* (L.) Kurz.

Roots

Stems

## ABSTRACT

**Background:** In Ayurveda, “Dashamoolarishta” is one of the important composite herbal formulations. Mainly, the root and root bark of *Oroxylum indicum* are used as one of the ingredients in its preparation. This leads to over exploitation of medicinal plants owing to excessive demand due to population expansion and its perceived importance in traditional herbal remedies.

**Objective:** For the conservation of biodiversity, the present investigation had an objective to prepare the extracts of different parts of *O. indicum* plant and to, compare the chemo-profiles as well as to study the biological activities of the prepared extracts.

**Materials and methods:** Hydro-alcoholic (HA) and aqueous (Aq) extracts of various plant parts were prepared and chemical investigation was done with the help of (LC-MS/MS). Further, *in vitro* biological activities such as immuno-stimulation (IS) using a cytokine bioassay in RAW264.7 and *in vitro* anticancer in TNF- $\alpha$  ELISA in THP-1 cells were studied.

**Results:** The mass spectral profile of the plant revealed the presence of markers such as oxoxylin A and chrysin in HA and Aq extracts of stem, leaf, bark and root. Cytokine release and TNF- $\alpha$  secretion was observed in both hydro-alcoholic and aqueous extracts.

**Conclusion:** Based on the results from the present study, it can be concluded that it is possible to replace the roots and the bark of *O. indicum* with the stem of young plants and leaves. It paves a way for the conserving the medicinal plants without uprooting and extinguishing the whole plant.

© 2021 The Authors. Published by Elsevier B.V. on behalf of Institute of Transdisciplinary Health Sciences and Technology and World Ayurveda Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

At present the demand for herbal products, secondary metabolites derived from the medicinal plants and the use of medicinal plants is growing swiftly worldwide [1,2]. According to International Union for Conservation of Nature and the World Wide Fund for Nature, there are about 50,000–80,000 species of plants which are used for the medicinal purposes around the world. Of these, around 15,000 species are going to become

extinct due to over harvestation and natural habitat destruction [3]. About 20% of resources from the wild sources have been exhausted due to the increase in the population and utilization of plants in large quantities [4].

There is an immediate need to create awareness regarding conserving and sustaining of medicinal plants for their usefulness. A detailed study of phytochemicals and pharmacological evaluation of various plant parts is required for this purpose. This is the requirement for the strategy of substitution of plants or plant parts [5].

In the recent past, exploitation of medicinal plants has been carried out owing to the population expansion and increased use of conventional herbal medicines. This resulted in delayed growth and slow reproduction and thus making the medicinal plants prone

\* Corresponding author.

E-mail: [aswatharam@gmail.com](mailto:aswatharam@gmail.com)

Peer review under responsibility of Transdisciplinary University, Bangalore.

to extinction. To overcome this, there is a need to enforce conservation of plant materials, large scale cultivation or to substitute parts of the plant such as young stem and leaves instead of roots without destroying the whole plant. It is necessary to evaluate the resemblance and variations among plant parts with respect to their chemical principles and biological properties [5,6].

Similar studies in India are carried out and exemplified for *Musta* and *Nagaramusta*. The first one *Cyperus rotundus* L. (*Musta*) can be used in place of *Aconitum heterophyllum* Wall. ex Royle (*Ativisha*) based on the approach and theory concerning phytochemical and pharmacological evaluation [7]. The latter example is where, Ayurvedic practitioners use *Cyperus scariosus* R. Br. (*Nagaramusta*) as alternates for *Musta* [8].

*Oroxylum indicum* (L.) Kurz. (Family: Bignoniaceae) commonly known as Indian trumpet flower or broken bones is an important plant traditionally used in polyherbal formulations such as “*Chyawanprash*”, “*Dashamoolarishta*” and “*Kutajarishtha*”, etc., [5,6,9]. The tree being overused and vulnerable, is facing the risk of getting endangered. The tree may become extinct in near future and would thus enter red data book [10]. In our work leaf, stem, root and bark were considered for chemical analysis. The aerial parts such as leaf and young stem may be considered as substitute for the vital parts of the plant i.e., roots and bark for the conservation of the species. The current study has also focused on the sustainability of plant using aerial parts such as leaves and young stems which can regrow from the trees. Since, harvesting flowers and fruits affect the seed formation and regeneration process, we used only young stems and leaves in our study.

The plant had been reported to contain several constituents such as flavonoids, iso-flavonoids, triterpenoids, steroids and others. Of these constituents, Oroxylin A and Chrysin as the major flavonoids [11]. The Ayurvedic Formulary of India (AFI) has suggested the use of stem bark as substitutes for roots of *Dashamoolarishta* [12]. Therefore, the present study was aimed at comparing the leaves and young stems with roots and bark of *O. indicum* with respect to their LC-MS profiles. The plant is already reported to possess immunostimulatory activity via enhancement of humoral immunity and phagocytosis in the body [13]. The reported pharmacological activities include antimicrobial, anti-inflammatory, anti-arthritis, anticancer, anti-ulcer, hepato-protective, anti-diabetic, antidiarrheal and anti-oxidant were exhibited by its extracts and isolates [14]. Hence, the present study also aimed at the screening of extracts for immuno-stimulatory and anti-cancer activities by *in vitro* assays.

## 2. Materials and Methods

### 2.1. Plant materials

Leaves (NPD/569/15); young stems (NPD/404/15); bark (NPD/549/15) and roots (NPD/739/15) of *O. indicum* (L.) Kurz. were collected from various parts of Karnataka, India and Andhra Pradesh, India and it was authenticated by Dr. Kannan R., Ph.D, Principal Scientist, The Himalaya Drug Company, Bangalore, Karnataka, India and voucher specimens were deposited. The plant name has been checked with <http://www.theplantlist.org> (Accession date: 16.05.2015).

### 2.2. Chemicals and solvents

Ultra-pure water (Elga, Veolia water, Paris, France) and LCMS grade acetonitrile/methanol (J.T. Baker, Center Valley, PA, USA) were used. LR. grade ethanol was purchased from Chanshu Hongsheng Fine Chemicals Co. Ltd., China. Extracts were concentrated using a vacuum rotary evaporator (Büchi R-215, Switzerland). LC-

MS analysis was carried out using HPLC SHIMADZU LC-20AD which is coupled with an API-2000 mass spectrometer-MS/MS, [Applied Biosystem/MDS SCIEX, Canada]. Cell culture media and biochemical reagents were obtained from Himedia, Mumbai, India.

### 2.3. Extraction

300 g each of young stems, leaves, barks and roots of *O. indicum* (L.) Kurz. were taken individually in a macerator and extraction was carried out separately with 70% ethanol in de-mineralized water and 100% de-mineralized water with 2 L for 24 h and this process was repeated thrice. Extracted solution was filtered through Whatman filter paper No. 1 and three washes were combined, dried *in vacuo* to provide the hydro-alcoholic (young stems, OHAS; leaves, OHAL; bark, OHAB; roots, OHAR) and aqueous (young stems, OAS; leaves, OAL; barks, OAB; roots, OAR) extracts respectively and the yields are presented in Table 1.

The aqueous and hydro alcoholic extract, we processed to produce the final product which was available in dry powdered form. Before use with cells, either dry powder was weighed and reconstituted in distilled water as per the concentration required and then added to the culture for treatment. The vehicle used for all cell culture studies was sterilized distilled water.

### 2.4. Liquid chromatography tandem mass spectroscopy (LC-MS/MS)

LC-MS comprises of an HPLC (Shimadzu LC-20AD) coupled with an API-2000 mass spectrometer-MS/MS, [Applied Biosystem/MDS SCIEX, Canada]. 2 mg/mL concentration of the sample was prepared in LC-grade methanol and injected via the SIL-HTC Shimadzu autosampler.

The MS machine parameters and the experimental conditions were followed to obtain MS/MS spectrum. An API-2000 mass spectrometer along with electron spray ionization (ESI) interface was applied. Batch acquisition as well as data processing were regulated by Analyst 1.5 version software. Curtain gas (CUR) 25 psi; Focusing potential (FP) -400 V; Entrance potential (EP) -10 V; Ionization source temperature 420 °C; Ion source Gas 1 (GS1) 50 psi; ion source Gas 2 (GS2) 60 psi and the chromatographic conditions mentioned below.

The deprotonated molecular ion  $[M-H]^-$  of Oroxylin A and Chrysin obtained at  $m/z$  283.1 and  $m/z$  253.1 Further, both parent ion subjected to product ion scan yielded  $m/z$  267.8 and  $m/z$  142.7 at collision energy -25 V and -35 V, respectively.

The separation was achieved through Maisch GmbH C18 Reprosil-Pur Basic (100 × 3 mm, 3 μm) column and column temperature 40 °C was maintained through Shimadzu CTO-10 AS VP column oven. Mobile phase was delivered at flow rate of 0.5 mL/min through Shimadzu LC-20 AD series binary gradient pump with Shimadzu DGU-20A3 degasser. The mobile phase composed of solvent-A which contains water with 0.1% formic acid as well as solvent-B as a mixture of acetonitrile and water in 70:30 ratios. The solvent B was linearly ramped from 20% to 50% in 8 min and was further ramped to 80% in 7 min. The solvent B was brought to initial concentration in 3 min and re-equilibrated for

**Table 1**  
Extractive yield (% w/w) of *Oroxylum indicum* extracts.

S. No.	Plant part	Hydro-alcoholic ext.	Aqueous ext.
1.	Leaves	3.8	4.2
2.	Young stems	3.1	4.1
3.	Bark	1.2	3.3
4.	Root	3.3	4.0

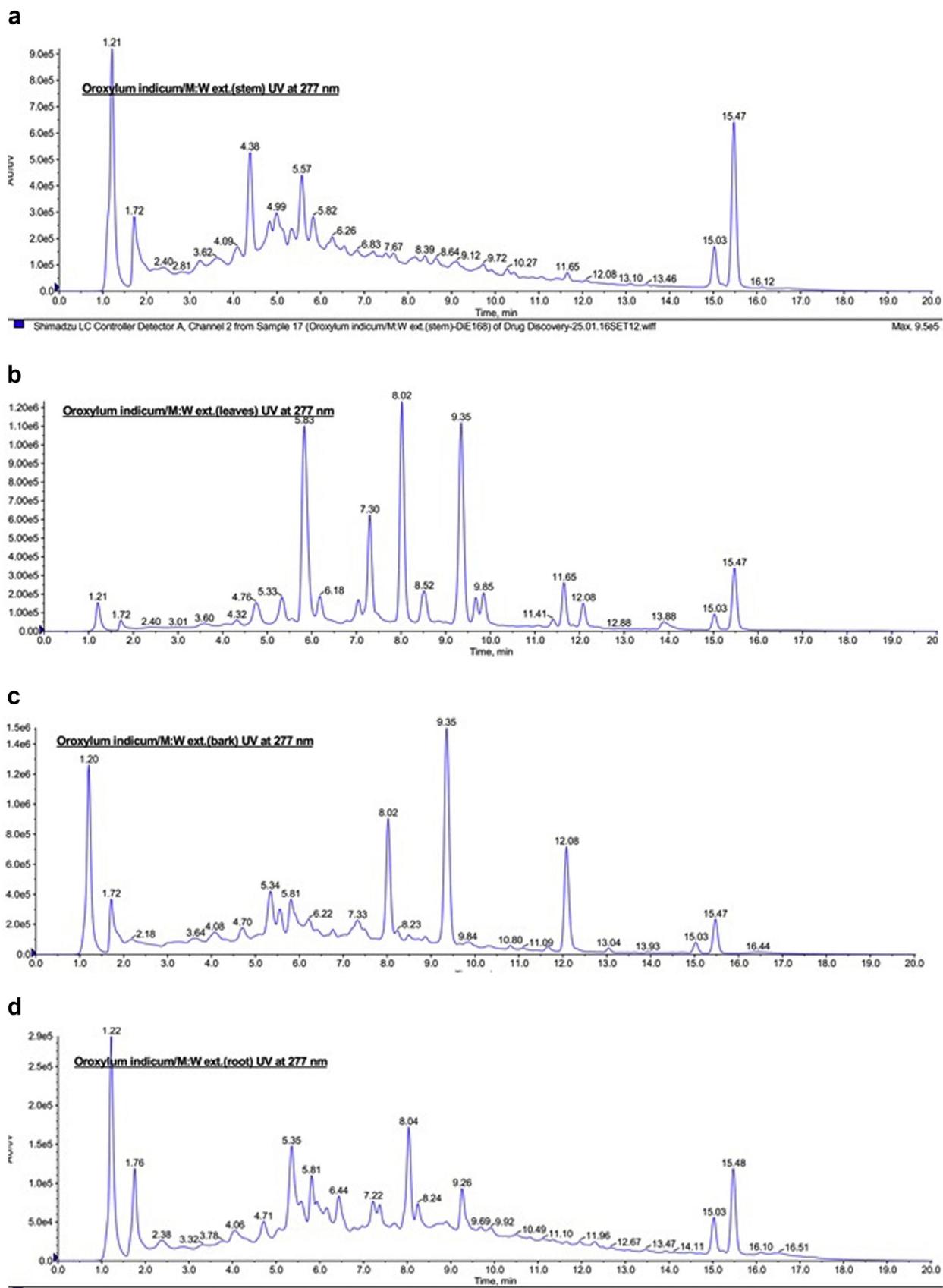


Fig. 1. a–d) LC-MS chromatograms of *Oroxyllum indicum* extracts at 277 nm and Mass fragmentation with the R<sub>t</sub> 15.03 (Chrysin) and 15.47 (Oroxylin A).

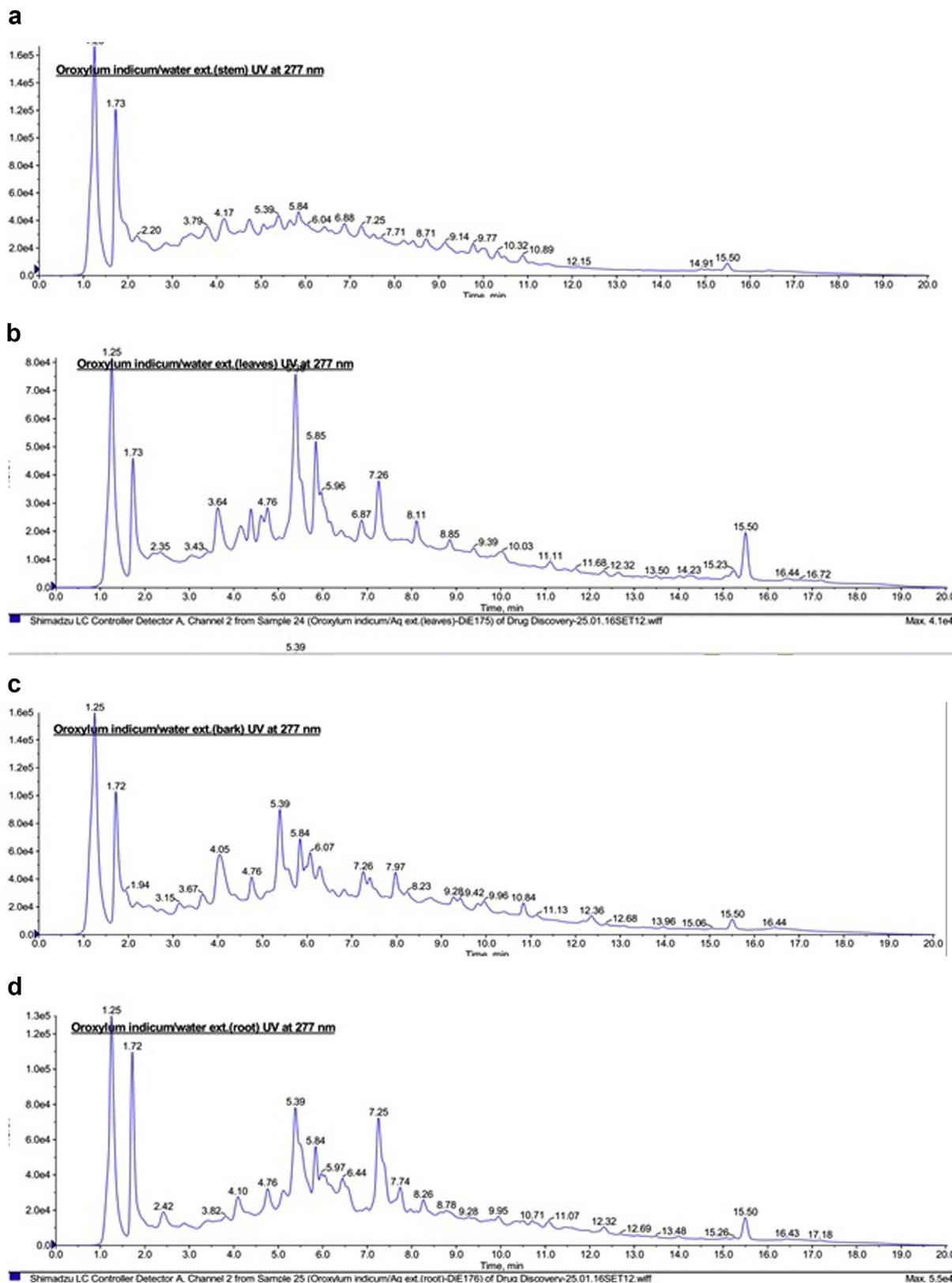
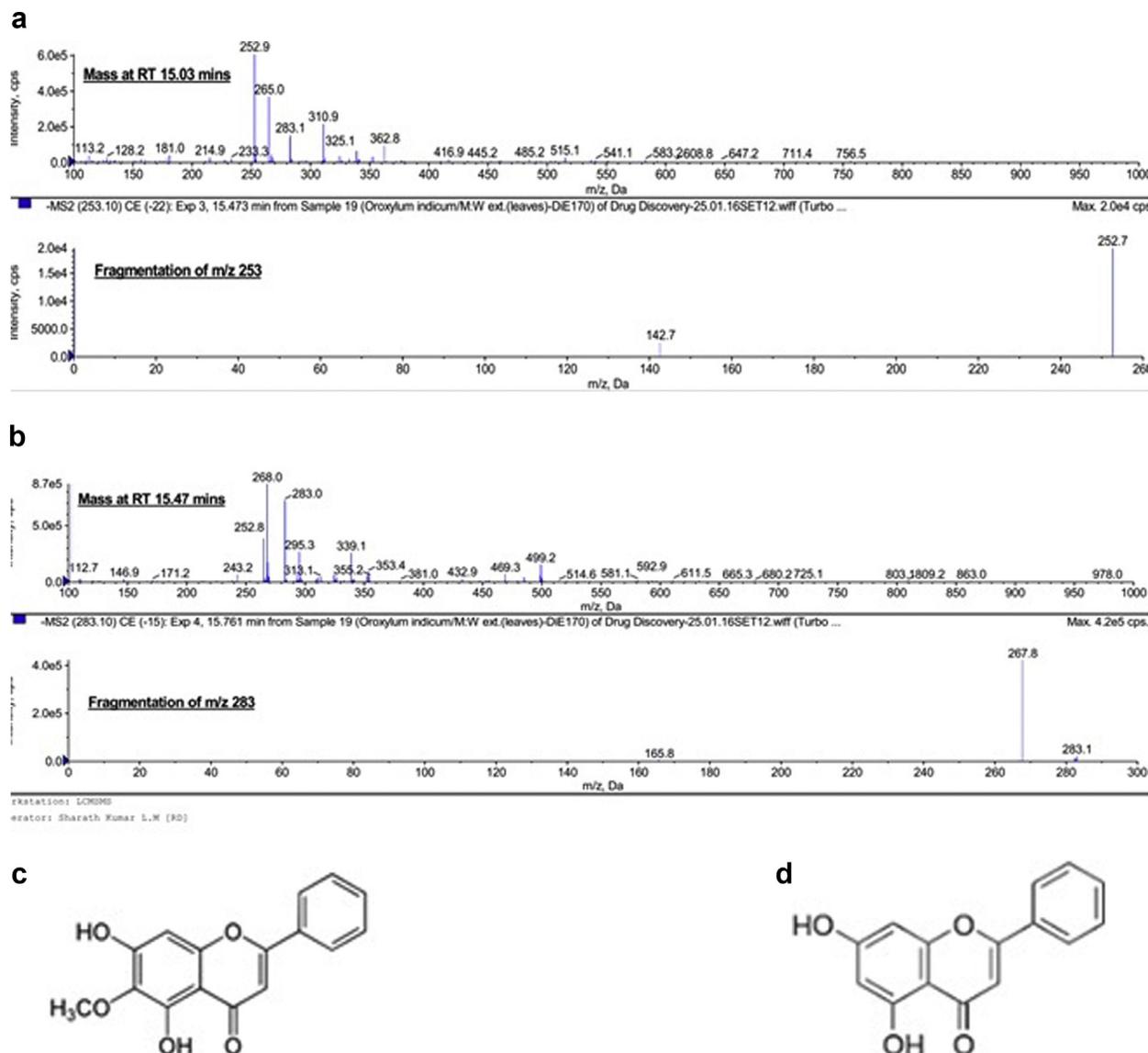


Fig. 2. a–d) LC-MS chromatograms of *Oroxyllum indicum* extracts at 277 nm and Mass fragmentation with the  $R_f$  15.03 (Chrysin) and 15.47 (Oroxylin A).

an additional 2 min. Chromatographic Peak was monitored at 277 nm and 324 nm. The injection volume 20  $\mu$ L was injected through Shimadzu SIL-HTC autosampler and 20 min. was the total analysis run time.

### 2.5. Cell culture

RAW 264.7 cells (macrophage derived), THP-1 and L929 cells were acquired from National Center for Cell Science (NCCS), Pune,



**Fig. 3.** a–d) LC-MS chromatograms of *Oroxyllum indicum* extracts at 277 nm and Mass fragmentation with the  $R_t$  15.03 (Chrysin) and 15.47 (Oroxylin A) and the chemical structures of major chemical constituents.

India and it was grown in 96 well plates in DMEM HG, RPMI and DMEM low glucose correspondingly with 10% FBS and were incubated at 37 °C with 5% CO<sub>2</sub>.

## 2.6. Cell viability assay

RAW 264.7 cells and THP-1 cells were grown in 96 well plates in DMEM HG and RPMI media respectively, with 10% FBS and it was incubated for assay purpose. The initial stock solution of test product (10 mg/mL) was prepared by dissolving in DMEM HG medium with 2% FBS and subsequent dilutions were prepared to achieve concentrations of 1000, 500, 250, 125, 62.5, 31.25 and 15.62 µg/mL. The dilutions (100 µL/well) were added to the RAW264.7 and THP-1 cells and the plates were incubated at 37 °C with 5% CO<sub>2</sub>. Cell control and positive control (SDS) were also maintained. The morphological changes in the cells were observed after 24 h of incubation using an inverted microscope and cell viability was assessed by MTT assay where 10 µL of MTT (5 mg/mL) solution was added into each well and incubated for 4 h at 37 °C. Once the cell supernatant was

discarded, the dye bound to the cell was extracted by adding 100 µL of DMSO into each well. Absorbance was measured at 540 nm and percentage toxicity was assessed from the absorbance values of the groups treated with drug and untreated with drug.

## 2.7. Tumor necrosis factor (TNF)- $\alpha$ stimulatory assay and ELISA

TNF assay in RAW 264.7 cells was carried out as described previously [15,16]. RAW264.7 cells were treated with *Oroxyllum* extracts at two non-toxic concentrations for 24 h and the levels of TNF- $\alpha$  in the cell culture supernatant was measured on L929 cells. Cell control and positive standard (LPS -1 µg/mL) was also maintained. The TNF- $\alpha$  induced toxicity in L929 cells was determined by MTT assay.

THP-1 cells ( $1 \times 10^6$ /mL) were treated with test samples at 100 and 200 µg/mL and it was incubated for 24 h at 37 °C with 5% CO<sub>2</sub>. Cell control as well as LPS control (1 µg/mL) were maintained. Cell free conditioned media were assayed for TNF- $\alpha$  by commercially available kit (Krishgen Biosystems, India).

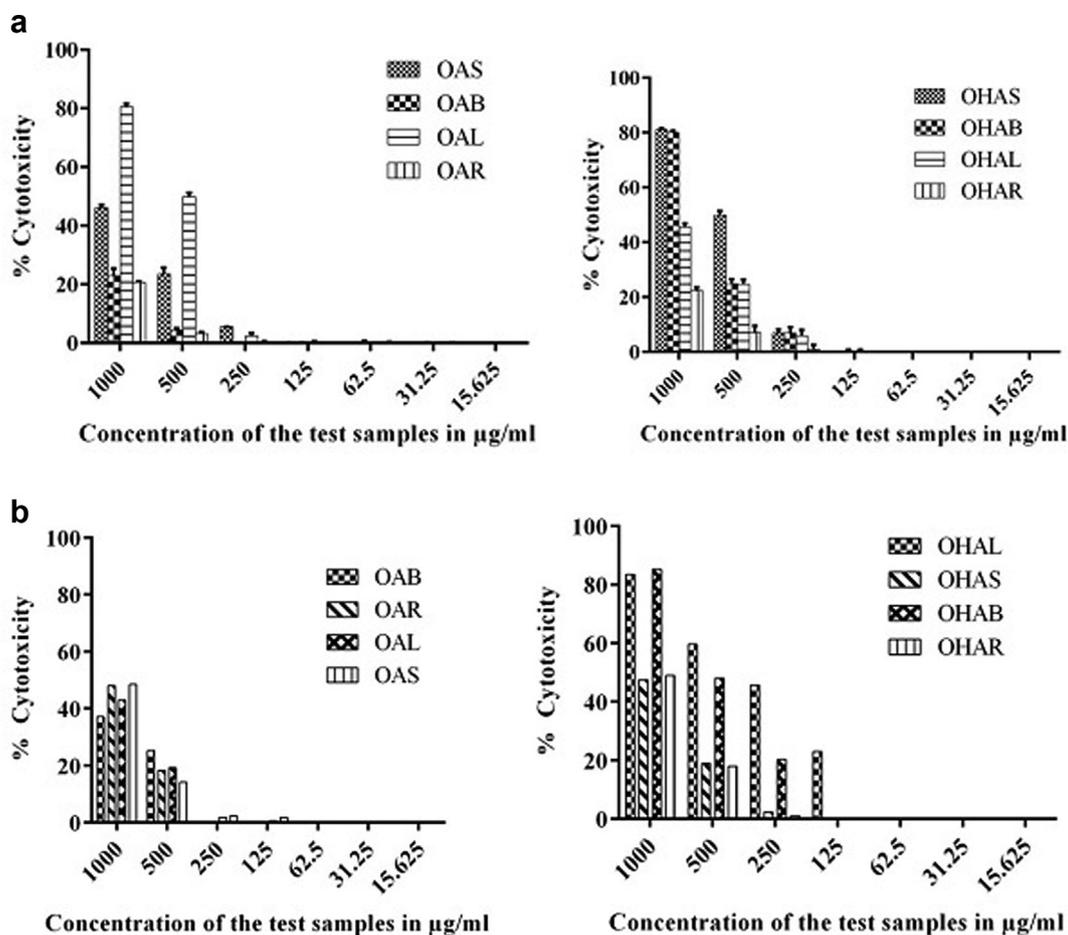


Fig. 4. a and b) Cytotoxic effect of extracts on RAW 264.7 and THP-1 cells.

### 2.8. Statistical analysis

Results were depicted as mean ± S.E.M. and statistical analysis was conducted by One-way ANOVA with Dunnett’s post-test using Graph Pad Prism version 4.01 for Windows, GraphPad Software, San Diego California USA. ‘P’ value less than 0.05 was regarded as statistically significant.

### 3. Results and discussion

Roots of *O. indicum* (L.) Kurz. rich in flavonoids are commercially exploited and generally, its aqueous extracts are used in polyherbal formulations such as “Chyawanprash”, “Dashmoolarishta” and “Kutajarishtha” etc. that results in step-down and rooting out of this plant. Therefore, it is necessary to collect and substitute the parts of the plant such as leaves and young stems rather roots and bark [5,6]. *O. indicum* (L.) Kurz. reported to have immunostimulatory activity via enhancement of humoral immunity phagocytosis of the body [13]. Cytokines maintain tissue homeostasis and mediate immunological responses during chronic and acute infections [17]. A variety of cytokines are released by immune cells, against infectious pathogens such as virus, bacteria, fungus, toxins etc. depending on various protective mechanisms. The cytokines include tumor necrosis factor (TNF), interferons, interleukins, colony stimulating factors (CSF) and transforming growth factors (TGF) [18]. Drug candidates known to alter the immune responses by modulating immune signalling molecules are of importance in various therapeutic

conditions [19]. Natural products of herbal origin with immunomodulatory activity has been reported for a long time and is generally considered safe and used as adjuvants in modulating immune responses [20].

In the present study, chemical profile of the young stems, leaves, barks and roots of *O. indicum* (L.) Kurz. were compared by LC-MS/MS. Oroxylin A and Chrysin belongs to the flavone class and responsible for varied pharmacological activities attributing to stem, leaf, root and bark of this plant. Flavonoid compound Oroxylin A (5’7-dihydroxy-6-methoxy-2phenyl-4H-1-benzopyran-4-one) [21] was reported to possess anti-cancer activity in human ovarian cancer [22], human breast cancer cells [23], induces differentiation of t (8; 21)-positive Kasumi-1 and primary acute myeloid leukemia cells [24]. Another important constituent is Chrysin which is a hydroxylated flavone [25] and it is capable of killing lung, breast, cervical, liver, leukemia, colon, nasopharyngeal, prostate, glioblastoma, thyroid and pancreatic cancer cells in both in vitro and in vivo models [26]. Therefore, Oroxylin A and Chrysin were chosen as biomarkers for the current study. Extracts were also evaluated for immunostimulatory activity using ELISA in RAW 264 cells.

Results revealed that there was not majorly difference in the aqueous extraction yield of plant materials (~4%) except bark (3.3%). Hydro-alcoholic and aqueous extracts of stems, barks, leaves and roots were found to be qualitatively chemical similar and revealed the presence of marker compounds such as oroxylin A (R<sub>f</sub> 15.47) and chrysin (R<sub>f</sub> 15.03) at 277 nm (Figs. 1–3a–d).

Extracts showed varied toxicity to RAW 264.7 and THP-1 cells and the Cytotoxic effect of extracts on RAW 264.7 and THP-

1 cells (Fig. 4a and b). The aqueous extracts were mostly non-toxic to RAW 264.7 and THP-1 cells at test concentrations (15.62–1000 µg/mL) whereas the hydro-alcoholic extracts of bark showed moderate toxicity for RAW264.7 and THP-1 cells. Hence, two non-toxic concentrations (100 and 200 µg/mL) were used for further studies.

In the present study, the toxicity exhibited by L929 cells was used as the measure to assess the TNF-α release by the drug treatment. In other words, the viability of L929 cells was inversely proportional to the TNF-α levels secreted to the culture medium by RAW264.7 cells as a response to the drug.

Extracts elicited cytokine production in RAW264.7 cells. The percentages of cytokine release in aqueous extracts were 41.30 ± 5.60, 25.50 ± 4.90, 21.40 ± 4.20 and 20.30 ± 2.90% for OAS, OAL, OAR and OAB at 200 µg/mL, respectively. The percentages of cytokine release in hydro-alcoholic extracts were 57.60 ± 4.10, 52.90 ± 9.40, 35.30 ± 2.0 and 13.70 ± 2.70% for OHAB, OHAL, OHAS and OHAR at 200 µg/mL, respectively (Fig. 5a and b). In the present study, LPS-a known TNF-α stimulator was used as positive control; which at 1 µg/mL showed a 309.85 pg/mL elevation of TNF-α production.

The TNF-α in the cell culture supernatant was quantitatively estimated using a commercially available ELISA kit. The untreated THP-1 cells exhibited a basal release of TNF-α at a concentration of 11.28 (±0.29) pg/mL. The THP-1 cells treated with LPS (1 µg/mL) showed an increase in the TNF α levels, which was 54.63 ± 3.12 pg/mL. At 200 µg/mL, maximum TNF-α secretion was found in OHAL

extracts (98 ± 5.8 pg/mL) followed by OHAR (70.90 ± 2.50 pg/mL), OHAS (66.10 ± 8.90 pg/mL) and OHAB (16.60 ± 4.40 pg/mL). Among the aqueous extracts, OAS showed the maximum TNF-α secretion followed by OAB, OAR and OAL. The percentages of TNF-α release in aqueous extracts were 175.70 ± 14.10, 143.20 ± 17.60, 128.30 ± 10.3 and 112.80 ± 0.60 for OAS, OAB, OAR and OAL at 200 µg/mL respectively. The Immuno-stimulatory effects of aqueous and hydro alcoholic extracts are shown in Fig. 5a and b.

In the *in vitro* experiment, the objective was to study the effects of extracts on anti-cancer activity. “Tumor Necrosis Factor” (TNF-alpha) was recognized as the serum mediator of innate immunity having the ability of inducing hemorrhagic necrosis in tumor and it has the capacity to directly kill tumor cells *in vitro* [27]. Immuno-stimulatory cytokines like TNF-α, IL-1β, MIP-1α act as effective messenger molecules in an immune response. A well balanced secretion of these cytokines under normal conditions ensures proper functioning of the immune system. An agent which stimulates the secretion of immunostimulatory cytokines resulting in dendritic cell activation and subsequently enables efficient functioning of T-cells is said to possess strong immunostimulatory activity. In the present study, a substantial increase was observed in secretion of cytokines and TNF-α from RAW 264.7 and THP-1 cells after 24 hours of treatment with extracts, indicating the immuno-stimulatory potential of *O. indicum*. This could be attributed to the constituent, a polyphenolic compound chrysin which block TNF-α triggered ICAM-1 expression by inhibiting ERK, JNK and P38 in epithelial cells [28,29].

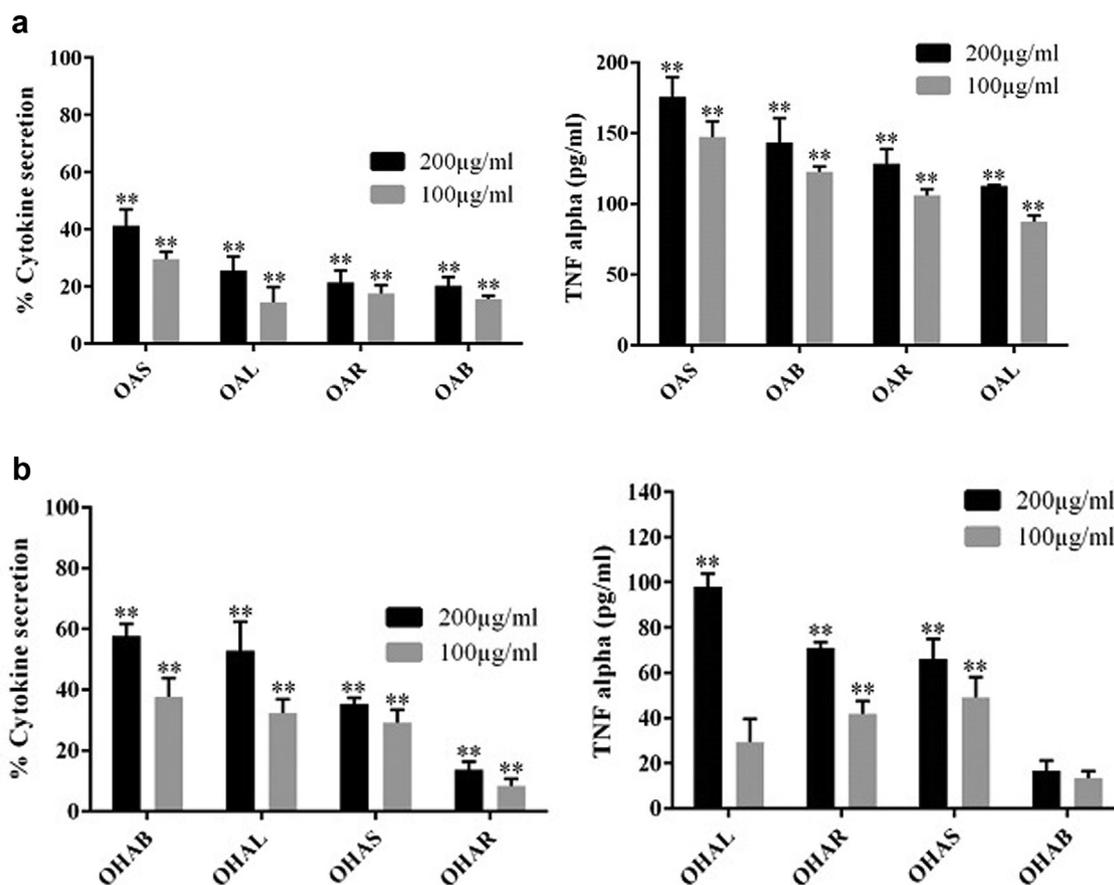


Fig. 5. a) Immuno-stimulatory effects of aqueous extracts on RAW 264.7 (left) and THP-1 (right) cells. OAB: Bark; OAL: Leaves; OAS: Stem and OAR: Root (\*\*p < 0.05). b) Immuno-stimulatory effects of hydro alcoholic extracts on RAW 264.7 (left) and THP-1 (right) cells. OHAB: Bark; OHAL: Leaves; OHAS: Stem and OHAR: Root (\*\*p < 0.05).

#### 4. Conclusion

The present study is an attempt to evaluate the young stems, bark, leaves and roots of *O. indicum* based on their phyto-chemical and pharmacological parameters. Liquid chromatography mass spectrometric analysis confirmed the presence of oroxylin A ( $R_t$  15.47) and chrysin ( $R_t$  15.03) in aqueous and hydro-alcoholic extracts in the plant parts studied. All the extracts showed mild to moderate cytokine secretion and TNF- $\alpha$  release. Thus, the leaves and young stems can be explored as a substitute to bark and roots for its therapeutic benefits in pharmaceutical industries. The experimental results also warrant further mechanistic studies to evaluate and compare the immune-modulatory activity of the different plant parts of this plant. This investigation also aids in biodiversity conservation. The leaves and young stems can be used as substitute to bark and roots, thereby preventing mass destruction of *O. indicum*.

#### Grants

Nil.

#### Author contributions

Deeksha Rai: The experiment was carried out as PG program student at the R & D Centre, at Himalaya Drug Company, Bangalore.

H.N. Aswatha Ram: As guide for Deeksha Rai, student of MPharm program, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, MAHE, Manipal.

K. Neeraj Patel: Concept of the study and Technical Input.

U.V. Babu: Concept and drafting of the manuscript.

L.M. Sharath Kumar: LC-MS analysis and analytical part.

R. Kannan: Authentication and Selection of the Plant material.

#### Acknowledgement

The authors sincerely thank Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, India and The Himalaya Drug Company, Makali, Bangalore, India for providing the necessary facilities to carry out this work.

#### References

- [1] Nalawade SM, Sagare AP, Lee CY, Kao CL, Tsay HS. Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. *Bot Bull Acad Sin (Taipei)* 2003;44(2):79–98.
- [2] Cole IB, Saxena PK, Murch SJ. Medicinal biotechnology in the genus *Scutellaria*. *In Vitro Cell Dev Biol Plant* 2007;43(4):318–27.
- [3] Bentley R. Medicinal plants. London: Domville-Fife Press; 2010.
- [4] Ross IA. Medicinal plants of the world: chemical constituents, traditional and modern medicinal uses. New York, NY: Humana Press; 2005.
- [5] Zschocke S, Rabe T, Taylor JL, Jäger AK, Van Staden J. Plant part substitution—a way to conserve endangered medicinal plants? *J Ethnopharmacol* 2000;71(1–2):281–92.
- [6] Jena AK, Karan M, Vasisht K. Plant parts substitution based approach as a viable conservation strategy for medicinal plants: a case study of *Premna latifolia* Roxb. *J Ayurveda Integr Med* 2017;8(2):68–72.
- [7] Venkatasubramanian P, Kumar SK, Nair VS. *Cyperus rotundus*, a substitute for *Aconitum heterophyllum*: studies on the Ayurvedic concept of Abhava Pratinidhi Dravya (drug substitution). *J Ayurveda Integr Med* 2010;1(1):33.
- [8] Nagarajan M, Kuruvilla GR, Kumar KS, Venkatasubramanian P. Pharmacology of *Ativisha*, *Musta* and their substitutes. *J Ayurveda Integr Med* 2015;6(2):121.
- [9] Aparna S, Ved DK, Lalitha S, Venkatasubramanian P. Botanical identity of plant sources of *Dasamula* drugs through an analysis of published literature. *Ancient Sci Life* 2012;32(1):3.
- [10] Samatha T, Rama Swamy N. Conservation of an endangered medicinal forest tree species, *Oroxylum indicum* L. Kurz, through in vitro culture: a review. In: Khasim SM, Long C, Thammasiri K, Lutken H, editors. *Medicinal plants: biodiversity, sustainable utilization and conservation*. Singapore: Springer; 2020. p. 769–83.
- [11] Jagetia GC. A review on the medicinal and pharmacological properties of traditional ethnomedicinal plant sonapatha. *Oroxylum indicum* *Sinusitis* 2021;5(1):71–89.
- [12] Formulary – single Drugs, List of single drugs of plant origin. The Ayurvedic formulary of India. 2nd ed., vol. 1. New Delhi: Government of India, Ministry of Health and Family Welfare; 2000. p. 307–30.
- [13] Gohil P, Zaveri M, Jain S. Immunomodulatory activity of n-butanol extract of *Oroxylum indicum*. *Pharm Biol* 2008;46(12):914–9.
- [14] Dinda B, Sil Sarma I, Dinda M, Rudrapaul P. *Oroxylum indicum* (L.) Kurz, an important Asian traditional medicine: from traditional uses to scientific data for its commercial exploitation. *J Ethnopharmacol* 2015;161:255–78.
- [15] Sandeep Varma R, Ashok G, Vidyashankar S, Nandakumar KS, Patki PS. Bresol inhibits phosphodiesterase 4 gene expression and modulates the levels of select mediators of inflammation in human monocytic cells. *J Immunol* 2011;8(4):315–23.
- [16] Sandeep Varma R, Ashok G, Vidyashankar S, Patki P, Nandakumar KS. Anti-inflammatory properties of Septilin in lipopolysaccharide activated monocytes and macrophage. *Immunopharmacol Immunotoxicol* 2011;33(1):55–63.
- [17] Liu E, Moriyama H, Abiru N, Miao D, Yu L, Taylor RM, et al. Anti-peptide autoantibodies and fatal anaphylaxis in NOD mice in response to insulin self-peptides B: 9-23 and B: 13-23. *J Clin Invest* 2002;110(7):1021–7.
- [18] Roitt I, Brostoff J, Male D. Immunology. 5th ed. Philadelphia: Mosby; 1998.
- [19] Gautam M, Saha S, Bani S, Kaul A, Mishra S, Patil D, et al. Immunomodulatory activity of *Asparagus racemosus* on systemic Th1/Th2 immunity: implications for immunoadjuvant potential. *J Ethnopharmacol* 2009;121(2):241–7.
- [20] You HJ, Choi CY, Kim JY, Park SJ, Hahm KS, Jeong HG. Ursolic acid enhances nitric oxide and tumor necrosis factor- $\alpha$  production via nuclear factor- $\kappa$ B activation in the resting macrophages. *FEBS Lett* 2001;509(2):156–60.
- [21] Lu L, Guo Q, Zhao L. Overview of oroxylin A: a promising flavonoid compound. *Phytother Res* 2016;30(11):1765–74.
- [22] Shen JJ, Zhu XF, Xu J, Wang ZF, Gu WJ, Chen Y. Oroxylin A exerts anticancer effects on human ovarian cancer cells via the PPAR $\gamma$ -dependent reversal of the progesterone receptor membrane component 1/2 expression profile. *Oncol Rep* 2020;43(4):1309–18.
- [23] Sun X, Chang X, Wang Y, Xu B, Cao X. Oroxylin A suppresses the cell proliferation, migration, and EMT via NF- $\kappa$ B signaling pathway in human breast cancer cells. *BioMed Res Int* 2019;23:2019. 9241769.
- [24] Hui H, Zhang X, Li H, Liu X, Shen L, Zhu Y, et al. Oroxylin A, a natural anticancer flavonoid compound, induces differentiation of t (8; 21)-positive Kasumi-1 and primary acute myeloid leukemia cells. *J Canc Res Clin Oncol* 2016;142(7):1449–59.
- [25] Mehdi SH, Nafees S, Zafaryab M, Khan MA, Alam Rizvi MM. Chrysin: a promising anticancer agent its Current trends and future Perspectives. *Eur J Exp Biol* 2018;8:16.
- [26] Yu XM, Phan T, Patel PN, Jaskula-Sztul R, Chen H. Chrysin activates Notch 1 signaling and suppresses tumor growth of anaplastic thyroid carcinoma in vitro and in vivo. *Cancer* 2013 15;119(4):774–81.
- [27] Josephs SF, Ichim TE, Prince SM, Kesari S, Marincola FM, Escobedo AR, et al. Unleashing endogenous TNF-alpha as a cancer immunotherapeutic. *J Transl Med* 2018;16(1):1–8.
- [28] Xagorari A, Roussos C, Papapetropoulos A. Inhibition of LPS-stimulated pathways in macrophages by the flavonoid luteolin. *Br J Pharmacol* 2002;136(7):1058–64.
- [29] Chen CC, Chow MP, Huang WC, Lin YC, Chang YJ. Flavonoids inhibit tumor necrosis factor- $\alpha$ -induced up-regulation of intercellular adhesion molecule-1 (ICAM-1) in respiratory epithelial cells through activator protein-1 and nuclear factor- $\kappa$ B: structure-activity relationships. *Mol Pharmacol* 2004;66(3):683–93.