Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 14-25
Received: 07-09-2019
Accepted: 10-10-2019

Samit Poudel
Pokhara University, School of Health and Allied Sciences, Nepal

Biswash Sapkota
Rajiv Gandhi University of Health Sciences, Bangalore, India

Purushottam Timilsina
Rajiv Gandhi University of Health Sciences, Bangalore, India

Kalpana Parajuli
Pokhara University, School of Health and Allied Sciences, Nepal

Atisammodavardhana Kaundinnyayana
Pokhara University, School of Health and Allied Sciences, Nepal

Corresponding Author:
Samit Poudel
Pokhara University, School of Health and Allied Sciences, Nepal

Phytochemical analysis, *in vitro* antioxidant activity and *in vivo* anti-inflammatory and analgesic activities of selected medicinal plants of Nepal

Samit Poudel, Biswash Sapkota, Purushottam Timilsina, Kalpana Parajuli and Atisammodavardhana Kaundinnyayana

Abstract
Nepalese medicinal plants were selected to study phytochemical presence, antioxidant, anti-inflammatory and analgesic activity of plants. Extraction was carried out by double maceration by ethanol and water. Phytochemical screening was performed by standard methods. Total phenol content was determined by Folin Ciocalteu method, flavonoid content was determined by aluminium chloride colorimetric method. The antioxidant activity was tested by DPPH free radical scavenging assay. *In Vivo* anti-inflammatory and analgesic activity was performed by carrageenan induced rat hind paw edema and hot plate methods respectively. The phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, saponins, phenols, terpenoids, glycosides and carbohydrates. The ethanolic and aqueous extract of *Viscum album* showed total phenols content, total flavonoids content and has significant antioxidant activity. Similarly, the aqueous extract of *Viscum album* has dose dependent analgesic activity. Thus, medicinal plants possess different compounds with valuable antioxidant properties which can contribute to the development of modern medicines.

Keywords: Anti-inflammatory, Antioxidant activity, DPPH, Medicinal plants, Phytochemicals

Introduction
The rich sources of medicinal plants make Nepal a highly potential zone for globalization of herbal medicine [1]. Medicinal plants have been known for millennia and are considered as a rich source of pharmaceutical agents for the prevention and treatment of diseases and ailments. Approximately, 80% of the world's population, directly or indirectly rely on traditional medicine based on herbal source of drugs.
Since ancient time, plants have been used as major source of drug [2]. Almost all parts of plant, flower, leaf, root, stem, fruit, seed, bark, latex and cone are used traditionally to treat various body ailments [3]. Modern medicines are also based on indigenous knowledge and ethnopharmacological practices which have become back bone for noble drug discovery [4].
During the last few decades there has been an increasing interest in the study of medicinal plants. So, exploration of Nepalese medicinal plants is needed to provide the scientific proof for the traditional uses of Nepalese medicinal plants.
Solena heterophylla was a perennial climber belonging to family Cucurbitaceae commonly known as Golkaankree. It is distributed throughout Nepal at 1600-3200 m, east to west. Leaf simple, alternate, stalked, 3.5-17 cm long, 2-14 cm broad, cordate at the base, tendril opposite to the leaf, shape variable, sometimes 3-5 lobed. Flower unisexual, yellowish white. Fruit obovoid, bright red when ripe. Flowering and fruiting occur in July-August [1].

Uses
Paste of root and fruit were taken to treat indigestion, throat infection. Ripe fruits are used in curing abdominal ulcer, root juice in peptic ulcer. Whole plant was given to cattle to increase lactation [1]. The tubers, leaves and seeds are extensively used in traditional system for various ailments like hepatosplenomegaly, spermatorrhoea, appetizer, cardiotonic, diuretic and thermogenic, haemorrhoids and invigorating. Behemic acid, columbin, lignoceric acids are the important compound found in this plants [5].
Abelmoschus esculantu was originally classified under the genus Hibiscus in the family Malvaceae. Okra (English name) known as Bindi in Nepal; and is an important vegetable which is widely distributed from Africa to Asia [6]. Okra is an annual and perennial herbaceous plant, growing to 2 m tall.

The leaves are 10–40 cm long and broad, palmately lobed with 3-7 lobes, the lobes are very variable in depth, from barely lobed, to cut almost to the base of the leaf. The flowers are 4–8 cm diameter, with five white to yellow petals, often with a red or purple spot at the base of each petal. The fruit is a capsule, 5–20 cm long containing numerous seeds [7, 8]. K, Na, Mg and Ca were found to be the principle elements, with Fe, Zn, Mn and Ni also present [9]. The natural phenolic content of okra seeds has been reported [10-12].

Uses

Okra seeds could serve as alternate rich sources of protein, fat, fiver and sugar [13-15]. Mature pods of Okra contain a mucilaginous substance; young shoots and leaves are edible[16]. Seeds are effective aphrodisiac and antispasmodic, and used in tonics. Also useful in treating intestinal disorders, urinary discharge, nervous disorders, hysteria, skin diseases etc. In India, roots, leaves (rarely), and seeds of ambrette are considered valuable traditional medicines [17].

Viscum album is a Large, semiparasite on trees belonging to Family Santalaceae, commonly called Hadchur and mentioned in Ayurveda, Siddha, and Chinese medicinal system for treatment of various disorders. Its leaves are sessile, flat, oblong, or obovate. Berry are usually globose, white. It is distributed from tropical- temperate. The flowers are very dioecious, sessile, 3-5 in a cluster, bracts concave is observed only for a short span of time, between December and January [18].

Uses

Almost every part (stem, bark, leaves, root and fruit) of this plant possesses pharmacologically active constituents with diverse sets of biological activity. Berry are laxative, tonic, aphrodisiac, diuretic, cardio tonic. Plant is given in enlargement of spleen, in case of wound, tumors, diseases of the ear. Plant paste is applied on dislocated bones, bark paste on muscular swellings [19]. In Nepal, the plant is traditionally used for curing sprains, bone fractures and dislocation and used as fodder [20]. Viscum album whole plant with Kaempferia rotunda rhizome and Bergenia ciliata stern is grounded to paste and applied on the affected areas to treat sprains, bone fractures, wounds and boils by the Nepalese communities [21].

Curcuma longfolia is a errect perennial herb about 1 m high belonging to Zingiberaceae family commonly called Haledo and mainly found in the Himalayas and Khasi Hills. It is distributed from tropical to Subtropical. Leaves in groups of 6-10 on pseudostems, about 40cm long, broadly lanceolate, acuminate, entire, bright green with distinct midrib, base sheathing. Flowers yellowish in pairs in the axiles of the bracts, 1 opening before the other. Tuber and rhizome are applied as paste, powder, decoction and infusion [22].

Uses

Rhizome is acrid, thermogenic, emollient, anodyne, anti-inflammatory, vulnery, depurative, strong antiseptic, appetizer, anthelmintic, laxative, diuretic, expectorant, haematinic, styptic, antiperiodic, alternative, alexiteric, detergent, febrifuge, opthalmic and tonic. It is used in all kinds of poisonous affections, ulcers and wounds [22].

Fraxinus floribunda (FF) Wallich belonging to Oleaceae family commonly called Laankuri and is a large tree mainly found in the Eastern Himalayas, Khasi Hills, and Sikkim. It is distributed throughout Nepal to Subtropical. Leaves are odd-pinnate whose leaflets has 3-5 pairs, or petiolate. Flower is pedicellate, small, white and polygamous. Fruits a winged, 1 seeded. It mainly used plant parts are tender stem and flower which derived aromatic oil from flowers, juice of stem [22].

Uses

The leaves and bark of the plant is considered as popular ethnomedicine used by the herbal healers in some villages of Sikkim for treating bone fracture, dislocation, and gout [23]. The leaves were found to possess potential anti-inflammatory and antinociceptive activity [24] but there was not a single report of anti-inflammatory activity on the bark of the plant in spite of being frequently used in folk medicine. Stem juice/ resin is a mild laxative.

Cissampelos pareira is a climbing herb with perennial root stock belongings to family Menispermaceae commonly called Batulpate. It is distributed throughout Nepal to about 3000 m, common in moist, shady places; also, pantropical. Leaf stalked, 2.5-8cm long, 2-7.5 cm wide, or biculate or reniform, usually peltate, mucronate, base cordate or turnicate, or more or less tomentose both sides. Flower minute, yellowish clustered in the axils or on long axillary stalks. Fruit a drupe, subglobose, red when ripe. Flowering occurs in April-June, Fruiting occurs in July-November. It propagated by seeds or root off shoot [22].

Uses

Juice of the plant, about six teaspoons twice a day, is given after delivery to stop bleeding and to counteract the loss of blood. It is tonic and diuretic. Its juice about three teaspoons three times is taken three times a day, in the cases of fever of indigestion. A decoction of the leaves is applied to soothe the pain of dislocated bones. The juice is applied to treat skin diseases and is taken internally as a cooling medicine for gonorrhoea. The root paste is used for snakebites, swelling of the gums and peptic ulcers [22].

Achyranthes aspera is a herb grown upto 30-90 cm high belonging to family Amaranthaceae commonly known as ‘Apaamaarg’ or ‘Ultokuro’. It is widely distributed Pantropical to tropical- Temperate region. Leaves are opposite, 2-12 cm long, rounded at the apex, elliptic, abovate, petiole short. Flower are small, greenish white color. Whole plant parts is applied in the form of decoction and juice [22].

Uses

Plant is purgative, diuretic, astringent, used in dropsy and piles. Leaf juice is used in stomachache, piles, skin eruptions, roots for pyrrhoea and cough. Seed power is used in bleeding piles; brain tonic. Root extract is used to treat menstrual disorders and dysentery. Plant decoction is used in pneumonia, cough, kidney stone, renal dropsy and anasarca. It is one of the constituents of Cystone, which is used to cure urinary tract infection [22]. Severe stomach pain. Whole plant of *Achyranthes aspera* along with leaves of *Aerva lanata* is crushed and taken with a little misri (sugar crystal). Excessive bleeding after menstruation. Paste of *Cuscuta reflexa* is made separately with paste of whole plant of *Achyranthes aspera*. *Achyranthera aspera* paste is warmed and mixed with paste of *Cuscuta reflexa* and tied to vaginal area before sleeping for 7 days [25].

Periploca calophylla is the trailing shurb belonging to family Apocynaceae commonly called Sikaari laharo. It is distributed throughout Nepal to Subtropical-Temperate region. Leaves stalked, 3.5-8.5 cm long, 0.3-1.7 cm wide, lanceolate, long acuminate, lathery, shiny. Flower pinkish in cymes. Fruit a follicle, cylindrical.

Uses

Whole plant parts paste is applied to set dislocated bone. The major chemical constituents isolated from the plants of this genus were cardiac glycosides and C-steroidal glycosides, which had the activities of cardiac and antitumor have complex chemical compounds and notable pharmacological activities [26].

Nycthanthes arbor-tristis (NAT) is a Shrub or small tree, up to 9 m high belonging to family Oleaceae commonly known as'Rudilo' or 'Bidilo' or 'Paarijaat' is a well-documented plant. It is a native of India, distributed wild in the sub-Himalayan region and often cultivated for its fragrant flowers. Leaves petioled; 10-12 cm long, 5-6 cm wide, ovate, acute, entire or with distant teeth. Flower is very fragrant, cyme. The indigenous people of both Nepal and India use Nyctanthes arbor-tristis to cure various ailments alongwith its use in Ayurveda, Siddha and Unani systems of medicines [27-8]. Whole plant part is applied as Juice, decoction, essential oil.

Uses

The leave juice and decoction are commonly used in traditional system of medicine to treat rheumatism, arthritis, and inflammatory disorders. The whole plant is used for the treatment of cancer, leishmaniasis, various inflammatory disorders, root for fever, sciatica, and anorexia; bark as an expectorant, leaf to control diabetes and as a cholagogue, diaphoretic and anthelmintic. The bark decoction is used to treat arthritis and malaria, to purge intestinal worms, and as a tonic and laxative [29]. Plant is acrid, bitter, antibacterial, anodyne, anti-inflammatory, digestive, anthelmintic, depurative, sudorific, expectorant, cholagogue, anthelmintic, laxative, and tonic. Leaves are useful in fever and rheumatism. Seeds are very useful in baldness, scurvy and affection of the scalp [22].

Cynodon dactylon is a Perennial, creeping grass belonging to family Poaceae commonly known as 'Dubo' or 'Situ' or 'Panjaa' or 'Durbadal'. It is widely distributed in all warmer countries and often cultivated in Subtropical-Temperate climate. Leaves short, subulate glaucous, narrow, flat Spikes green or purplish and spikelets 2-2.5 mm. The indigenous people of both Nepal and India use *Nyctanthes arbor-tristis* to cure various ailments along with its use in Ayurveda, Siddha and Unani systems of medicines [27-8]. Whole plant paste and juice are applied in different aliments.

Uses

Plant is a reputed remedy in epistaxis hematuria and scabies. Plant juice is applied in fresh cuts and wounds, used in dropsy, anasarca, hysteria, epilepsy, insanity, catarrhal ophthalmia. Root infusion is taken orally in bleeding piles. Crushed roots mixed with curds is used in chronic gleets. Rhizome is used in urinary and bladder complaints, cystitis, nephritis, also recommended in gouts and rheumatism. The roots were made into a paste with water and taken internally against colds, coughs and fevers. Scientific investigations have to be carried out with the roots of the plant [22].

Oxalis corniculata is a diffused hairy herb belonging to family Oxalidaceae commonly known as 'Chari amilo' or 'Tinpaatiya'. It is almost cosmopolitan and often cultivated in Tropical-Temperate climate. Leaves 3-foliate, long-petiole; Flowers yellow, in long stalked umbles; Capsules linear-oblong, to mentose. Whole plant paste, powder, extract oxalate, Malic acid, Tartaric acid and Citric acid fresh juice are applied in different aliments [22].

Uses

Plant is astringent, emmenagogue, antiseptic, digestive, carminative, liver tonic, diuretic, constipating, febrifuge, antibacterial, antiseptic. Boiled with buttermilk, it is a remedy for indigestion and diarrhea in children. Fresh plant juice cures dyspepsia, piles, anemia and tympanites, scurvy, and biliousness and for removing corns, warts and other excrescences of the skin. Leaf infusion is used to remove opacities of cornea [22].

Drynaria propinaua is foliage dying epiphytic or lithophytic fern belonging to family commonly known as chauta jor. It is almost found in Himalaya and often cultivated in Tropical-Temperate climate. Rhizome wide, creeping, with brown linear lanceolate scale. Fronds glabrous, 30-50 cm long, 15-20 cm wide, with distinct stipes, lobes serrate, reaching down near the rachis. Sori yellowish brown in a single row along the mid vein. Rhizome paste are widely applied on different aliments [22].

Uses

Rhizome paste is applied to treat backache and dislocated bone. This paste is considered good for sprains and is also applied over forehead to relieve headache; also used as an antidote to food poison [22].

Materials and Methods

Material

Solvents / Chemicals

The chemicals and solvents were used for the experiment were of analytical grade and provided by School of Health and Allied Sciences, Pokhara University.

Plant Materials

The entire experimental work was performed in the laboratories of School of Heath and Allied Sciences, Pokhara University from May 2018 to October 2018. The authentic sample of plants and crude drugs were preserved in the Laboratory of Pharmacognosy, School of Health and Allied Sciences, Pokhara University. Experimental plant materials were collected from chitwan and Lamjung district of Nepal during the month of May 2018. The details on plant sample collection have been shown in Table 1.

Table 1: List of Plants Used for the Experiment

Scientific Name	Local Name	Parts used	Family	Crude Drug Voucher No.
Solenaheterophylla	Golkaankree	Leaf	*Cucurbitaceae*	PUCD-2018-31
Abelmoschus esculantus	Bindi	Root	*Malvaceae*	PUCD-2018-32
Viscum album	Hadchur	Bark	*Sapindaceae*	PUCD-2018-33
Curcuma longfolia	Haledo	Rhizome	*Zingiberaceae*	PUCD-2018-42
Fraxinus floribunda	Laakuri	Bark	*Oleaceae*	PUCD-2018-34
Cissampelos pareira	Batule pate	Leaf and stem	*Menispemmaaceae*	PUCD-2018-35
Achyranthes aspera	Datiwan or Ultokuro	Stem and leaf	*Amaranthaceae*	PUCD-2018-36
Periplocacalphylla	Sikaarilaharo	Stem	*Asclepidaceae*	PUCD-2018-37
Nyctanthes arbor-tristis	Paarijaat	Leaf	*Oleaceae*	PUCD-2018-38
Cynodon dactylon	Dubo	Whole plant	*Poaceae*	PUCD-2018-39
Oxalis corniculate	Chari amilo	Whole plant	*Oxalidaceae*	PUCD-2018-41
Drynaria propinaua	Chautajor	Bulb	*Polypodiaceae*	PUCD-2018-40

Experimental Animals

Healthy adult Swiss albino rats weighing between 180 and 250 g of 3 weeks of age were procured from Nepal Plant Resource Center (Banaspati Bibhag) Kathmandu. Rats of different sex (male and female) were used in the experiment. They were housed in standard polypropylene cage under standard environment conditions (at normal room temperature and 12 hours natural light and dark cycle) within the University premises. Animals were kept for acclimatization in laboratory of Pokhara University and fed for 4 weeks with standard diet and water *ad. Libitum.* Animals were fasted for 12 hours before the experiment.

Ethical Consideration

An ethical approval was obtained from institutional Review Committee (IRC) Pokhara University Research Center (PURC) before all the animal experiment. All the research works involving use of animals were performed following IRC and NHRC rules and regulations. The research activities as well as the sacrifice of the animals after the research were conducted in ethical manner.

Methods

Collection and Identification of Plant Materials

Selected medicinal plants (Table 1) were collected from Chitwan and Lamjung District and processed for preservation and further examination. The herbaria were prepared and identified with the help of experts in Botanical Garden, Godawori and by comparison with literature.

Extraction

Extraction of selected plant samples were carried out by maceration. The dried and crushed crude drugs (30 g) were macerated with ethanol (240 ml) and water (240 ml) separately in the ratio 1:8 (w/v) for 48 hours at room temperature. Then, the extract obtained were filtered and the filtered extract obtained from first and second maceration were mixed thoroughly and allowed for evaporation on rotatory evaporator at 50-150 mbar pressure at 90 rpm and 5 ^{o}C chilling temperature till the solvent fully evaporates. Thus, obtained extracts were stored in glass vial and kept inside the vacuum desiccators at pressure 60 mbar containing silica crystals for complete drying. The weights of extract were noted on daily basis till the dry weight remains constant.

Calculation of Extract Yield Percentage

To calculate the extract yield percentage, first of all, weight of dried extract was measured.

Then, extract yield percentage was determined by using following formula:

Extract Yield Percentage: [(Weight of dry extract /Weight of crude drug sample) × 100%]

Phytochemical Screening

Phytochemical screening of various extracts was carried out to reveal the presences of secondary metabolites according to the method of Bhatnagar *et al.* [30] The qualitative results were expressed as (+) for the presence and (−) for the absence of phytochemicals.

Determination of Antioxidant Activity

The DPPH free Scavenging assay was carried out according to the method of Kim *et al.* [31] 2ml of different concentration of extract solution (1µg/ml, 10 µg/ml, 100µg/ml) of each plant sample were mixed with 2ml of DPPH solution(60µM). The mixture was allowed to stand in dark condition for 30 minutes for complete reaction. Finally, the absorbance of each plant samples was measured at 517 nm by using UV spectrophotometer. Radical scavenging activity of each sample was calculated by using following formula:

Radical scavenging (%) = $[(A_0 - A_1 / A_0) \times 100\%]$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample extracts

Control is the test solution without sample. Ascorbic acid was taken as standard. Similar procedure was followed with ascorbic acid solution of concentration (1µg/ml, 10µg/ml and 100µg/ml).

The antioxidant activity of each sample was expressed in terms of IC_{50} (concentration required to inhibit DPPH radical formation by 50%). The scavenging activity (%) was then plotted against concentration and from the graph, Inhibitory Concentration 50 (IC_{50}) value was calculated by using linear regression analysis with Microsoft Office Excel 2007.

Determination of Total Phenol Content

The total phenolic content of the extract was determined by the Folin Ciocalteu method [32-3]. 1ml of crude extract (1 mg/ml) was mixed with 5 ml with distilled water and 1ml of Folin Ciocalteu reagent. After standing for 5 min, 1 ml of 10% (w/v) sodium carbonate was mixed and shaken. The mixture was allowed to stand for 60 min, and absorbance was measured at 725 nm. The calibration curve was prepared using gallic acid as the standard of 50 mg/l, 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l and 500 mg/l (Figure 18). The total phenolic content was calculated from the calibration curve and the results were expressed as mg of gallic acid equivalent (GAE) per gram dry weight of extract [34].

Determination of Total Flavonoid Content

Aluminium chloride colorimetric method was used for determination of flavonoid content according to the method of Kaur and Juneja 2014. 1 ml of each extract solution (1mg/ml) was mixed with 4ml of distilled water. Then, 0.3 ml of 5% sodium nitrite was added. After 5 minutes, 0.3 ml 20% aluminium chloride was added and allowed to stand for 6 minutes. Then, 2ml of 1M sodium hydroxide was added. The mixture was shaken and absorbance was measured at 510 nm using UV spectrophotometer. The calibration curve was prepared using quercetin as the standard of concentration 50mg/l, 100 mg/l, 200mg/l, 300 mg/l, 400 mg/l and 500mg/l. Total flavonoid content was calculated from the calibration curve and results were expressed as mg quercetin equivalent per gram dry extract weight [35-7].

***In Vivo* Determination of Anti-inflammatory Activity**

Carrageenan-Induced Paw Edema in Rats

Rats were divided into five groups each consisting of 6 rats as shown in the Table 2.

Table 2: Groups with Different Treatment Protocol for Anti-inflammatory Activity

Groups (n=6)	Treatment
Group I (Control group)	Distilled water
Group II	*Viscum album* (50 mg/kg)
Group III	*Viscum album* (100 mg/kg)
Group IV	*Viscum album* (200 mg/kg)
Group V (Standard group)	Diclofenac (50 mg/kg)

Experimental Procedure

The anti-inflammatory activities of plant extracts were evaluated by using carrageenan induced rat paw edema model according to Rao *et al*. with slight modifications [38]. The inflammatory response was induced by sub-plantar injection of 0.1 ml of 1% (w/v) sterile carrageenan in saline into the right hind paw of rats. The standard drug, diclofenac and plant extracts were administered per orally to the experimental animals 1 hr before carrageenan administration. The volume of the injected paw was measured with a plethysmometer before and after 1 hr, 2 hr, 3 hr and 4 hr of the injection of carrageenan. The edema inhibition (%) was calculated as percentage of the difference of inflammation index (Ii) according to the following formula:

$$\% \text{ Edema inhibition} = \frac{(\text{control group Ii} - \text{test group Ii})}{\text{control group Ii}} \times 100\ \%$$

The inflammation index was calculated as the difference between the final volume of the carrageenan injected paw (V_t) and the initial volume of the same paw before injecting it (V_0). *i.e.*, inflammation index (Ii) = V_t - V_0

***In Vivo* Determination of Analgesic Activity**

Hot Plate Method

Rats were divided into five groups each consisting of 5 rats as shown in the Table 3.

Table 3: Groups with Different Treatment Protocol for Analgesic Activity

Groups (n=5)	Treatment
Group I (Control group)	Distilled water
Group II	*Viscum album* (50 mg/kg)
Group III	*Viscum album* (100 mg/kg)
Group IV	*Viscum album* (200 mg/kg)
Group V (Standard group)	Diclofenac (50 mg/kg)

Experimental Procedure

Hot plate method was done to determine analgesic activity of plant extracts by Shanmukananda with some modification [39-40]. At first, hot plate temperature was maintained at 55±1ºC. Then animals treated with 0.5 ml of distilled water or extract (50 mg/kg, 100 mg/kg and 200 mg/kg) or standard drug Diclofenac (50 mg/kg) orally were placed on the hot plate and covered with a glass beaker to avoid heat loss. Each rat also acted as its own control. Cut off time was set up 15 sec. The latency time was recorded before the administration of extracts/standard drug and after 60 min, 120 min and 180 min of extracts/standard drug administration. The latency is defined as the reaction time taken by each mouse to respond to licking of the fore paws or jumping.

Percentage Analgesic Activity= $\{(P_0-P_1)/P_1\} \times 100\%$

Where, P_0= Pre-treatment Latency, P_1= Post treatment Latency

Statistical Analysis

Results were expressed as mean ± Standard deviation (SD). Statistical analysis was performed using student's t-test. $P<0.05$ was considered statistically significant. All the statistical analysis was carried out through Microsoft Excel 2007.

Results

Extraction Yield Value

The extraction yield of the plant sample in ethanol and water is given in Table 4. The yield percentage was relatively higher in aqueous extract in comparison to that of ethanolic extract. Among the different plant sample extracts yield percentage was found relatively higher in *S. heterophylla*, *N. arbortristis*, *V. album* and *F. floribunda.*

Table 4: Extraction Yield Value of Different Sample in Ethanol and Water

Plant name	Part used	Family	Yield% (Ethanol)	Yield% (Water)
Solena heterophylla	Leaf	*Cucurbitaceae*	3.75	18.37
Abelmoschus esculantus	Root	*Malvaceae*	6.95	6.30
Viscum album	Bark	*Sapindaceae*	6.42	11.41
Curcuma longfolia	Bulb	*Zingiberaceae*	5.56	10.94
Fraxinus floribunda	Bark	*Oleaceae*	8.95	11.26
Cissampelos pareira	Leaf and stem	*Menispemmaaceae*	3.43	10.64
Achyranthes aspera	Leaf and stem	*Amaranthaceae*	4.14	11.84
Periploca calophylla	Stem	*Asclepidaceae*	2.72	9.12
Nycthanthes arbor-tristis	Leaf	*Oleaceae*	16.04	16.98
Cynodon dactylon	Whole plant	*Poaceae*	7.88	7.53
Oxalis corniculate	Whole plant	*Oxalidaceae*	6.02	10.28
Drynaria propinaua	Rhizome	*Polypodiaceae*	8.88	9.25

Phytochemical Screening

Results obtained for qualitative screening of phytochemicals in different plant extracts are presented in Table 5. Preliminary phytochemical screening performed as per standardized procedures showed the presence of varied degree of phytoconstituents present in the aqueous and ethanolic extracts. Among the different plant samples alkaloids, terpenoid and glycoside content were found both in water and ethanol extract of plant sample *V. album*, *F. floribunda* and *P. calophylla* content was found in both ethanolic and aqueous extract of studied plant samples.

Table 5: Phytochemical Screening of Different Plant Samples in Ethanol and Water

Phtochemicals Plants samples	Alkaloids				Carbohydrate				Flavonoids		Terpenoids		Glycosides		Phenol		Saponin		Tannin	
	Mayers		Wagner		Molisch		Benedicts		Alkaline reagent test		Salkowski		Modified Borntrager's test		Ferric Chloride test		Foam test		Gelatin test	
	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W
S. heterophylla	+	–	–	+++	–	–	–	–	–	++	+	+	++	++	–	++	+	+	+	–
A.esculantus	++	–	++	–	–	++	+	–	++	+++	++	–	++	++	–	+	–	–	+	++
V. album	+	+	–	–	+	+	++	++	–	+	+++	+	+	++	++	+++	–	–	++	–
C. longfolia	+	–	–	+	–	+	–	–	++	+++	++	++	++	++	–	+	+	–	–	++
F. floribunda	+	–	–	+	+	+	–	+	–	–	++	+	+	++	++	+++	–	–	–	–
C. pareira	+++	+	+++	–	–	–	–	–	++	–	++	++	++	++	+	+++	+	–	+	–
A. aspera	–	–	–	–	–	++	–	–	–	++	+	+	++	++	–		–	+	–	–
P. calophylla	+++	++	+++	+++	–	+	–	–	++	+++	++	+	+	++	–	+++	–	+	+	++
N. arbor-tristis	–	–	–	–	–	++	–	++	–	–	+	–	++	++	–	+++	–	+	–	–
C.dactylon	+	–	–	–	+	+	–	–	+	+++	+	+	++	++	++	++	–	+	–	++
O.corniculata	+	–	–	++	–	+	–	+	–	–	+++	++	++	++	++	++	–	–	+++	–
D.Propinaua	–	++	–	–	+	–	–	++	++	+	++	+	+	++	++	+	–	–	+	–

Note: *E= Ethanol, W= Water;* +++ Strongly present; ++ Present; + Weakly present; – Absent

Determination of Antioxidant Activity

DPPH Radical Scavenging Activity

Percentage radical scavenging activity with IC_{50} value of different samples in ethanolic and aqueous solvent is shown in Table 6. Among the ethanolic extract of different plant samples, DPPH radical scavenging activity of *Fraxinus Floribunda* (IC_{50}: 5.4 µg/ml), *Viscum album*(IC_{50}:6.47µg/ml) and *Periploca calophylla* (IC_{50}: 11.69µg/ml) were found to be significant whereas *Abelmoschus esculantus* (IC_{50} value >100 µg/ml) possessed very low DPPH radical scavenging activity with reference to ascorbic acid standard (IC_{50}: 4.06 µg/ml).

Table 6: IC_{50} value of Ethanolic and Aqueous Extract of Different Plant Samples with Reference to Ascorbic Acid

Plant Sample/Standard ↓ Concentration →	IC_{50} (µg/ml) Ethanol	IC_{50} (µg/ml) Aqueous
Solena heterophylla	162.69	189.17
Abelmoschus esculantus	174.82	196.47
Viscum album	6.47	3.65
Curcuma longfolia	92.86	129.22
Fraxinus floribunda	5.4	3.21
Cissampelos pareira	37.82	29.82
Achyranthes aspera	189.13	123.67
Periploca calophylla	11.69	9.03
Nycthanthes arbor-tristis	61.64	90.14
Cynodon dactylon	178.01	144.17
Oxalis corniculata	184.65	103.15
Drynaria propinaua	95.13	120.83
Ascorbic acid	4.06	4.06

Data are expressed as mean ± standard deviation (n=3)

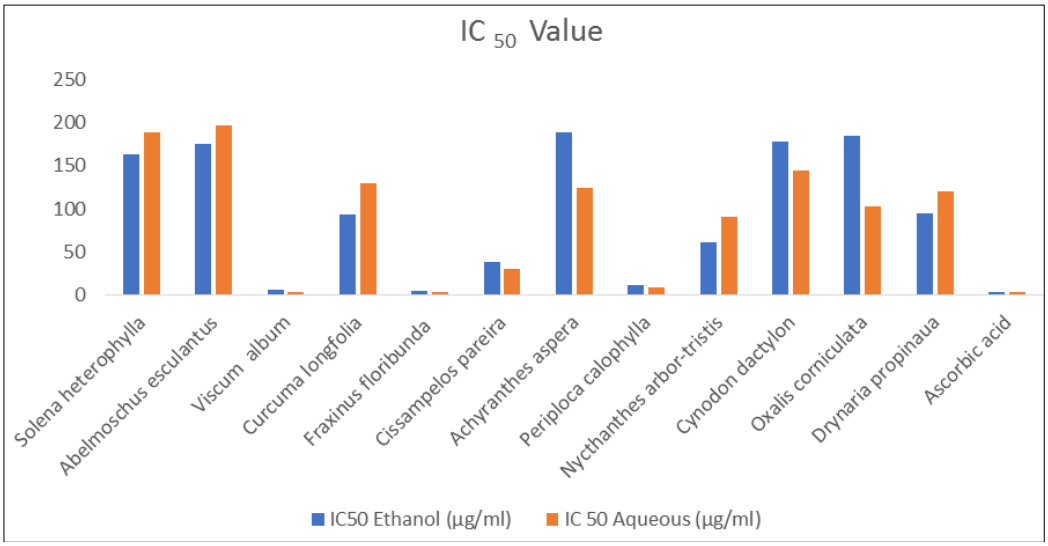

Fig 1: IC_{50} value of Ethanolic and Aqueous Extract of Different Plant Samples with Reference to Ascorbic Acid

Total Phenol Content

The total phenolic content of aqueous and ethanolic extracts was estimated by Folin Ciocalteu's method using gallic acid as standard. The maximum absorption was observed at 725 nm. The gallic acid solution of concentration (50-200mg/l) confirmed to Beer's Law at 725 nm with a regression co-efficient (R^2) = 0.979 (Figure 18). Total phenol content was calculated with the help of calibration curve of Gallic acid as standard and expressed as mg GAE/g dry extract weight (Table 7 and Figure 2).

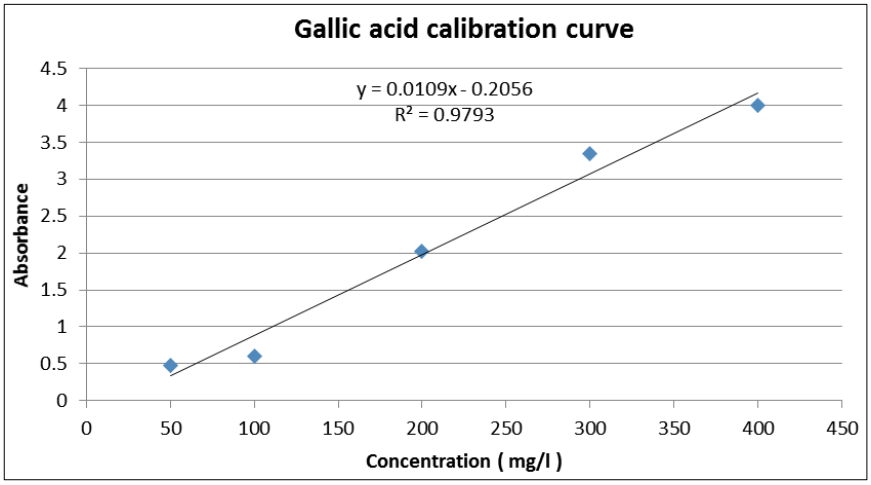

Fig 2: Calibration curve of Gallic acid

Total phenol content of ethanol and water extract of different plant samples are shown in Table 10. Among ethanolic extract, total phenol content was found highest in *Viscum album* (333.93±0.004 mg GAE/g dry extract weight) and was found lowest in *solena heterophylla* (22.67±0.003 mg GAE/g dry extract weight). Whereas, among different aqueous extracts, total phenol content was found highest in *Viscum album* (359.70±0.01 mg GAE/g dry extract weight) and lowest in *curcuma longfolia* (33.07±0.01mg GAE/g dry weight

Table 7: Total Phenol Content Expressed as Mg Gallic Acid Equivalent per Gram Dry Extract Weight

Plant Samples	Phenol Content (mg GAE/g dry extract weight)	
	Ethanol	Water
Solena heterophylla	22.67±0.003	41.90±0.03
Abelmoschus esculantus	44.20±0.002	42.93±0.00
Viscum album	333.93±0.004	359.70±0.01
Curcuma longfolia	69.97±0.004	33.07±0.01
Fraxinus floribunda	189.90±0.004	204.37±0.06
Cissampelos pareira	141.67±0.009	157.23±0.11
Achyranthes aspera	40.53±0.007	62.90±0.04
Periploca calophylla	71.87±0.013	74.63±0.06
Nycthanthes arbor-tristis	55.23±0.003	49.30±0.01
Cynodon dactylon	133.03±0.009	49.90±0.02
Oxalis corniculate	89.13±0.006	59.57±0.02
Drynaria propinaua	68.90±0.008	131.47±0.01

Note: Data are expressed as mean ± standard deviation (n=3)

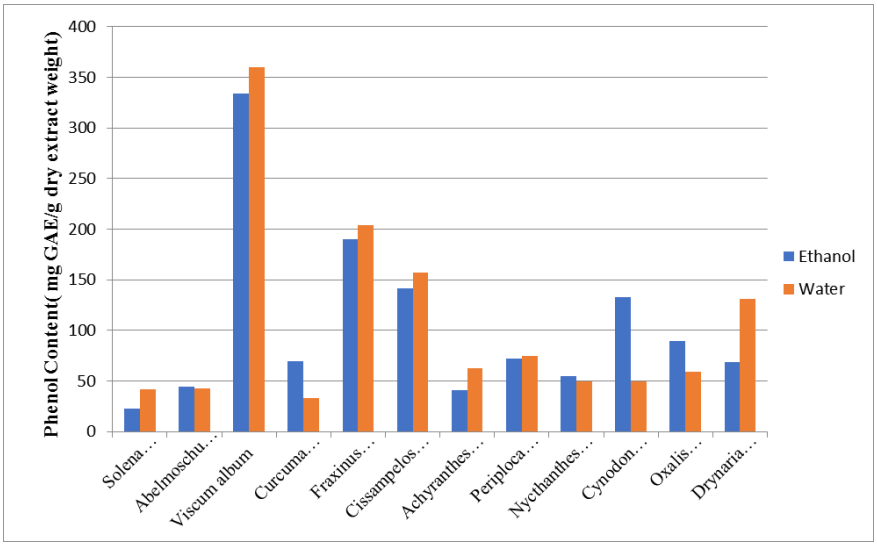

Fig 3: Total Phenol Content of Ethanolic and Aqueous Extract of Different Plant Samples

Total Flavonoid Content

The total flavonoid content for aqueous and ethanolic extracts was measured with the aluminium chloride colorimetric assay using quercetin as standard. The quercetin solution of concentration (50-500mg/l) conformed to Beer's Law at 510 nm with a regression co-efficient (R^2) = 0.994. The plot has a slope (m) = 0.00023 and intercept = 0.0302. The equation of standard curve is y = 0.00023x + 0.03022 (Figure 20). Total flavonoid content in different plants samples were calculated with the help of calibration curve of Quercetin as standard and expressed as mg QE/g dry extract weight (Table 8 and Figure 4).

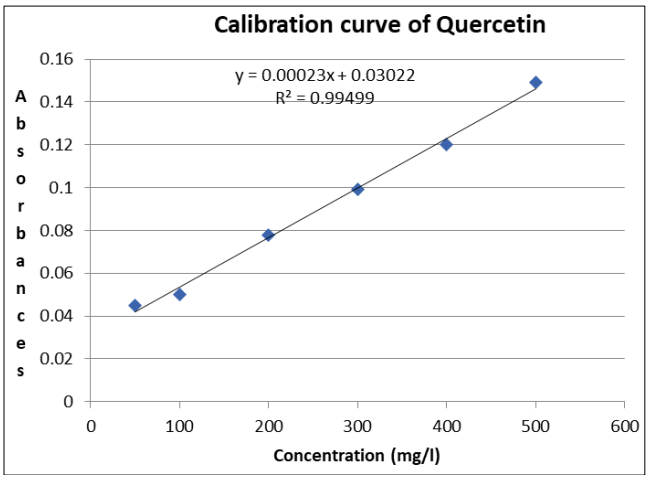

Fig 4: Calibration Curve of Quercetin

Total flavonoid content was found higher in ethanolic extract in comparison to the aqueous extract. Among ethanolic extract total flavonoid content was found highest in *Viscum album* (1365.8±0.009mg QE/g dry extract weight) and was found lowest in *Cynodon dactylon* (123.8±0.003mg QE/g dry extract weight). Whereas, among aqueous extract total flavonoid content was found highest in *Viscum album* (1378.8±0.01 mg QE/g dry extract weight) and lowest in *Abelmoschus esculantus* (29.5±0.01 mg QE/g dry extract weight).

Table 9: Total Flavonoid Content Expressed as Quercetin Equivalent Per Gram Dry Extract Weight

Plant Samples	Total Flavonoid content (mg QE/g dry extractweight)	
	Ethanol	Water
Solena heterophylla	474.5±0.008	264.3±0.00
Abelmoschus esculantus	176.0±0.009	29.5±0.01
Viscum album	1365.8±0.009	1378.8±0.01
Curcuma longfolia	309.3±0.005	312.1±0.01
Fraxinus floribunda	1252.8±0.015	1239.6±0.02
Cissampelos pareira	405.0±0.006	442.5±0.00
Achyranthes aspera	418.0±0.003	212.1±0.01
Periploca calophylla	496.3±0.005	423.7±0.01
Nycthanthes arbor-tristis	610.8±0.006	94.7±0.01
Cynodon dactylon	123.8±0.003	77.3±0.00
Oxalis corniculate	194.8±0.016	330.9±0.01
Drynaria propinaua	236.9±0.003	678.8±0.01

Note: Data are expressed as mean ± standard deviation (n=3)

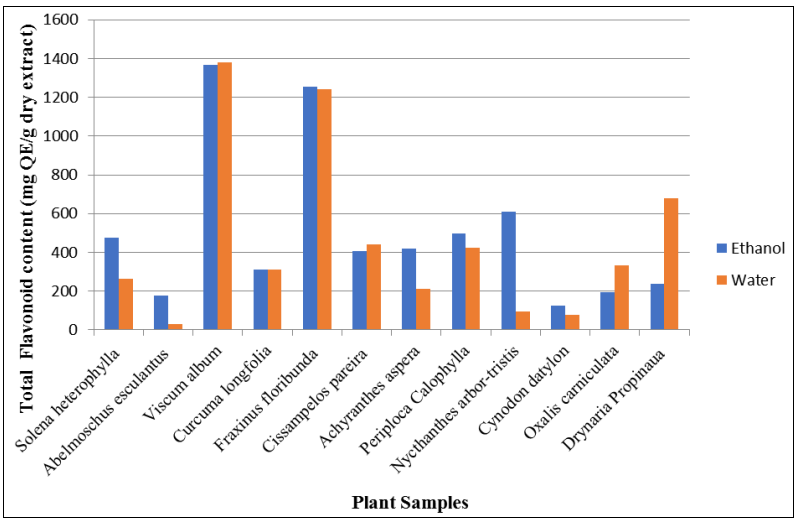

Fig 5: Total Flavonoid Content of Ethanolic and Aqueous Extract of Different Plant Samples

Anti-inflammatory Activity

Carrageenan Induced Paw edema

The *in vivo* anti-inflammatory activity of aqueous extract of *V.album* was compared with standard drug Diclofenac and control in hourly basis up to four hours after the induction of inflammation. The result of anti-inflammatory activity was expressed in mean ± standard deviation of Inflammation index as shown in Table 10. The percentage edema inhibitory activity of rat paw edema by standard and extract was observed as shown in Table 11. Similarly, Figure 6 illustrates dose dependent inhibition of rat paw edema was observed in plant samples and standard. *V. album* showed greater percentage inhibition of rat paw edema and was found almost significant with reference to standard drug Diclofenac. At 4-hour, *V. album* (200 mg/kg) showed 58.89% inhibition of rat paw edema. Standard drug Diclofenac (50 mg/kg), *V. album* (100 and 200 mg/kg) showed significant decrease in rat paw edema from 2 hr onwards.

Table 10: Inflammation Index of Rat Paw Edema with Reference to Diclofenac Standard

Groups (n=5)	Inflammation Index			
	1 hr	2 hr	3 hr	4 hr
Control group	0.26±0.07	0.315±0.057	0.322±0.075	0.302±0.134
V. album (50 mg/kg)	0.187±0.032*	0.217±0.052*	0.21±0.053*	0.157±0.062*
V. album (100 mg/kg)	0.172±0.026*	0.202±0.025*	0.18±0.063*	0.143±0.055*
V. album (200 mg/kg)	0.162±0.024*	0.193±0.029*	0.165±0.023*	0.123±0.023*
Diclofenac (50 mg/kg)	0.138±0.04*	0.165±0.042*	0.15±0.046*	0.118±0.06*

Note: Data expressed as mean ± standard deviation (n=6), *p<0.05, when compared to the control group.

Table 11: Percentage Inhibition of Paw Edema Volume with Reference to Diclofenac Standard

Groups (n=5)	% Inhibition of Paw Edema Volume			
	1hr	2hr	3hr	4hr
Diclofenac (50 mg/kg)	46.79	48.44	53.13	60.56
V. album (50 mg/kg)	28.21	32.29	34.38	47.78
V. album (100 mg/kg)	33.97	36.98	43.75	52.22
V. album (200 mg/kg)	37.82	39.58	48.96	58.89

Note: Data expressed as mean ± standard deviation (n=6).

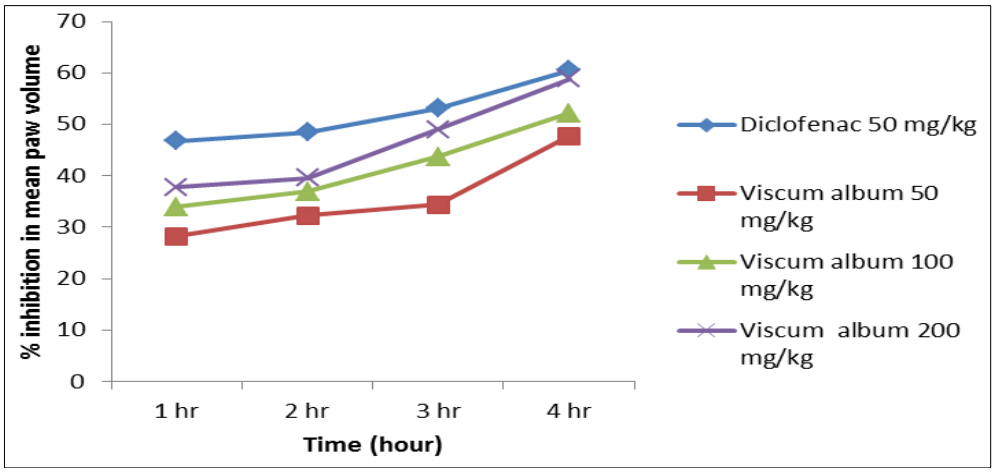

Fig 6: Percentage Inhibition of Rat Paw Edema with Reference to Diclofenac Standard

Analgesic Activity

Hot Plate Method

The results of analgesic activity of standard drug and plant extracts are shown in Table 12. The analgesic activity of aqueous extract of *V. album* at different doses and standard drug diclofenac was found to exhibit significant activity when compared to the control. There was dose dependent increase in reaction time of *V. album* extracts and Standard drug Diclofenac. The maximum possible analgesic 58.91% for a dose of 200 mg/ kg b.wt. followed by 45.62% for a dose of 100 mg/ kg b.wt. and 41.41% for a dose of 50 mg/ kg b.wt. as compared with diclofenac 50 mg/ kg b.wt. that showed 59.33% at 180 min of drug administration (Figure 7)

Table 12: Reaction Time in Various Experimental Groups via Hot Plate Method

Groups	Reaction Time in Seconds			
	0 min	60 min	120 min	180 min
Control (Distilled Water)	8.02±3.14	10.11±3.04	10.50±3.34	9.31±3.21
V.album (50 mg/kg)	8.83±3.75	16.61±3.67*	19.83±4.02*	17.88±4.16*
V. album (100 mg/kg)	9.87±3.35	17.99±2.78*	20.57±3.09*	18.75±2.88*
V. album (200 mg/kg)	9.08±2.73	19.37±3.24*	22.92±1.50	21.50±2.2*
Diclofenac (50 mg/kg)	7.25±1.5	21.16±5.3*	23.26±5.18*	21.58±3.79*

Note: Data are expressed as mean ± standard deviation (n=5),*p<0.05, when compared to the control group

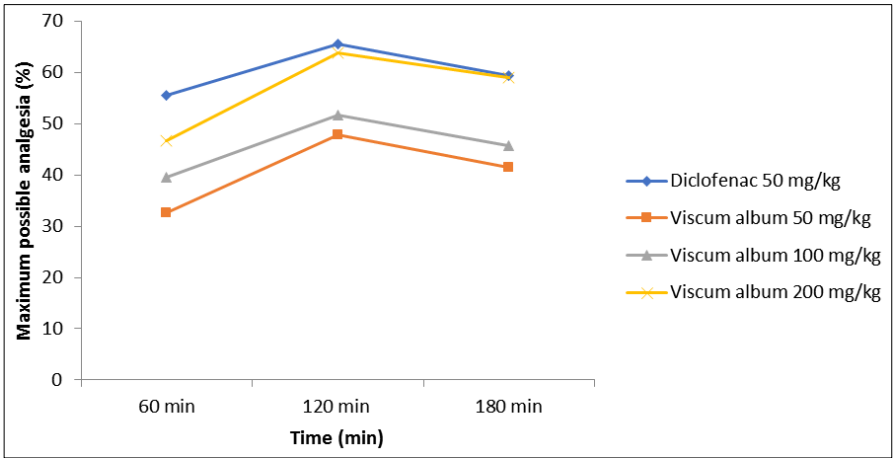

Fig 7: Graphical Representation of Percentage Analgesic Activity of Different Plant Samples with Reference to Diclofenac Standard

Discussion

The present study was designed to screen phytochemicals and investigate total phenolic content (TPC), total flavonoid content (TFC), antioxidant and to evaluate anti-inflammatory and analgesic properties of plant having the best antioxidant activity and phytochemical analysis.

In our study preliminary phytochemical screening of ethanolic and aqueous extract of the selected twelve different plants revealed the presence of various bioactive components like alkaloids, carbohydrates, flavonoids, terpenoids, glycosides, phenols, saponin, and tannin in different parts of the selected plants. Variation in phytochemical constituents was observed in different plant samples. These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities [41].

Among the different plant samples tannins was found in both ethanolic and aqueous extracts of *A. esculantus* and *P. calophylla* whereas found only in ethanolic extracts of *S. heterophylla*, *V. album*, *C. pareira*, *O. corniculata* and *D. propinaua*. Aqueous extract of *C. longfolia* and *C. dactylon* consists of tannin. Tannins have been reported for its wound healing properties, these are Anti-inflammatory and analgesic and antioxidant [42]. Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes [43]. Saponins and tannins are double polar as compared to other secondary metabolites. It was confirmed from the study that ethanolic solvent is more appropriate than other solvents in extracting secondary metabolites of plants [44-6].

The antioxidant activity can be measured by several methods. Among them DPPH free radical scavenging activity is used to measure the antioxidant activity. In the present study, DPPH radical scavenging activity of *F. floribunda* was found highest in both ethanolic and aqueous extract followed by *V. album*, *P. calophylla* and *C. pareira*. Concentration dependent DPPH radical scavenging activity was observed in different plant samples. Total phenolic and flavonoid content of both ethanolic and aqueous extract was also found higher in *V. album* and *F. floribunda*. Free radical scavenging activity of these plant samples may be due to the presence of such phytoconstituents. Phenolics and flavonoids are two naturally occurring phenolic compounds, which are particularly beneficial, acting as antioxidants and their polyphenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals [47].

Phenolic compounds were determined by Folin Ciocalteu (FC) method. It is rapid and easy to perform with low cost [32]. Polyphenols in plant extract react with FC reagent to form a blue phosphotungstic-phosphomolybdenum complex that can be quantified by UV visible light spectroscopy at 725 [42]. In our study, among the different plant samples, phenolic content was found highest in both ethanolic and aqueous extract of *V. album*. Phenolic content was also found varied in between aqueous and ethanolic extract of samples. In comparison to ethanolic and aqueous extract of different samples, phenol content of ethanolic extract of *F. floribunda*, *C. pareira*, *D. propinaua*, *P. calophylla*, *A. aspera*, *O. corniculata* were found higher than aqueous extract. In the present study it was also found that total phenolic content of plant samples such as *A. esculantus*, *C. longfolia*, *N. arbortritis*, *C. dactylon* and *O. corniculata* were found higher in aqueous extract than ethanolic extract. These types of variation may be due to the nature of the solvents used and the solubility of the phenols in the solvent.

The presence of flavonoids can explain the anti-inflammatory activity of this plant. In the present study, total flavonoid content was found higher in *V. album* followed *F. floribunda* in both ethanolic and aqueous extract. Total flavonoid content varied in different solvent and was higher in ethanolic extract in comparison to the aqueous extract.

Inflammation models induced by carrageenan are frequently used acute inflammation models mainly because they are well- researched and they exhibit a high degree of reproducibility. Inflammation Induced by carrageenan promotes a biphasic acute inflammatory response [48]. Initially, the inflammatory reaction to carrageenan (0–1 h) is caused by the release of histamine, serotonin, bradykinin, complement and reactive oxygen species. In the Second, accelerating the phase of swelling (2–4 h), an increased production of prostaglandins in the inflammatory area has been demonstrated. Phytochemicals like flavonoids (quercetin, rutin), phenolics (ellagic acid, gallic acid), citric acid, vanillic acid, tannins etc. may be the reason behind high TPC, TFC and better antioxidant activity [49]. This result also corresponds to the previously reported data on fruit demonstrating good antioxidants with high TPC and TFC [50]. In our study, based on its *In vitro* experiment among different plant samples *F. floribunda* and *V. album* plants were found the best antioxidant activity along with its phytochemical, *V. album* was selected for *in vivo* experiment because of its phytochemical constituents such as tannins and total phenolic and flavonoids content was found to be excellent.

In the present study, *V. album* showed dose dependent increase in analgesic activity. It was observed that percentage analgesic activity of *V. album* (200 mg/kg) at 180 min was 58.91% which was comparable to that of standard Diclofenac (59.33%). Flavonoids, Alkaloids and Tannins are reported to inhibit PGs synthesis [51]. Analgesic effects have already been established in flavonoids, tannins and alkaloids therefore the anti-nociceptive effects observed in the extract may be attributed to its phytochemical constituents [52-3].

Conclusion

In the present study, among the twelve different plant samples, *V. album*, *F. floribunda*, *P. calophylla* and *C. pareira* showed potent antioxidant activity and high phenol and flavonoid content. *V. album* showed good Anti-inflammatory and analgesic activity in compared to Diclofenac in animal model. An Anti-inflammatory and analgesic activity of the plant *V. album* might be due to the presences of active chemical constituents such as phenol, flavonoid, tannin and terpenoids. Hence, further investigation should be carried out to determine the exact mechanism behind this anti-inflammatory and analgesic activity and to isolate the active constituents responsible for the pharmacological activities.

References

1. Kunwar RM, Burlakoti C, Chowdhary CL, Bussmann RW. Medicinal Plants in Farwest Nepal: Indigenous Uses and Pharmacological Validity. Medicinal and Aromatic Plant Science Biotechnology. 2010; 4:28-42.
2. Newman DJ, Cragg GM, Snader KM. Natural Products as Sources of New Drugs over the Period 1981-2002. Journal of Natural Product. 2003; 66:1022-37.
3. Pan SY, Zhou SF, Gao SH, Yu Zl, Zhang SF, Tang MK *et al*. New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics. Evidence-Based Complementary and Alternative Medicine, 2013, 1-25.
4. Devkota SR. Ethno-medicinally used Anti-microbial and pain-relieving plants in the Kaski district, western Nepal. World Journal of Pharmaceutical Sciences. 2014; 10:1240-1248.
5. Rastogi RP, Mehrotra BN. A compendium of Indian medicinal plants CDRI, Lucknow and Publications & Information Directorate, New Delhi, 1993.
6. Hossen MA, Jahan I, Mamun MAA, Sakir JAMS, Shamimuzzaman M, Uddin MJ *et al*. CNS depressant and analgesic activities of*Abelmoschus esculentus* Linn. Molecular and Clinical Pharmacology. 2013; 4(1):44-52.
7. Gad N, Moez A, Kandil H. Response of Okra (Hibiscus esculentus) growth and productivity to cobalt and humic acid rates. Int J Chem Tech Res. 2015; 8(4):1782-91.
8. Verma VD, Saifullah M, Rabbani MG. () Evaluation and characterization of okra (*Abelmoschus esculentus* L. Moench.) genotypes. SAARC Journal of Agriculture. 1993; 7(1):92-99.
9. Moyin-Jesu EI. Use of plant residues for improving soil fertility, pod nutrients, root growth and pod weight of*Abelmoschus esculentum* L. Bioresource technology. 2007; 98(11):2057-2064.
10. Arapitsas P. Identification and quantification of polyphenolic compounds from okra seeds and skins. Food Chemistry. 2008; 110(4):1041-1045.
11. Huang Z, Wang B, Eaves DH, Shikany JM, Pace RD. Phenolic compound profile of selected vegetables frequently consumed by African Americans in the southeast United States. Food Chemistry. 2007; 103(4):1395-1402.
12. Adelakun OE, Oyelade OJ, Ade-Omowaye BIO, Adeyemi IA, Van de Venter M. Chemical composition and the antioxidative properties of Nigerian Okra Seed (*Abelmoschus esculentus* Moench) Flour. Food and Chemical Toxicology. 2009; 47(6):1123-1126.
13. Oyelade OJ, Ade-Omowaye BIO, Adeomi VF. Influence of variety on protein, fat contents and some physical characteristics of okra seeds. Journal of Food Engineering. 2003; 57(2):111-114.
14. Deters AM, Houghton PJ, Hylands PJ, Mensah AY, Hensel A. *In vitro* tests and ethnopharmacological investigations: wound healing as an example. Journal of ethnopharmacology. 2005; 100(1-2):100-107.
15. Adelakun OE, Oyelade OJ, Ade-Omowaye BIO, Adeyemi IA, Van de Venter M. Chemical composition and the antioxidative properties of Nigerian Okra Seed (*Abelmoschus esculentus* Moench) Flour. Food and Chemical Toxicology. 2009; 47(6):1123-1126.
16. Effiong GS, Ogbanm PS, Ibia IO, Adama AA. Evaluation of nutrients supplying potentials of Fluted Pumpkin (*Telfariaoccidentalis*, Hook F) and Okra (*Abelmoschus esculentus*, L Moench). Academic Journal of Plant Sciences. 2009; 2(3):209-214.
17. Dwivedi A, Gautamand G, Argal A. Evaluation of Anti-Inflammatory Activity of Leaves and Seed Extracts Of *Abelmoschus Moschatus* Medik. Life sciences leaflets, 2017, 48-54.
18. Janeyulu NR. Antiulcer activity of *Viscum articulatum* burm f. (viscaceae). International Journal of Current Pharmaceutical Research. 2011; 2:139-143.
19. Dikshit S. Angio spermicphyto parasites of the Darjeeling Himalayas and their ethnomedicinal importance. Environmental Science & Ecology. 2014; 32, 491–494.
20. Tamang P, Singh NB. Medical ethnobiology and indigenous knowledge system of the Lapcha of Fikkal VDC of Ilam Nepal. Journal of Institute of Science and Technology. 2015; 19:45-52.
21. Basnett H. Ethnomedicinal plants used for the treatment of sprain and fractures by the Nepalese community of east Sikkim, 2015.
22. Manandhar NP. Plants and People of Nepal, Timber Press, Portland, 2002, 165-324.
23. Bijoy G. The Medicinal Plants of the Sikkim Himalaya, Chakung: Maples, 2002.
24. Lingadurai S, Nath LK, Kar PK, Besra SE, Joseph RV. Anti-inflammatory and anti-nociceptive activities of methanolic extract of the leaves of *Fraxinus floribunda* Wallich. African Journal of Traditional, Complementary and Alternative Medicines. 2007; 4(4):411-416.
25. Nawaz AMM, Hossain M, Karim M, Khan M, Jahan R, Rahmatullah M. An ethnobotanical survey of Jessore district in Khulna division, Bangladesh. American-Eurasian Journal of Sustainable Agriculture, 2009, 195-202.
26. Luo JR, Qian JF. Advances of Research on Periploca Plants, Faculty of Pharmacy, Dali University, Dali, Yunnan 671000, China, 2008.
27. Agrawal J, Pal A. *Nyctanthes arbor-tristis* Linn-A critical ethnopharmacological review. Journal of ethnopharmacology. 2013; 146(3):645-658.

28. Biswas KR, Rahmatullah M. Traditional medicinal practices of a Sardar healer of the Sardar (Dhangor) community of Bangladesh. The Journal of Alternative and Complementary Medicine. 2012; 18(1):10-19.
29. Puri A, Saxena R, Saxena RP, Saxena KC, Sriva Stava V, Tandon JS. Immunostimulant activity of *Nyctanthes arbor-tristis* L. Journal of Ethnopharmacology. 1994; 42(1):31-37.
30. Bhatnagar S, Sahoo S, Mohapatra AK, Behera DR. Phytochemical Analysis, Antioxidant and Cytotoxic Activity of Medicinal Plant *Combretum roxburghii* (Family: Combretaceae). International Journal of Drug Delivery and Research. 2012; 4:193-202.
31. Kim DO, Chun OK, Kim YJ, Moon HY, Lee CY. Quantification of polyphenolics and their antioxidant capacity in fresh plums. Journal Agriculture Food Chemistry. 2003; 51:6509-6515.
32. Blainski A, Lopes GC, De Mello JCP. Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. Molecules. 2013; 18(6):6852-6865.
33. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method Enzymol. 1999; 299:152-178.
34. Sankhalkar S, Vernekar V. Quantitative and Qualitative analysis of Phenolic and Flavonoid content in *Moringa oleifera* Lam and *Ocimum tenuiflorum* L. Pharmacognosy research 2016; 8(1):16.
35. Malešev D, Kuntić V. Investigation of Metal-Flavonoid Chelates and the Determination of Flavonoids Via Metal-Flavonoid Complexing Reactions. Journal of the Serbian Chemical Society. 2007; 72:921-939.
36. Eghdami A, Sadeghi F. Determination of Total Phenolic and Flavonoids Contents in Methanolic and Aqueous Extract of a *Chillea millefolium*. Organic Chemistry Journal. 2010; 2:81-84.
37. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis. 2002; 10(3).
38. Rao Y, Fang S, Tzeng Y. Anti-inflammatory activities of flavonoids isolated from *Caesalpiniapulcherrima*. *Journal of Ethnopharmacology*. 2005; 100(3):249-53.
39. Wani TA, Kumar D, Prasad R, Verma PK, Sardar KK, Tandan SK *et al*. Analgesic activity of the ethanolic extract of *Shorea robusta* resin in experimental animals. Indian journal of pharmacology. 2012; 44(4):493.
40. Jannot Y, Felix V, Degiovanni A. A centered hot plate method for measurement of thermal properties of thin insulating materials. Measurement Science and technology. 2010; 21(3):035106.
41. Negi R, Negi YK, Uniyal V, Saxena S, Bisht S. Phytochemical Analysis and Antibacterial Activity of Three Indigenous Plants of Garhwal Himalaya AgainstSome Pathogenic Microorganisms. Journal of Pharmacy Research Kharar. 2012; 5:1583-1586.
42. Schofield P, Mbugua D, Pell A. Analysis of Condensed Tannins: A Review on Animal Feed Science and Technology. 2001; 91:21-40.
43. Doss A, Mubarack HM, Dhanabalan R. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. Indian Journal of Science and Technology. 2009; 2(2):41-43.
44. Ahmad A, Kaleem M, Ahmed Z, Shafiq H. Therapeutic Potential of Flavonoids and Their Mechanism of Action against Microbial and Viral Infections: A Review. Food Research International. 2015; 77:221-235.
45. Cowan MM. Plant products as antimicrobial agents. Clinical microbiology reviews 1999; 12(4):564-582.
46. Emad AM, Gamal EGE. Screening for antimicrobial activity of some plants from Saudi folk medicine. *Global* Journal of Research on Medicinal Plants & Indigenous Medicine. 2013; 2(4):189.
47. Harborne JB. General procedures and measurement of total phenolics. Methods in plant biochemistry. 1989; 1:1-28.
48. Li L, Nisar N, Lu S, Khin NC, Pogson BJ. Carotenoid metabolism in plants. Molecular plant. 2015; 8(1):68-82.
49. Li L, Nisar N, Lu S, Khin NC, Pogson BJ. Carotenoid metabolism in plants. Molecular plant. 2015; 8(1):68-82.
50. Labh SN, Shakya SR, Kayasta BL. Extract of Medicinal lapsi *Choerospondias axillaris* (Roxb.) exhibit antioxidant activities during *in vitro* studies. Journal of Pharmacognosy and Phytochemistry. 2015; 4(3):194.
51. Kaur G, Kaur H, Tiwari P, Kumar B, Kaur M. Phytochemical screening and extraction: a review. *Internationale pharmaceutica sciencia*. 2011; 1(1):98-106.
52. Musa MA, Cooperwood JS, Khan MOF. A review of coumarin derivatives in pharmacotherapy of breast cancer. Current medicinal chemistry. 2008; 15(26):2664-2679.
53. Zulfiker AHM, Rahman MM, Hossain MK, Hamid K, Mazumder MEH, Rana MS. *In vivo* analgesic activity of ethanolic extracts of two medicinal plants-*Scoparia dulcis* L. and *Ficus racemosa* Linn. Biology and Medicine. 2010; 2(2):42-8.