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Ethnomedicinal Survey and a Quantitative Analysis of Bioactive Plants Used by Sonowal Kachari Community of Assam

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Abstract

Background
In recent times, cases of infectious and malignant diseases are increasing in an exponential manner. The treatments to these diseases face drawbacks of specificity and high costs. Due to this, many patients seek alternative or complementary methods of treatment. Ethnomedicine is the first and foremost choice in this regard. However due to cultural belief no proper documentation with scientific validation is available. The present study intended to validate the potentiality of the ethnomedicinal plants of Sonowal Kachari community of Assam, quantitatively and qualitatively along with a few bioactivity studies of oxidative stress and antioxidant defences in a murine immune model.

Methods
The study was based on extensive ethnomedicinal field survey for 1 year period from 2015-2016. Data was analysed and most potent plant was short listed using disease consensus index (DCI), phytochemical screening and 2,2-diphenylpicrylhydrazyl (DPPH) assay. An effective dose was determined by calculating IC50 with 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction (MTT) assay in murine splenic macrophages. Bioactivity were studied in lipopolysaccharide (LPS) stimulated murine splenic macrophages for oxidative stress and antioxidant parameters.

Results
From the preliminary quantitative and qualitative screening it was found that *Oroxylum indicum* methanol extract (OIM) has a potent antioxidant activity. Based on IC50 (inhibitory concentration for 50% viability) value a working dose of 4.76 µg/ml was determined. The oxidative stress and antioxidant parameters show high free radical scavenging activity of OIM.

Conclusion
Systemic pharmacological trials in present study validate the active bioactive potential of the ethnomedicinal plant as per scientific standards.

Keywords: Ethnomedicine, Oxidative stress, Antioxidant, Sonowal Kachari.

1. Introduction

With recent advancement of technologies, countries with strong health care systems have the advantage of early diagnosis and advanced treatment of life-threatening diseases like cancer leading to better survival rates among the patients (1). However, cancer still remains the second leading cause of death around the world, resulting in nine million diagnosed cases and around five million deaths each year (2). Till date available treatment of cancers suffers from drawbacks of toxicity and non-specificity. Due to various drawbacks and high costs of therapy, many cancer patients seek alternative and/or complementary methods of treatment. Ethnomedicine is the first and foremost choice in this regard.

Natural products account for more than half of all modern medications in clinical use. Many plant derivatives have been employed with variable levels of effectiveness and to provide a better treatment for various cancers. A wide range of plant extracts have been shown to have anticancer and/or anti-carcinogenic properties. Currently, the chemotherapeutic treatment of tumors involves a number of plant-based compounds that are either in use or in clinical studies. Plant-based ethnobotanical leads are among the most effective agents in the hunt for anticancer drugs. There is a growing interest to study the natural drugs which claim to have anti-inflammatory and anticancer effects. Moreover, if such natural drugs are found to possess immunomodulatory properties, this could act as a double-edged sword employing both chemotherapy and immune targeting of cancer cells.

Sonowal Kachari, one of the largest plain tribes of Assam, is confined to the remote areas of Brahmaputra Valley. The ethno-botanical lore of this tribe is very rich. Knowledge on effective medicines acquired by them through experience is usually passed on orally as a guarded secret of certain families (3) and due to this reason ethno-medicinal plants used by this community have remained unexplored and unreported. A few field surveys have been carried out (4) covering documentation of several treatments for different ailments except cancer. Moreover, there are no reports on qualitative and quantitative bioactivity studies related to the documented ethnomedicines of this community so far. Therefore, in the present investigation, we intended to validate the potentiality of the ethnomedicinal plants of Sonowal Kachari community for the bioactive activities by using quantitative tools and test the selected medicinal plant for such bioactivity studies.

2. Materials and methods

2.1. Chemicals

2,2-diphenylpicrylhydrazyl (DPPH), 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Histopaque-1077 were procured from Sigma Aldrich (USA). Gibco Roswell Park Memorial Institute (RPMI) 1640 Medium and 5,5'-dithiobis (2-nitrobenzoic acid) (DNTB) or Ellman's reagent were obtained from Thermo Fisher Scientific (India). Nitro blue tetrazolium (NBT) was procured from Sisco Research Laboratory, India. Pyrogallol was obtained from Fisher Scientific. The organic solvents for plant extract preparation were obtained from Himedia (India). All other chemicals and reagents used for different analysis were pure analytical grade and were procured from local suppliers.

2.2. Ethics approval

Mice were maintained, treated and sacrificed in accordance with the guidelines set by the Institutional Ethics Committee (IEC), Assam University, Silchar, India under the approval number of IEC/AUS/2015-027 dt.4/9/15

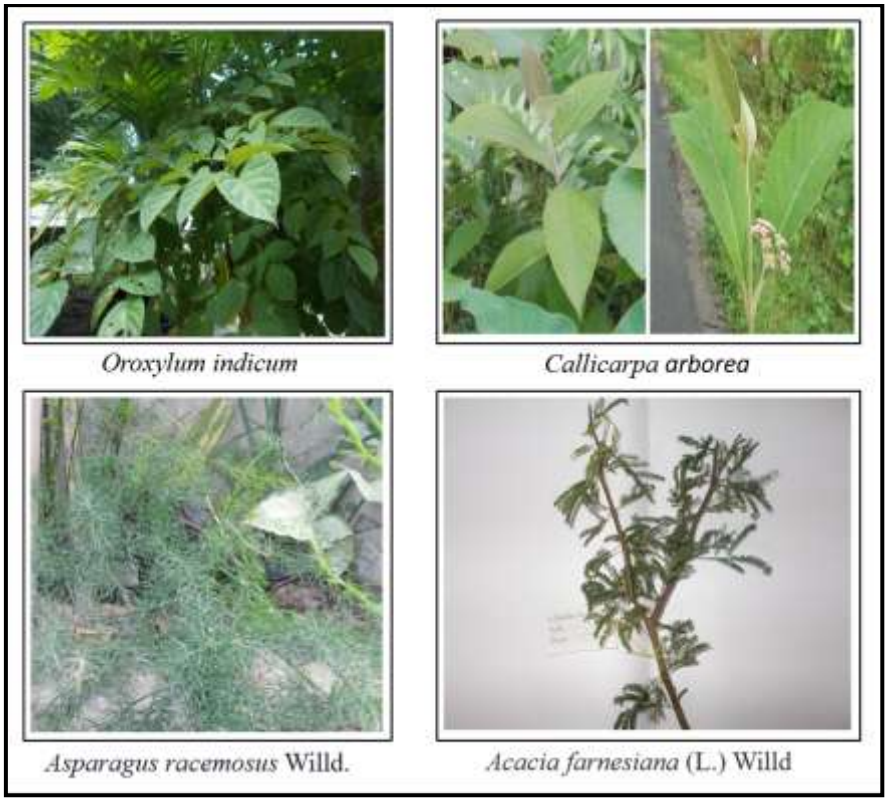
2.3. Area of study

The field survey was carried out in Sonowal Kachari inhabited areas, mostly remote border areas of Dibrugarh, Tinsukia, Dhemaji, Lakhimpur and Jorhat districts of Assam.

2.4. Collection of data

The field survey was carried out from 2015 to 2016 for a period of one year and data on plants used to treat tumor were collected through semi-structured questionnaires (5). The traditional medicine practitioners were selected by using snowball technique. The questions were divided into two groups, of which one was for general information about the informant like age, sex, education etc. and the other was for quantitative evaluation. The plant samples were collected from different areas as mentioned in the section entitled as area of study and initially identified using various literature *viz*. Flora of Assam (6) Flora of British India (7). For further verification, herbarium specimen was deposited in Botanical Survey of India, North Eastern Circle, Shillong.

Figure 1 Photographs of a Few Plants Collected During Field Survey.



2.5. Quantitative screening of plants based on disease consensus index (DCI)

Data was collected from the informants and evaluated quantitatively using disease consensus index (DCI). This index was used to select highly used plant specimens used to treat specific chronic disease in a specific community (5). It is a comparison based on mathematical aspect (limit theory), the ideal answers of informant reports (Cc) and the ideal answers for each species (Vx).

$$DCI = \left(\sum_{i=1}^{\infty} Vxi / Cc \; mVx \right) Pm - 0.1$$

Where, "x" is any species, "mVxi" is the sum of the individual values obtained for one species within the community; it evaluates knowledge and mentions. "mVx" is the statistical mean of the individual values for one species; it evaluates knowledge. "Cc" is the correlation coefficient, defined as the maximal number of informants who refer to a species; it evaluates mentions. "$Pm^{-0.1}$" is the compensation factor, and analyses the dispersion for one species, considering the mode of preparation and parts used.

2.6. Preparation of crude plant extracts using Soxhlet method

Five plants with highest quantitative scoring i.e., DCI, were selected for the further study. Different plant parts were collected as per the recommendation of traditional healers of the community used to treat tumors, and then shade dried and powdered. The crude extracts of each sample were prepared using Soxhlet method with petroleum ether (PE), ethyl acetate (EA), acetone (Ac) and methanol (Me). The extracts were then dried using desiccator.

Figure 2 Photographs of Soxhlet Extraction Method Using Different Organic Solvents

2.7. Qualitative Phytochemical Screening

Standard qualitative procedures of Trease and Evans (8), Sofowora (9) and Harbone (10) were followed to prepare PE, EA, Ac and Me extracts from each selected plants and were tested to estimate the presence of important bioactive compounds, mainly secondary metabolites.

2.8. Measurement of antioxidant activity by 2,2-diphenylpicrylhydrazyl (DPPH) assay

A slightly modified protocol of (11) was used to determine *in vitro* antioxidant activity of the selected plant extracts. The dried plant extracts were dissolved in methanol to prepare different concentration of solutions. DPPH methanol solution ($6X10^{-5}$ mol/L) was added to the plant extracts and incubated for 30 mins. Methanolic solution of DPPH was taken as control. Absorbance was taken at 515 nm. The antioxidant activity was calculated by the following formula

$$\% \text{ of antioxidant activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Sample}} \times 100$$

The antioxidant potential was expressed as IC_{50} value and plant extract with highest antioxidant potential i.e., lowest IC50 value was selected for bioactivity studies.

2.9. Bioactivity studies

2.9.1. Experimental animals

For *in-vitro* bioactivity studies, 6 to 8 weeks old male Swiss albino mice, body weight of 20±2g were purchased from Pasteur Institute, Shillong, India. Animals were kept in the departmental animal facility under the guidelines of Institutional Ethical Committee (IEC) (Assam University, Silchar, Assam).

2.9.2. Isolation of Splenic macrophage

Splenic macrophages were isolated through density gradient centrifugation at 1500 rpm for 30 mins using Histopaque 1077 (Sigma, USA). The cells were then allowed to adhere to the plastic surface of the Petri dish for 1 hour in 37°C, 5% CO_2 incubator. The non-adherent cells were removed and adherent cell were washed once and re-suspended in culture media at a density of 10^6cells/ml (12) and further used in biochemical assays.

2.9.3. Dose standardization by MTT assay

Isolated splenic macrophages were divided into 11 experimental groups with 3 replicates (10^6 cells/ml per replicate). Ten (10) doses of selected plant extracts were prepared with the lowest dose of 1µg/ml to the highest dose of 100µg/ml. One untreated group was taken as control. 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) reduction assay was use for the determination of cell viability (13) and IC50 (inhibitory concentration for 50% viability) value was calculated to find an effective dose for further experiments.

2.9.4. Biochemical assays

Effect of the selected plant extract on lipopolysaccharide (LPS) induced oxidative stress and its antioxidant efficacy was studied using a dose lower than the IC50 value for three different groups *viz.* control (C), LPS treated (LT) and LPS with plant extract treated (LPET). Following the protocol of Marklund and Marklund (14), superoxide anion (o_2^-) release was estimated. Estimation of antioxidative

enzymes like Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH) was done following the methods of McCord (15), Bergmeyer (16) and Ellman (17) respectively.

2.10. Flow chart representation of the experiment

Our entire experimental process is depicted in a precise manner with the help of flow diagram (Figure 5)

2.11. Statistical analysis

The quantitative phytochemical screening of selected plants was done by one plant value i.e., a kind of binary evaluation- "1" or "yes" for knowledge and "0" or "no" for lack of such knowledge about the questions asked. Qualitative phytochemical screening, *in vitro* antioxidative activity were measured in triplicates.

All *in vitro* bioactivity studies were carried out in triplicates. The values were expressed as mean ± standard error mean (SEM) and analyzed with non-linear regression Student's t test and one way ANOVA. Significance were marked at $p<0.05$ and the significance level has been mentioned in the respective graphs. Statistical calculations were performed in SPSS v20 and data representations were done in the GraphPad Prism 8 software

3. Results

3.1. Collection of data and quantitative screening based on disease consensus index (DCI)

Types of plants used by the Sonowal community for treating tumors are quite less in number. Therefore, plants used in other chronic pathological conditions like piles, boils, wounds which if left untreated might leads to the development of cancer were also considered in our study. In this field survey 45 types of plants were reported by 31 informants. The mode of application of the plant parts along with its scientific name followed vernacular name and DCI were tabulated in Table 1.

DCI was used to evaluate collected data quantitatively and five plants with highest DCI were selected for further studies. We found that *Oroxyllum indicum* has highest DCI value of 0.611 followed by *Abelmoschus moschatus* 0.355, *Zanthozylum nitidum* 0.341, *Eupatorium odoratum* 0.311 and *Callicarpa arborea* 0.292.

3.2. Qualitative phytochemical screening

The extracts of *A. moschatus*, *C. arborea*, *E. odoratum*, *O. indicum* and *X. nitidum* were prepared using the prescribed plant parts with PE, EA, Ac and Me (Table 2) and employed for qualitative screening. From qualitative phytochemical screening it was found that the methanol extracts of *A. moschatus* (AMM), *C. arborea* (CAM), *O. indicum* (OIM), *Z. nitidum* (ZNM) and ethyl acetate extract of *Z. nitidum* (ZNE) showed the presence of maximum no. of phytochemicals (Table 3). Therefore, all five selected extracts were subjected for further analysis.

Table 1 List of scientific name, family and vernacular names with disease consensus index (DCI) of the plants along with the ailment type, parts used and forms of preparation used by Sonwal Kachari tribe of Assam, North- East India. Plants with high DCI were highlighted in the column. Among forty-five plants five plants with high DCI were selected for the continuation of the research.

Sl. No	Scientific name	Family	Vernacular Name	Disease type	Part used	Forms of preparation and use (Pm)	no. of part use	∑Vxi	mVxi	DCI
1	***Abelmoschus moschatus***	Malvaceae	Gorokhia koroi	Boils, Breast cancer, Wound healing	Root paste	one form: paste	1	11.9	0.915	**0.355**
2	***Aegle marmelos* (L.) Corrêa**	Rutaceae	Bel	Piles	Ripe fruit	one form: eaten with milk	1	3.6	0.514	0.064
3	***Ageratum conyzoides* L.**	Asteraceae	Gundhuwa bon	Cut and wounds	Leaves	one form: paste	1	7.6	0.760	0.192
4	***Albizia lebbeck* (L.) Benth.**	Fabaceae	Siris Gos	Cancer	Leaves and roots	two form: paste, decoction	2	7.4	0.740	0.195
5	***Alstonia scholaris* (L.) R.Br.**	Apocynaceae	Chatiana	Boils	Latex of the leaves	one form: latex	1	2.5	0.500	0.043
6	***Amorphophallus paeoniifolius* (Dennst.) Nicolson**	Araceae	Ol kochu	Cancer	Stem	one form: paste	1	6.6	0.825	0.179
7	***Asparagus racemosus* Willd.**	Asparagaceae	Sat mul	Gall stone	Root decoction	one form: decoction	1	6.6	0.943	0.202

8	***Boerhavia diffusa* L.**	Nyctaginaceae	Purnanawaa	Cancer	Whole plant	one form: paste	1	4.2	0.700	0.098
9	***Bonnaya ciliata* (Colsm.) Spreng.**	Linderniaceae	Horu kasidoria	Cut and wounds	Leaves	one form: paste	1	4.1	0.820	0.111
10	***Bryophyllum pinnatum* (Lam.) Oken**	Crassulaceae	Duporteng a	stone	Leaves	one form: raw eaten	1	4.2	0.525	0.076
11	***Caesalpinia bonducella* (L.) Roxb.**	Caesalpiniaceae	Leta guti	Wounds	Seed paste	one form: seed paste	1	5.8	0.725	0.140
12	***Callicarpa arborea* Roxb.**	Lamiaceae	Bon mala, Tongloti	Cancer, Cut, Wounds, Boil	Root, bark decction	one form: decoction	1	12.2	0.718	**0.292**
13	***Calotropis procera* (Aiton) Dryand.**	Apocynaceae	Akon	Boils, cancerous wounds	Leave paste	one form: paste	1	3.6	0.600	0.073
14	***Cannabis sativa* L.**	Cannabaceae	Bhung goss	Boils, wounds	Leave juice	one form: juice	1	3.4	0.850	0.095
15	***Citrus limon* (L.) Osbeck**	Rutaceae	Kaji nemu	Cancer	Fruit	one form: burned juice	1	3	0.750	0.075
16	***Clerodendrum infortunatum* Gaertn.**	Lamiaceae	Dhopat tita	Skin cancer	Roots	one form: paste	1	3	0.600	0.061
17	***Colocasia esculenta* (L.) Schott**	Araceae	Pani kochu	Cuts and wounds	corm	one form: juice	1	6.8	0.756	0.170
18	***Croton tiglium* L.**	Euphorbiaceae	Koni Bih	Breast cancer, Boils, Wound healing	Seed paste	one form: seed paste	1	1.5	0.500	0.026
19	***Curcuma zedoaria* (Chri**	Zingiberaceae	Borahu	Piles	Rhizom pills	one form:	1	3.7	0.529	0.067

	stm.) Roscoe				oral	pills				
20	**Dactyloctenium aegyptium (L.) Willd.**	Poaceae	Bobosa bon	Piles	Whole plant, external use	one form: external	1	6.3	0.788	0.164
21	***Drymaria cordata* Willd. ex Schult.**	Caryophyllaceae	Lai jabori	Cuts and wounds, Mumps	whole plant	one form: paste	1	8.5	0.850	0.237
22	***Eupatorium odoratum* L.**	Asteraceae	Jarmani bon	Cuts and wounds, cancer, Scurvy	Leaves, Root paste	one form: paste	1	11.5	0.821	**0.311**
23	***Ficus religiosa* L.**	Moraceae	Ahot gos	Cut and wounds	Bark with tortois shell burn together. Ash is used	one form: ash	1	6	0.600	0.122
24	***Grewia serrulata* DC.**	Malvaceae	Kukur huta	Cut, Wounds	Leave paste external	one form: external	1	6	0.857	0.168
25	***Litsea salicifolia* Hook.f.**	Lauraceae	Dighloti	Boils	Leave paste	one form: paste	1	3.9	0.557	0.074
26	***Manihot esculenta* Crantz**	Euphorbiaceae	Himolu alu	Cancer	Bark, Exdue paste, oral	one form: paste	1	5.8	0.829	0.158
27	***Melastoma malabathricum* L.**	Melastomataceae	Phutuka	Boils	Young leave paste	one form: paste	1	6	0.857	0.168
28	***Mesua ferrea* L.**	Calophyllaceae	Nahar	Piles	Bark infusion oral	one form: infusion	1	5.2	0.520	0.093
29	***Mimosa pudica* L.**	Fabaceae	Lajuki lota	Cancer	Leave , root	two form:	2	7.4	0.822	0.215

					juice	paste, juice				
30	***Moringa oleifera* Lam.**	Moringaceae	Sajina	Cancer	Bark, Root, Leaves	two form: paste, decoction	2	7.8	0.600	0.170
31	***Oldenlandia corymbosa* L.**	Rubiaceae	Bonjaluk	blister in tongue	Leaves	one form: raw eaten	1	6.3	0.900	0.185
32	***Oroxylum indicum* (L.) Benth. ex Kurz**	Bignoniaceae	Bhatghila	Cancer, Wounds	Bark, Leave	two form: paste, decoction	2	19.6	0.891	**0.611**
33	***Paederia foetida* L.**	Rubiaceae	Bhedailota	Piles	Leaves, Buds	one form: boiled paste	1	4.6	0.575	0.090
34	***Perilla ocymoides* L**	Lamiaceae	Sookloti	Mouth ulcer, Gynaecological problems	Root, leaves	two form: paste, decoction	2	5.8	0.483	0.104
35	***Phyllanthus virgatus* G.Forst.**	Phyllanthaceae	Pani amlokhi	Anti cancer	Fruite juice	one form: juice	1	8.8	0.800	0.232
36	***Piper nigrum* L.**	Piperaceae	Jaluk	Anti cancer	Fruit paste decoction, oral	one form: oral	1	7.6	0.633	0.163
37	***Plumbago zeylanica* L.**	Plumbaginaceae	Boga agechita	Piles	Root with Turmeric= Paste	one form: paste	1	2.1	0.700	0.049
38	***Rubus moluccanus***	Rosaceae	Jetuli puka	Boils	Leaves, bud	one form: paste	1	7	0.778	0.180

39	***Stephania hernandiifolia* var. discolor (Blume) Miq.**	Menispermaceae	Tubukilota	Septic infection and boils for opening	Leave paste	one form: paste	1	5.2	0.743	0.128
40	***Syzygium cumini* (L.) Skeels**	Myrtaceae	Jamuk	Mouth cancer	Leaves	one form: paste	1	8.4	0.840	0.232
41	***Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.**	Combretaceae	Arjungos	Cancer, Diabets, Asthma	Bark	one form: decoction	1	9.4	0.723	0.226
42	***Trichosanthes palmata* L.**	Cucurbitaceae	Kuwabhaturi	Boils	Root & seed paste	one form: paste	1	8.2	0.820	0.221
43	***Tridax procumbens* L.**	Asteraceae	Bishalyakarani	Cuts and wounds	Leaves	one form: paste	1	8	0.889	0.232
44	***Zanthoxylum nitidum* DC.**	Rutaceae	Tejmui	Cancer	Whole plant	one form: paste	1	9.8	1.089	**0.341**

Table 2 Five types of plants were tabulated here in a descending order of their DCI value. Based on the information collected from the medicinal man of Sonowal Kachari tribe of Assam, North-East India, mentioned plant parts were used to formulate four types of crude extract. Total yield of crude extract obtained from each type of preparations mentioned in the table below.

Sl no.	**Plant Name**	**Part used**	**Plant Extract Name**	**Initial weight taken (gm)**	**Final weight obtain (gm)**
1	***Oroxylum indicum (L.) Benth. ex Kurz (Bhatghila)***	Leaves	Petroleum Ether Extract (**OIP**)	100	2.17
			Ethyle acetate Extract (**OIE**)		1.56
			Acetone extract (**OIA**)		1.03
			Methanol extract (**OIM**)		1.25
2	***Abelmoschus moschatus (Gorokhia koroi)***	Root	Petroleum Ether Extract (**AMP**)	100	1.36
			Ethyle acetate Extract (**AME**)		1.06
			Acetone extract (**AMA**)		0.94
			Methanol extract (**AMM**)		1.01
3	***Zanthozylum nitidum* DC *(Tejmui)***	Whole plant	Petroleum Ether Extract (**ZNP**)	100	1.98
			Ethyle acetate Extract (**ZNE**)		1.76
			Acetone extract (**ZNA**)		1.55

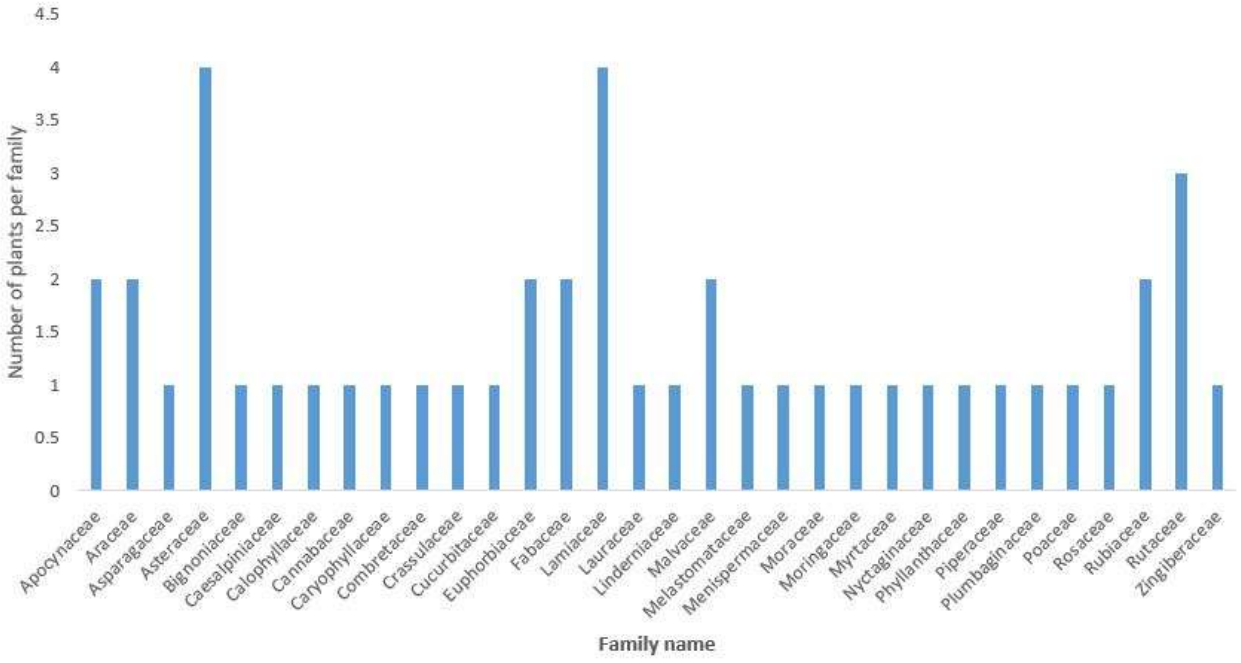
			Methanol extract (**ZNM**)		1.49
4	***Eupatorium odoratum L. (Jarmani bon)***	Root	Petroleum Ether Extract (**EOP**)	100	0.96
			Ethyle acetate Extract (**EOE**)		1.32
			Acetone extract (**EOA**)		1.03
			Methanol extract (**EOM**)		1.21
5	***Callicarpa arborea* Roxb *(Bon mala, Tongloti)***	Bark	Petroleum Ether Extract (**CAP**)	100	2.25
			Ethyle acetate Extract (**CAE**)		2.02
			Acetone extract (**CAA**)		1.73
			Methanol extract (**CAM**)		1.8

Table 3 Result of qualitative phytochemical screening of four types of extract of *Oroxyllum indicum* (OIP, OIE, OIA and OIM), *Abelmoschus moschatus* (AMP, AME, AMA, AMM), *Zanthozylum nitidum* (ZNP, ZNE, ZNA, ZNM), *Eupatorium odoratum* (EOP, EOE, EOA, EOM) and *Callicarpa arborea* (CAP, CAE, CAA, CAM) is presented in the table below. Important secondary metabolites/ phytochemicals (flavonoids, steroids, alkaloids, coumarins, proteins, saponins, phlobatannins, volatile oils, tannins and phytosterols) were identified in each extract. "+" is used for the presence and "-" is used for the absence of above-mentioned secondary metabolites in the extract. Highest no. of phytochemicals were obtained in OIM (methanol extract of *Oroxyllum indicum*), AMM (methanol extract of *Abelmoschus moschatus*), ZNA (acetone extract of *Zanthozylum nitidum*), ZNM (methanol extract of *Zanthozylum nitidum*) and CAM (methanol extract of Callicarpa arborea).

Qualitative Phytochemical Screening										
Extract name	**Flavonoids**	**Steroids**	**Alkaloids**	**Coumarins**	**Proteins**	**Saponins**	**Phlobatannins**	**Volatile oils**	**Tannins**	**Phytosterols**
OIP	+	+	-	+	-	-	-	-	-	+
OIE	+	-	-	-	-	+	-	-	+	-
OIA	+	-	+	+	-	+	-	-	-	-
OIM	+	+	+	-	-	+	+	+	+	+
AMP	-	-	-	-	-	-	-	-	-	+
AME	+	+	-	-	-	+	-	-	+	-
AMA	+	-	+	-	-	-	-	-	+	+
AMM	+	-	-	-	+	+	-	-	+	+
ZNP	-	-	+	-	-	-	-	-	-	-
ZNE	+	-	+	+	+	+	-	+	-	-
ZNA	-	-	+	-	-	+	-	+	-	-
ZNM	+	-	+	+	+	-	+	-	+	-
EOP	+	+	+	-	-	-	-	-	+	-

EOE	+		+	-	+	-	-	-	+	-
EOA	+	+	-	-	-	-	+	-	+	-
EOM	+	-	+	-	-	-	+	-	+	-
CMP	-	-	-	-	-	+	-	-	-	-
CME	+	+	+	-	-	-	-	-	-	-
CMA	+	-	-	-	-	-	-	-	-	-
CMM	+	+	+	-	-	+	-	-	+	+

Figure 3 Bar diagram showing the distribution of the plants in various families.

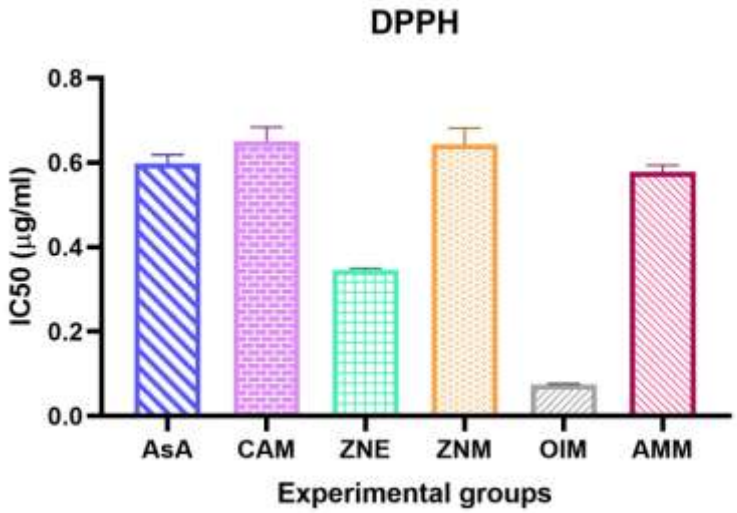


3.3. DPPH assay

DPPH is the stable free radical used to test the free radicle scavenging activity and to evaluate antioxidant potential of plant extract. In our studies OIM extract showed highest antioxidant potential with IC_{50} of 0.074 mg/ml followed by ZNE 0.348 mg/ml, AMM 0.591 mg/ml, CAM 0.615 mg/ml and ZNM 0.619 mg/ml (Figure 4). Therefore, OIM extract was selected for bioactivity study in murine splenic macrophages.

Figure 4 DPPH free radical scavenging activity of six types of plant extracts taking AsA (ascorbic acid) as standard. CAM (methanol extract of *Callicarpa arborea*), ZNE (ethyl acetate extract of *Zanthozylum nitidum*), ZNM (methanol extract of *Zanthozylum nitidum),* OIM (methanol extract of

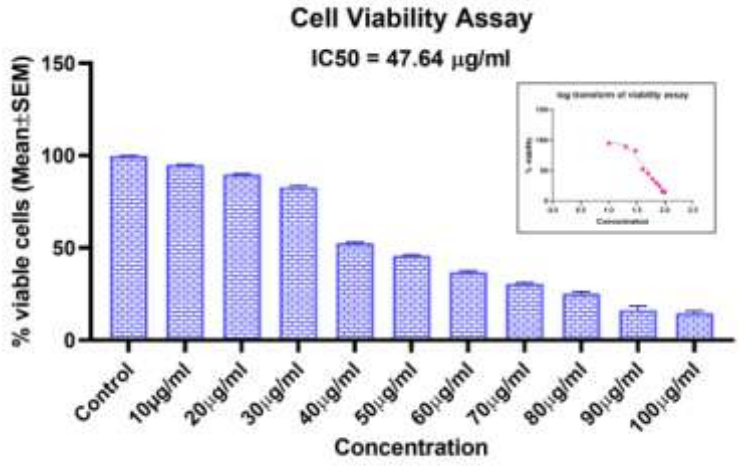
***Oroxyllum indicum*) and AMM (methanol extract of *Abelmoschus moschatus*). DPPH scavenging activity is expressed as IC50 value. Data represented as mean ± S.E.M values (n=3) with three replicates of each group.**



3.4. Standardization of dose

MTT is a yellow-coloured water soluble tetrazolium salt which can be reduced by viable cells with active metabolism into purple insoluble formazan, i.e., colour formation is inversely proportional to the cytotoxicity. In our experiment we found the IC_{50} of OIM is 47.64 µg/ml (Figure 5). From the reports by US NCI plant screening program, a crude plant extract can exhibit *in vitro* cytotoxicity effect with an $IC_{50} \leq 20$µg/ml (18). Therefore, OIM with higher IC_{50} value is less toxic to the normal splenic macrophages in our experimental studies. Hence, we take a dose of 4.76 µg/ml for our further studies.

Figure 5 Graph depicted the % of cell viability with the increasing dose of the OIM extract (methanol extract of *Oroxyllum indicum*) and in the inset non-linear regression curve of the cell viability assay (MTT) is given. 10 doses of OIM extract were selected for the assay. At the concentration of 47.64 µg/ml, OIM extract showed 50% of cell viability. Further experiments were done taking one tenth dose of the IC50 value i.e. 4.76 µg/ml.

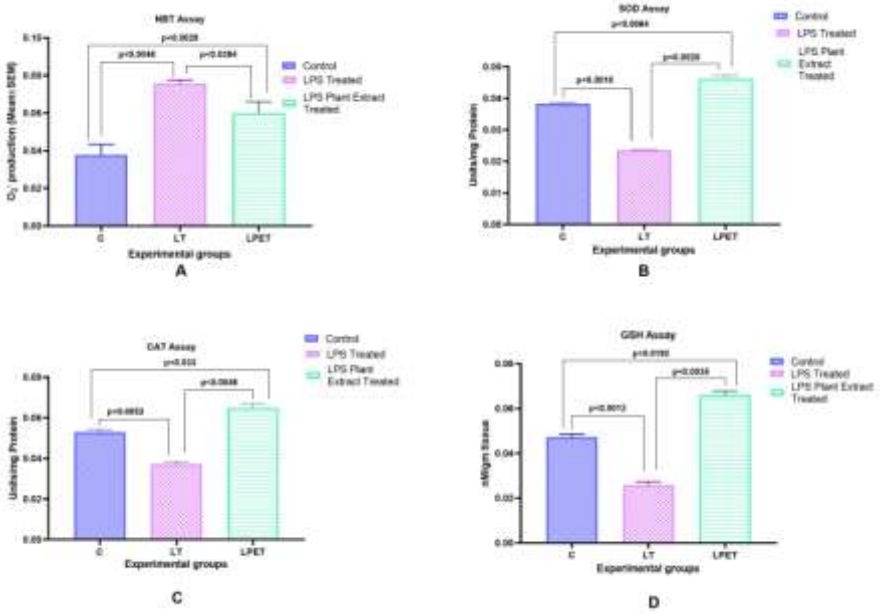


3.5. Effects of OIM extract on in vitro oxidative stress

The LPS induced oxidative stress in LT group (0.0756±0.002) results in rapid increase of O_2^- anion release as compared to control (0.0378±0.002), however the LPET group (0.0596±0.001) showed reduction in superoxide anion release after OIM extract treatment (Figure 6A).

The exposure of LPS lower down the release antioxidant enzyme SOD (Figure 6B), however plant extract enhances its production in the LPET group. The similar pattern was seen in cases of other antioxidat enzymes *viz*. CAT (Figure 6C) and GSH (Figure 6D).

Figure 6 ***In vitro*** **efficacy of OIM extract (methanol extract of** ***Oroxyllum indicum*****) on LPS stimulated experimental model was evaluated via A. nitroblue tetrazolium assay (NBT) and the correlation with antioxidant production was observed through B. superoxide dismutase assay (SOD), C. catalase assay (CAT), D. reduced glutathione assay (GSH). The values are expressed as mean±S.E.M. (n=6) of each group.**



4. Discussion

Malignant tumors are one of the major causes of death in present century. Despite of having a number of treatments for the cause, due to the side effects of used drugs especially chemotherapeutic drugs, non-specificity and high-cost people seek alternative therapy. In this regard plant-based medicines are the first and foremost choice. Many plant-based medicines have been clinically tested and incorporated in modern medical system. The source of these phytomedicines are the ethnomedicines of various tribes, as these have been screened and qualified by the experiences of many generations for their easy accessibility, efficiency with lower side effects. Only drawback of these ethnomedicinal system is that they are not documented properly and passed to next generation "orally". Sonowal Kachari tribe of Assam is a native tribe with rich ethnobotanical lore and have same drawback as other tribes on their ethnomedicinal practices. No. of field survey for the documentation of traditional knowledge of this tribe are few in numbers (4), (19), (20), however their qualitative and quantitative analysis was not done yet. Therefore, the present study is designed to document the ethnomedicinal

knowledge of this tribe qualitatively, quantitatively and estimate their bioactivity in murine splenic macrophages.

During the survey it was found that Sonowal Kacharis' have some supernatural belief regarding some persistent disease like cancer, tuberculosis etc. Therefore, they do not treat this disease directly. As cancer has multistage developmental form, pre-cancerous lesions to malignant tumor stage (21), therefore data for treatment of chronic pre-cancerous lesions, cuts and wounds etc. have also been collected for the experimental purpose.

From the quantitative analysis it was found that *A. moschatus, C. arborea, E. odoratum, O. indicum* and *Z. nitidum* have the highest DCI. These plants were also reported for their ethnomedicinal uses in various other tribes around the world (22), (23), (24), (25), (26).

Secondary metabolites of plants are the main contributor for bioactivity. These metabolites act as an antioxidant, immunomodulator, antimicrobial, anticancer and anti-inflammatory agents in various pathological illness. Present study of plants with highest quantitative value reveals the presence of different secondary metabolites. Among 20 different extracts OIM, AMM, ZNA, ZNM and CAM found to have highest number of phytochemicals including flavonoids, alkaloids, tannins etc. and may be responsible for their antioxidative efficacy. The instantaneous and effective way for the assessment of antioxidant activity of bioactive compounds is to study DPPH free radical scavenging activity. The presence of phenolic components such as flavonoids, polyphenolic acids etc. increases the antioxidative activity, which is expressed as IC_{50}. Lower the IC_{50} value, higher the antioxidative activity. In this study, methanol leaves extract of OIM showed highest antioxidative activity among other different tested extracts. Moirangthem *et.al* (2013) (27) reported the positive correlation between the flavonoids and antioxidants in the methanol bark extract of *O. indicum*. Similar report has been given by Mishra *et. al* (2010) (28). Swamy *et al.* (2007) (29) quantifies total phenolic and total flavonoid components of various solvent extract of different parts of *O. indicum* due to their vast therapeutic uses. Roy *et.al* (2007) (30) has reported *in vitro* inhibition of HL-60 cells by baicalein, a flavonoid of *O. indicum*. Thus, providing considerable relevance with our experimental finding.

Prolong oxidative stress leads to chronic inflammation, which causes many chronic diseases like cancer, diabetes, cardiovascular disease etc. Due to oxidative stress extensive amount of free radicals and reactive oxygen species (ROS) are produced in the cell leading to cell death. Antioxidant plays an important role in reducing the oxidative stress. The cell has developed various enzymatic (SOD, CAT and GPx) and non-enzymatic (vitamin C and E, GSH etc.) antioxidants to scavenge oxidative stress (31). The antioxidative efficacy of a compound must be tested in a biological system to validate biochemical results. In the present study antioxidative efficacy of OIM was tested in a LPS stimulated immune-compromise model of murine splenic macrophages. LPS a cellular component of gram negative bacteria is a classic inflammasome activator (32). In recent study, of Halawa *et. al* (2013) (33) they reported the oxidative stress and apoptosis inducing properties of LPS. It also been reported for promoting metastasis in cancer cells (34), (32). In this study, similar result with Halawa *et. al* (2013) (33) was observed for LPS treated group i.e. release of high amount of superoxide anion (O_2^-), which was reduced after OIM treatment and its effect on the cell number was observed under the microscope (Figure 7). This shows that OIM can reduce oxidative stress. The elevated level of antioxidative enzymes SOD, CAT and GSH indicates the activation of the antioxidant defence system in the immune-compromised cells by OIM. Hence, supporting the results of the phytochemical analysis and DPPH assay.

Figure 7 Two sets of microscopic view of murine splenic macrophages before (A) and after (B) the treatment with OIM extract (methanol extract of *Oroxyllum indicum*) in a lipopolysaccharide (LPS) stimulated samples. (C) Quantification of cells of above mentioned experimental groups. No. of cells were significantly higher in OIM extract treated group. OIM extract reduced oxidative stress induced via LPS and restored cell numbers.

5. Conclusion:

Thus, from the study it is found that Sonowal Kachari tribe is rich in its ethnomedicinal knowledge and protective about their cultural belief. The medicinal plants used by the community have high amounts of phytochemicals that believe to enhance their medicinal efficacy. Systemic pharmacological trial for antioxidative enzymes with highest phytochemical containing extract i.e., OIM validate estimated biochemical results. Further systemic analysis is needed to validate the indigenous use of medicinal plants and incorporation of the traditional knowledge in modern healthcare system. This will add newer frontiers to medicinal research. Moreover, such type of ethnomedicinal studies help in the preservation of undocumented knowledge of indigenous tribes.

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