ORIGINAL ARTICLE

Evaluation of *in vitro* antioxidant activity, phytochemical screening of selected medicinal plants of Nepal and *in vitro* anti-inflammatory Activity of *Morus alba* L. and *Colebrookea oppositifolia* J.E. Smith

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Abstract

Introduction: Oxidative stress and inflammation are pathophysiological condition of mammalian tissues in response to infectious organisms, toxic chemical substances, physical injury or tumor growth, and leading to local accumulation of plasma fluid and blood cells. The main aim of the present study was to evaluate *in vitro* antioxidant and *in vivo* anti-inflammatory activity of *Morus alba* L., *Imperata cylindrica* (L.) Beauv, *Colebrookea oppositifolia* J.E. Smith and *Crinum amoenum* Roxb. **Materials and Methods:** Phytochemical screening of the entire sample was carried out by different chemical tests. The *in vitro* antioxidative activity was evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging activity method. Free radicals and reactive oxygen species are also implicated in inflammatory diseases. Therefore, two most potent plants extract, showing best free-radical scavenging activity, that is, *M. alba* (82%) and *C. oppositifolia* (79%) at 100 µg/mL concentration were chosen for *in vivo* anti-inflammatory analysis using 1% w/v carrageenan induced rat paw edema model. Diclofenac sodium was used as a standard drug at the concentration of 50 mg/kg of body weight. Aqueous and ethanolic leaves extract at the concentration of 100 mg/kg and 250 mg/kg were used as test drugs. Standard drug and all the extracts were administered 60 min before carrageenan injection. The paw volume was measured at 1, 2, 3, and 4 h after carrageenan injection, using a plethysmometer. **Results:** The phytochemical screening of all four plant species revealed that they contain terpenoids, saponins, alkaloids, and flavonoids. The ethanolic extract of *M. alba* root bark and *C. oppositifolia* leaves exhibited dose-dependent DPPH radicals inhibition with IC_{50} value of 51.37 µg/mL and 55.81 µg/mL, respectively, showing comparable potency with ascorbic acid (IC_{50}: 5.47 µg/mL). Both plant extract suppressed rat paw edema significantly ($P < 0.05$) in a dose-dependent manner, compared to the negative control group. The ethanolic extract of *Morus alba* and *Colebrookea oppositifolia* exhibited highest

INTRODUCTION

Medicinal plants have been recognized from ancient times as a major source of pharmaceutical preparations to prevent and treat diseases and ailments. According to the World Health Organization, more than 80% of the population within developing countries use herbal and other

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57.87% and 50.68% inhibition of paw edema volume, respectively, almost similar to the inhibitory effect of the standard drug Diclofenac sodium (63.39%). **Conclusion:** Thus, the root bark extract of *M. alba* and leaves extract of *C. oppositifolia* possess potent in vitro antioxidant effect and in vivo anti-inflammatory effect in carrageen induced Wistar rat model.

Key words: Anti-inflammatory activity, Antioxidant activity, Carrageenan, *Colebrookea oppositifolia*, Free radicals, *Morus alba*

traditional medicines to treat their common ailments.[1] Modern medicine is also based on indigenous knowledge and ethnopharmacological practices, which have become the backbone for noble drug discovery.[2] Free radicals are chemically reactive species, generated during cellular metabolism and have important roles in cell signaling, apoptosis, gene expression, and ion transportation.[3] However, their excessive generation leads to oxidative damage of membrane lipids, DNA, tissues, and biomolecules.[4] The disproportion between free radicals generation inside the body and its potentiality to prevent or treat their detrimental effects through counterbalance by antioxidants leads to oxidative stress.[5] Oxidative stress eventually leads to different pathological conditions, especially degenerative diseases such as aging, cancer, coronary heart disease, cataract, inflammation, neurodegenerative disorders, and other chronic complications. The human body and other organisms have developed an antioxidant defense system that includes enzymatic, dietary antioxidants, metal chelating, and free-radical scavenging activities to neutralize these radicals after they have formed.[3] The most efficient enzymatic antioxidants involve catalase, glutathione peroxidase, and nitric oxide synthase (NOS).[6] Vitamins E and C, carotenoids, natural flavonoids, and thiol antioxidants (lipoic acid, glutathione, and thioredoxin) are some examples of non-enzymatic antioxidants.[7] Some of the most effective synthetic antioxidants are butylated hydroquinone, propyl gallate, butylated hydroxyanisole, and butylated hydroxytoluene.[8] Due to various undesirable effects such as carcinogenic effects of synthetic antioxidants, researchers are getting interested in the study of different plant parts such as leaves, roots, barks, fruits, flowers, and seeds which may contain natural antioxidant constituents.[9]

The medicinal importance of a plant is due to the presence of some special compounds such as alkaloids, terpenoids, flavonoids, glycosides, resins, volatile oils, saponins, gums, and tannins which are known as phytochemicals. Phytochemical screening is a qualitative analysis of detecting the different classes of chemical compounds present in the given plant extract. This screening provides a basement to evoke active ingredients and therapeutic activity.[10]

Inflammation is a localized safeguarding pathophysiological response of mammalians cells/tissues, against infectious organisms, toxic chemical substances, physical injury, and tumor growth. Rapid increasing concentration of various endogenous biological molecules, including reactive oxygen species (ROS), prostaglandin E2, nitric oxide (NO), and cytokines is associated with the development of inflammation.[11] The inflammation can be of acute or chronic type, based on the nature of the stimulus, and effectiveness of the inflammatory reaction to remove injurious stimuli and damaged tissue.[12] In the process of the inflammation process, the first step is the action of macrophage immune cells to protect against causative factors and restrain infectious microorganism attacks by releasing anti-microbial agents and cellular signaling molecules. However, excessive release of cellular mediators by macrophages can result in the development of different diseases such as fibrosis, pneumonia, autoimmune complications, encephalitis, rheumatism, esophagitis, heart problems, and cancer, due to sever host cell damage.[13-16] In the present scenario, nonsteroidal anti-inflammatory drugs such as corticosteroids, salicylates, or narcotics such as opioid analgesics have been employed for the prevention of pain and inflammatory conditions. However, their serious adverse and toxic effects, and high cost for the formulation of novel synthetic drugs, have compelled us to screen the potential anti-inflammatory drugs possessing less toxic effects; from natural plant sources.

Morus alba L. is a perennial shrub or a medium sized tree with a cylindrical stem and rough, brown, vertically fissured bark, belonging to the family Moraceae and is popularly known as "Kyu Kafal" in Nepali language. It is distributed throughout the Nepal, Northern India, Bhutan, Western China, and Myanmar, in the range of 900–2400 m.[17] Conventionally, the leaves of the *M. alba* are used as expectorant, to treat fever, headache, sore throat, elephantiasis, and inflamed eyes. Fresh fruit juice is taken as tonic and mouth cleansing agent. The root bark is very much effective to cure tooth ache and constipation.[18] Apart from the ethnomedicinal uses, scientific investigations have demonstrated that this plant possess antihyperglycemic, antioxidant, anthelmintic, antimicrobial, anticancer, antistress, antimutagenic, hepatoprotective, immunoregulatory, anti-atherosclerotic, and skin toning activities.[18,19]

Imperata cylindrica (L.) Beauv is a perennial rhizomatus grass, belonging to family Poaceae and is popularly known as "Siru" in Nepali language. It is distributed throughout Nepal to about 2400 m in open field as a weed and also distributed widely in Afghanistan, Pakistan, India, Bhutan, Tibet, central Asia, Myanmar, and Mediterranean region.[17,20] Ethnomedicinally, decoction of root is popularly used to treat worm infection, diarrhea, dysentery, indigestion, and gastric troubles. Fresh juice of the root is used for asthma, jaundice, dropsy, and nosebleeds. A paste of the root is taken in fever.[17,21] Various biological activities such as antibacterial, astringent, diuretic, sialagogue, emollient, febrifuge, styptic, restorative, and anti-ageing properties have been reported from this grass.[22]

Colebrookea oppositifolia J.E. Smith is an evergreen small bushy shrub or tree, belonging to family Lamiaceae and is popularly known as "Dhurseli" in Nepali language. It is distributed throughout the Nepal to about 1800 m in open, dry, and rocky places. It is also found in Northern India, Bhutan, Southwestern China, and Indo-China.[17] In folk remedies, the plant is effective for epilepsy, peptic ulcer, dysentery, fever, conjunctivitis, as anti-fertility agent, urinary problems, wounds, dermatitis, etc. Scientifically, this plant has been demonstrated to exhibit diverse pharmacological activities such as cardio protective, anticonvulsant, antinociceptive, insecticide, anti-inflammatory, anthelmintic, antifungal, cytotoxic, antiulcer, and hepatoprotective.[23,24]

Crinum amoenum Roxb. is a perennial bulbous herb, belonging to family Amaryllidaceae. It is popularly known as "Himalyan *Crinum* Lily" in English and "Hade Lasun" in Nepali language. Himalayan *Crinum* Lily is a species of *Crinum* lily found in the Himalayas from central Nepal to Northeast India and Burma in open places at altitudes of 700–1700 m.[17,25] Conventionally, the rhizome is fed to cattle to treat dysentery. The paste of underground part is used in abscess/dehydration. The roots of some *Crinum* species are used to treat urinary infections, coughs and colds, renal and hepatic conditions, sores, sexually transmitted diseases, and backache. Although no scientific studies have been reported from the *C. amoenum* Roxb., the bulb extract of the *Crinum* species has diverse pharmacological activities such as antiviral, analgesic, antineoplastic, immunostimulant, wound healing, anti-inflammatory, anthelmintic, and antimicrobial effects.[26]

Despite widespread medicinal use of these plants in Nepal, there are limited studies reported in literature for their antioxidant and anti-inflammatory activity. Hence, the present study was undertaken to evaluate *in vitro* antioxidant and *in vivo* anti-inflammatory activity, along with phytochemical screening tests of ethanolic and aqueous extracts of selected plants to support their medicinal value.

MATERIALS AND METHODS

Chemicals and Drugs

Diclofenac sodium was purchased from SR Drug Laboratories Pvt. Ltd., Satungal, Kathmandu, Nepal, Carrageenan (HiMedia Laboratories Pvt. Ltd. LBS Marg, Mumbai, India), ascorbic acid (Nike Chemical, India), orthophosphoric acid, 1-Nepthylene ethylenediamine dihydrochloride, potassium iodide, and Iodine (HiMedia Laboratories Pvt. Ltd. Mumbai), ammonia solution (Nike Chemical India), HCl, H_2SO_4, Fehling's solutions A and B, ethanol, NaOH (Qualingens Fine Chemicals Division of GlaxoSmithKline Pharmaceutical Pvt. Ltd, Mumbai), and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) (Wako Pure Chemical Co. Ltd., Osaka, Japan).

Instruments

UV spectrophotometer model UV 1800 240V (Shimadzu Corporation, Kyoto, Japan), Plethysmometer model 7141 (UGO Basil SRL, Biological Research Application, Italy), Rotary evaporator (BUCHI Labortechnik AG, Switzerland), Refrigerator (LG company), Electronic balance (FA1104 Electronic Balance), and rectangular water bath (VIT company).

Plant Materials

Fresh and healthy root barks of *M. alba*, apical leaves of *C. oppositifolia*, bulbs of *C. amoenum*, and roots of *I. cylindrical* were collected from Lekhnath-12, Kaski district, Nepal; (tropical region, 515 m above the sea level) in July and August 2019. The plants were identified and confirmed by senior taxonomist Prof. Dr. Radhe Shyam Kayastha. The voucher specimens of all the plant samples were protected in the herbarium museum of the Department of Pharmaceutical Sciences, Pokhara University, Nepal. The details of collected plants and voucher ID are depicted in Table 1.

Plant Extract Preparation

Plant materials [Figure 1] were cleaned with distilled water. The remaining water was wiped with the help of a clean cloth. Plants parts were cut into small pieces and kept for shade drying at room temperature in a well-ventilated environment. The drying process was carried out for 15 days until the dry weight remained constant. After the completion of drying, all the plant materials were grinded to a fine powder with the help of a portable grinding machine. The comminuted powder mass was then moved through the sieve of mesh size 40. For the extraction, each powdered plant samples (100 g) were subjected to first cold maceration in 700 mL of ethanol and water separately for 24 h, followed by second maceration for another 24 h. Then, the filtrate obtained from the first and second maceration was mixed thoroughly and allowed for evaporation on rotatory evaporator (temperature < 50°C) until the solvent fully evaporated. Thus, obtained extracts were stored in a glass vial and kept inside the vacuum desiccator containing silica crystals for complete drying. The weight of extract was noted daily, until the dry weight remained constant. Thus, obtained extracts were preserved at 4°C until use.

Phytochemical Screening

All the plant extracts were investigated for the presence of alkaloid, saponin, terpenoid, tannin, cardiac glycosides, and flavonoid, according to previously established method.[27-30]

DPPH Radical Scavenging Assay

The antioxidant potency of all the plant extracts was investigated by DPPH free-radical scavenging activity assay,

Table 1: Information on Scientific names, local names, collected plant parts, voucher numbers and extractive yield of ethanol and water extract

S. No.	Scientific names	Local names	Parts used	Voucher number	Ethanol (Yield %)	Water (Yield %)
1	*Morus alba* L.	Kyu Kafal	Root bark	PUH-2019-19	2.30	2.01
2	*Imperata cylindrica* (L.) Beauv	Siru	Root	PUH-2019-20	11.07	10.10
3	*Colebrookea oppositifolia* J.E. Smith	Dhurseli	Leaves	PUH-2015-21	3.68	7.53
4	*Crinum amoenum* Roxb.	Hade Lasun	Bulb	PUH-2015-22	16.63	34.39

with the help of the established method by doing slight modification.[31,32] DPPH solution having a concentration of 0.1 mM was prepared. Three different concentrations of plant extracts (1, 10, and 100 µg/mL) were prepared. Then, 2 mL of prepared DPPH solution and 2 mL of each test sample were mixed with strong shaking and placed in the dark place, at normal room temperature. After 15 min, absorbance was recorded at 517 nm with the help of a UV spectrophotometer. Positive and negative controls were ascorbic acid and methanol, respectively. Every test was carried out 3 times. The percentage of radical neutralization was calculated using the following formula.

$$\%\ \text{Inhibition Percentage radical scavenged} = \left(\left[A_0 - A_1\right] / A_0\right) * 100\%$$

Where, A_0 represents the absorbance of methanolic DPPH solution and A_1 represents the absorbance of the test sample solution.

Animals and Ethical Approval

Female Wistar rats, weighing 150–210 g, about the age of 30 days were obtained from the Department of Plant Resources, Kathmandu, Nepal. Rats of the same sex (female) were used in the experiment to avoid the hormonal disturbances that occur within the physiological system of the animal which might affect the reliability of the result. They were housed in a standard polypropylene cage under normal room temperature (25±3°C) and 12 h natural light/dark cycle. Animals were fed for 3 weeks with a standard diet and water *ad libitum*. Animals were kept for fasting up to 12 h before the experiment but permitted free access to water. Throughout the experiment, internationally accepted ethical guidelines for the care of laboratory animals were followed and all ethical manners were considered under the "Ethical Guidelines for Care and Use of Animals in Health Research in Nepal, 2005."[33-35] All experimental rats were subjected to 35% CO_2 euthanasia after completing the experiment. The experimental protocol was verified by the Institutional Review Committee of Pokhara University (Ref: PU/IRC/058/39).

Acute Toxicity Study

Acute toxicity in rats was conducted by following the protocols developed by the Organization of Economic Cooperation and Development.[36] A total of six groups of female Wistar rats (n = 5) were formed. Among them, one group was a normal control group and the other five were test groups. The normal control group was treated with normal saline and all test groups were provided with plant extract of different concentrations (100, 250, 500, 1000, and 2000 mg/kg, p.o.,) at 10 mL/kg. Any symptoms of toxicity and mortality were observed every 1 h for the next 6 h and whole body weight was calculated after 1, 7, and 14 days of the treatment.

In vivo Acute Anti-Inflammatory Activity: Carrageenan Induced Rat Paw Edema Volume Measurement

The anti-inflammatory potency of ethanolic and aqueous extract of *M. alba* root and *C. oppositifolia* leaves was investigated by adopting rat paw edema model, according to previously established method with slight modification.[37] Wistar female rats were divided into seven groups (five rats in every group).

Group- I animals (normal control) received 500 µL of distilled water only.

Group-II animals (carrageenan control) received 0.5 mL distilled water p.o., 60 min before carrageenan injection.

Group-III, the standard group, was given p.o., an aqueous solution of 50 mg/kg Diclofenac sodium (50 mg/kg), 60 min before carrageenan injection.

Group-IV and V received p.o. ethanolic root extract of *M. alba* at the concentration 100 mg/kg and 250 mg/kg, respectively, 60 min before carrageenan injection.

Group-VI and VII received p.o. ethanolic leaves extract of *C. oppositifolia* at the concentration 100 mg/kg and 250 mg/kg respectively, 60 min before carrageenan injection.

After 60 min, a solution of 1% carrageenan prepared in normal saline was injected into the sub-plantar area of the right hind paw of every rat, in each test group. Before the development of paw edema, the paw volume of every rat was noted using a Plethysmometer. After the induction of edema, the measurements were taken up to 4 h, at every 1-h intervals. The percentage of increase in paw volume of carrageenan injected rats was interpreted as the development of edema. The inflammation index was calculated as the difference between the final volume of the carrageenan injected paw (*Vt*) and the initial volume of the same paw before injecting it (*V0*), that is, inflammation index (Ii) = *Vt*–*V0*. The edema inhibition (%) was calculated as a percentage of the difference of Ii according to the following formula.

$$\% \text{ Inhibition} = \frac{\text{Control group Ii} - \text{Test group Ii}}{\text{Control Ii}} \times 100$$

Statistical Analysis

All results were expressed as mean ± standard deviation. The mean values were calculated using Microsoft Excel 2019. The data from the carrageenan induced paw edema were analyzed by one-way analysis of variance followed by Dunnett's test using SPSS version 16 software. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical Screening

Qualitative exploration of phytochemicals is a pivotal footstep to gather the information about presence of pharmacologically effective secondary metabolites in the plants, revealing a crucial role toward the beneficial medicinal and physiological activities such as antiviral, antimicrobial, anticancer, antioxidant, antidiabetic, and antimicrobial activities.[38,39] Preliminary phytochemical investigation revealed the presence of varied degree of alkaloid, saponin, terpenoid, tannin, cardiac glycosides, and flavonoid, in all the studied plants. In this study, cardiac glycosides were absent in the ethanolic and aqueous root extract of *I. cylindrica.* Similarly, ethanolic and aqueous leaves extract of *C. oppositifolia* was devoid of tannins. In the aqueous *I. cylindrica root* extract and *C. oppositifolia* leaves extract, alkaloids were detected in very less amounts; however, alkaloids were absent in their ethanolic extract. Although flavonoid was abundantly present in ethanolic *M. alba* root bark extract, it was not detected in aqueous extract. The results are summarized in Table 2.

Antioxidant Activity

Among eight different plant extracts, significant antioxidant potency was exhibited by *M. alba* ethanolic root bark extract (EC_{50}–51.37 µg/mL) and *C. oppositifolia* ethanolic leaves extract (EC_{50}–55.81 µg/mL), comparable to the standard ascorbic acid (EC_{50}–5.47 µg/mL), as determined by DPPH radical scavenging assay technique. Aqueous extract of both plants was not effective to scavenge the free radicals. Furthermore, both ethanolic and aqueous extracts of *C. amoenum* bulb and *I. cylindrical* were reported to be very poor DPPH radical scavenger in this study. Antioxidant potency of *M. alba* root bark extract and *C. oppositifolia* leaves extract is almost comparable to the previous study.[8,40] No significant scientific study has been conducted regarding antioxidant activity of *I. cylindrica* and *C. amoenum.* Graphical representation of DPPH free-radical scavenging potency shown by all the investigated plant extracts is depicted in Figure 2.

Anti-inflammatory Activity

Table 3 and Figure 3 reveal the consequence of anti-inflammatory potency exhibited by orally administered ethanolic and aqueous extract of *M. alba* root bark and *C. oppositifolia* leaves, as compare to standard drug Diclofenac sodium, from carrageenan-induced rat paw edema model. Injection of carrageenan into the right hind paw of experimental female rats induced progressive paw edema throughout the experimental period. While observing the normal control Group-I, the volume of the paw in each rat was reported to be almost constant throughout the study, whereas carrageenan control Group-II manifested an increase in paw volume and was significant at $P < 0.05$ while comparing to the normal control group. In all the experimental rats, treated

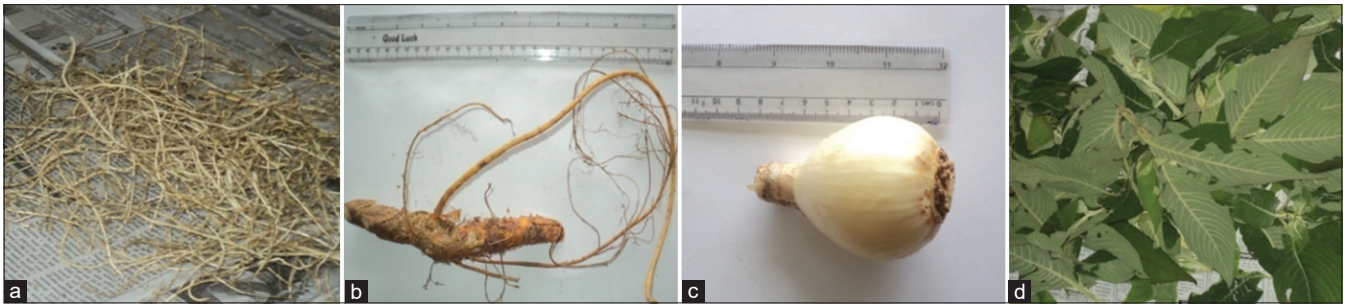

Figure 1: Different parts of selected medicinal plants (a) *Imperata cylindrica* roots, (b) *Morus alba* root, (c) *Crinum amoenum* bulb, (d) *Colebrookea oppositifolia* leaves

Table 2: Phytochemical screening of ethanolic and aqueous extracts of selected plants. Samples from the Nepal

S. No.	Test	MAEE	ICEE	COEE	CAEE	MAAE	ICAE	COAE	CAAE
1	Alkaloid	+	–	–	++	+++	+	+	+++
2	Saponin	+++	++	+	+++	+++	-	++	+++
3	Terpenoid	+++	+++	+	+++	++	++	++	++
4	Tannin	+	+++	–	+	–	+	–	++
5	Cardiac glycosides	+++	–	+	+	++	–	++	–
6	Flavonoid	+++	–	+	+	–	+	–	++

Indicators - +++: Abundantly present, ++: Adequately present, +: Less present, –: Absent, MAEE: *Morus alba* ethanolic extract, MAAE: *Morus alba* aqueous extract, ICEE: *Imperata cylindrica* ethanolic extract, ICAE: *Imperata cylindrica* aqueous extract, COEE: *Colebrookea oppositifolia* ethanolic extract, *COAE: Colebrookea oppositifolia* aqueous extract, CAEE: *Crinum amoenum* ethanolic extract, CAAE: *Crinum amoenum* aqueous extract

Table 3: Effect of ethanol and aqueous extract *Morus alba* and *Colebrookea oppositifolia* at the doses of 100 and 250 mg/kg and Diclofenac (50 mg/kg) in comparison to carrageenan control group in carrageenan-induced paw edema model using Plethysmometer

Time treatment (mg/kg)	Mean change in paw edema volume (ml) ± standard deviation and (% of edema inhibition)			
	After 1 h	After 2 h	After 3 h	After 4 h
Normal control (500 µL)	0.21±0.03	0.21±0.009	0.22±0.007	0.22±0.008
Carrageenan control (0.1 mL)	0.420*±0.003	0.518*±0.022	0.584*±0.015	0.432*±0.005
Diclofenac (50 mg/kg)	0.192±0.001* 54.38±0.32%	0.240±0.006* 53.66±1.2%	0.214±0.004* 63.39±0.63%	0.194±0.010* 55.09±2.36%
M. alba (100 mg/kg)	0.252±0.007* 40.00±1.61%	0.254±0.004* 50.96±0.72%	0.316±0.004* 45.89±0.64%	0.312±0.008* 27.08±1.19%
M. alba (250 mg/kg)	0.242±0.007* 42.38±1.61%	0.316±0.003* 39.18±0.61%	0.246±0.004* 57.87±0.64%	0.222±0.002* 48.61±0.56%
C. oppositifolia (100 mg/kg)	0.242±0.006* 42.38±1.43%	0.266±0.004* 48.64±0.72%	0.346±0.004* 40.75±0.64%	0.318±0.002* 26.38±0.56%
C. oppositifolia (250 mg/kg)	0.222±0.002* 47.143±0.58%	0.252±0.006* 51.35±1.16%	0.288±0.002* 50.68±0.39%	0.248±0.002* 42.59±0.56%

*Significant at $P<0.05$ compared with negative control group. Values are expressed as mean±standard deviation

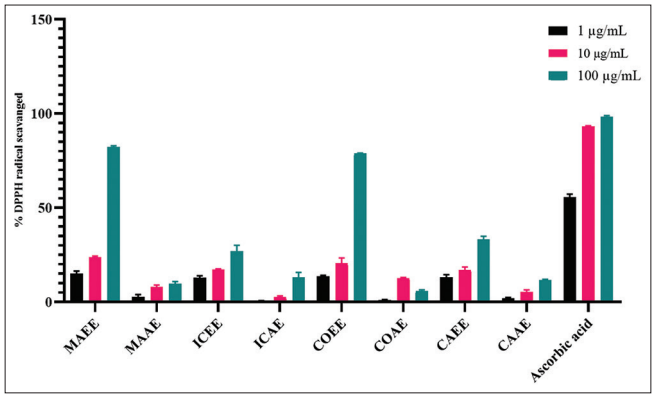

Figure 2: Bar diagram showing DPPH free-radical scavenging capacities of selected plants samples from Nepal. MAEE: *Morus alba* ethanolic extract, MAAE: *M. alba* aqueous extract, ICEE: *Imperata cylindrica* ethanolic extract, ICAE: *Imperata cylindrica* aqueous extract, COEE: *Colebrookea oppositifolia* ethanolic extract, COAE: Colebrookea oppositifolia aqueous extract, CAEE: *Crinum amoenum* ethanolic extract, CAAE: Crinum amoenum aqueous extract

with plant extract and standard drug, a progressive reduction in paw edema was noticed immediately after the oral ingestion. The reduction in paw edema was in proportion to the increased time. Ethanolic extract of both plants was found to be more effective than aqueous extract, in a dose-dependent manner. As shown in Figure 3, the highest inhibitory effect was displayed by ethanolic extract of *M. alba* root bark. From the curve (at 3 h), *M. alba* ethanolic extract (250 mg/kg) performed remarkable effect ($P < 0.05$) with the highest inhibitory potency on inflammation by 57.87%, which is almost comparable with standard drug Diclofenac sodium. Moreover, the administration of *C. oppositifolia* ethanolic leaves extract at the same dose also performed a prominent effect ($P < 0.05$) with the highest inhibitory potency on inflammation by 51.35%. Diclofenac sodium (50 mg/kg) performed the most prominent and highest anti-inflammatory effect by 63.39%.

Carrageenan-induced paw edema gives a close resemblance to occurrence of inflammation in human.

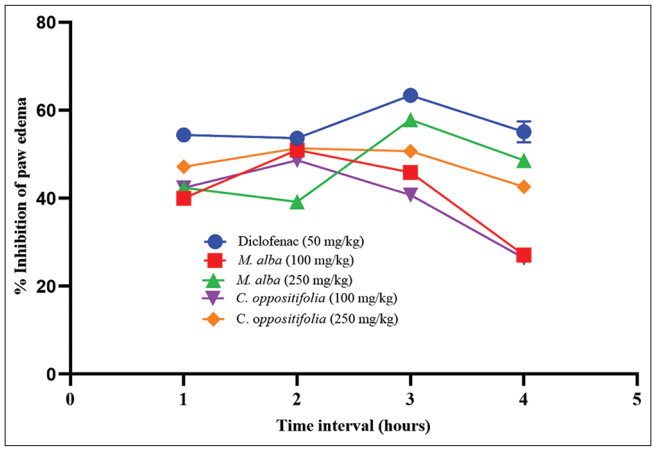

Figure 3: Graphical representations for percentage inhibition of mice paw volume by Diclofenac and, different plant extract, on time course curve. Each point indicates mean ± SEM (*n*=5), P<0.05 in comparison to the carrageenan control group

Hence, it is utilized as one of the most convenient method to investigate the possible drugs for acute inflammation. The inflammation induced by carrageenan is believed to be biphasic. First phase of inflammation is caused by release of histamine or serotonin and second phase is due to release of bradykinin, protease, prostaglandin, and lysosome.[11,37]

In this study, only the plants showing potent antioxidant activities (*M. alba C. oppositiflia*) exhibited measurable anti-inflammatory effect. The preliminary finding obtained from the investigation of these plants suggested that all the plants having ethno medicinal use may not confirm its therapeutic efficacy.[12,41] Thus, scientific investigation is crucial to minimize the unreasonable use of the herbal plants. The possible mechanism of anti-inflammatory effect of *M. alba* root bark extract may be due to cyclooxygenase inhibition and free-radical scavenging.[42] Its extract can block NO production by inhibiting inducible NOS over-expression in LPS-stimulated RAW264.7 cells (mouse macrophage cell line).[43] Anti-inflammatory potency of *C. oppositifilia* leaves extract was done for the first time in this study. In the previous study, it was reported that the methanolic root extract of *C. oppositifilia* inhibited the generation of pro-inflammatory mediators TNF-α, ROS, nitrite in lipopolysaccharide-stimulated RAW 264.7 cell lines. The presence of diverse bioactive compounds such as gossypin, acteoside, ferulic, and quercetin might be the possible mechanism for this effect.[44]

CONCLUSION

Thus, this study concluded that root bark extract of *M. alba* and leaves extract of *C. oppositifolia* possess potent *in vitro* antioxidant effect and *in vivo* anti-inflammatory effect in carrageen induced Wistar rat model. Further research should be carried out to determine the active compounds of these plants and their mechanism of actions.

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