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ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITIES OF METHANOLIC EXTRACT OF MEDICINAL PLANTS FROM BALOCHISTAN

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ABSTRACT

Methanolic extracts of selected medicinal plants were tested to evaluate *in vivo* anti-inflammatory and *in vitro* antioxidant potential. For anti-inflammatory activity, carrageenan induced paw edema method was applied using albino rats. The observations were carried out at different doses including 250 ml/kg and 500 ml/kg. The potent anti-inflammatory activity was depicted by *Viscum album* and *Withania coagulans* at 500 mg/kg dose. Although the extracts showed significant effect at both doses, stronger anti-inflammatory effect was obtained at 500 mg/kg dose. The plant extracts had better anti-inflammatory activity as compared to Diclofenac (50 mg/kg). Using DPPH analysis, the plant extract of *Sophora flavescens* and *Morus nigra* showed potential antioxidant effect. Although a potent result was obtained from each methanolic extract but it was significantly different from Vitamin C. Thus, it can reasonably be concluded that these plants showed significant responses against inflammation and oxidation processes and can be further investigated for the isolation of biological constituents.

Key words: anti-inflammatory, antioxidant, Balochistan, plant extracts, scavenging capacity.

INTRODUCTION

The anti-inflammatory drugs after have been found to have adverse effect which may include gastrointestinal problems (Singh, 1998) and kidney failure (Griffin *et al.*, 2000). Such side effects motivate scientists to undertake studies to find a new source to treat inflammation which has minimal adverse effects. Nature provide such source in the form of herbal plants because isolated compounds from natural sources are bioactive (Sandoval *et al.*, 2002) and thus possess many biological properties such as anti-inflammatory and anti-oxidant properties.

Inflammation is a defensive response of body cell against stress which body feels at time of any damage which can be due to any factor (biological, physical or chemical). Inflammation may have different forms, depending on the intensity of injury, such as loss of functionality of affected area, redness at spot of injury, heat, pain or swelling at affected area. It is a nonspecific body's internal defense response (Leelaprakash and Dass, 2011) which should be properly treated (Ewers, 1990).

The antioxidant potential of medicinal plants have significant importance because both inflammatory and oxidant activity are co-related. Oxidation process inside the body is responsible for production of free radicals which are responsible for damaging cells which may lead to inflammation of the body part. Thus it is necessary to remove these free radicals from organism's body. The target can be achieved by group of compound such as phenolic compounds and enzyme isolate from medicinal plant as these chemical constituents may have potential activity as an anti-oxidant agent. Such chemical agents not only cause the reduction in free radicals but also cause lowering of energy level of existing free radicals which results in lower damage of cell by free radicals (Cutler, 1984).

Pakistan's history is rich with folklore uses of plants as medicine. About 12% of 6000 higher plants from flora of Pakistan are utilized as medicine (Nasir *et al.*, 2014). Plants found in Pakistan, especially in Balochistan, such as *Viscum album, Morus alba, Morus nigra, Withania coagulans, Datura alba* and *Sophora flavescens* have been reported with medicinal potential.

Viscum album (European mistletoe) belongs to *Loranthaceae* family and Viscum genus (Choudhary *et al.*, 2010). Many therapeutic applications have been reported with this medicinal plant including diabetes mellitus, chronic cramps, stroke, stomach, heart problems (Ohiri *et al.*, 2003), asthma and lowering of blood pressure (Orhan *et al.*, 2005).

Datura alba, also medicinal plant of Balochistan is in *Solanaceae* family and is medicinally important for anticancer activity and histopathological effect (Khan *et al.*, 2011). It has been reported that the plant has a healing

potential against burn wound (Priya *et al.*, 2002). Antiarrhythmic effect (Yong-gang *et al.*, 2010), antitumor (Ryu *et al.*, 1997) and antibacterial activity (Cha *et al.*, 2007) has been reported for *Sophora flavescens* the belongs to the family Fabaceae.

Morus album and *Morus nigra* belongs to family Moraceae with known pharmacological activity as diuretic, hypoglycemic, hypotensive (Nomura, 1999) anti-inflammatory and antipyretic activities (Asano *et al.*, 2001).

Withania coagulans is important for biological activities and belongs to *Solanaceae* family. The isolated compound from *Withania coagulans* has been reported to have a potential activity against cancer (Youn *et al.*, 2013), wound healing, hepato-protective, anti-inflammatory, anti-hyperglycaemic, hypolipidaemic, free radical scavenging, antimicrobial, cardiovascular, central nervous system depressant, immune-modulators, anti-tumour, cytotoxic (Maurya, 2010) and anti-oxidant activity (Mathur *et al.*, 2011).

The present research was conducted with a purpose to assess anti-inflammatory and anti-oxidant activities of Methanolic extracts of *Viscum album, Withania coagulans, Morus alba, Morus nigra, Dutra alba and Sophora flavescens* from flora of Balochistan, Pakistan, considering their capacity to repair damage cell and potential activity against inflammation.

MATERIALS AND METHODS

Chemicals

Diclofenac sodium (Courtesy of Sami pharmaceuticals Pvt. Ltd.), Carrageenan (0.1 mL w/v in normal saline), Methanol (Sigma Aldrich) and DPPH (Sigma Aldrich).

Sample collection and identification

The authentic plant species were collected from different areas of Balochistan, Pakistan, with special reference to Neelam valley (Azad Kashmir) and Main-Ghundi. The plant specimens were deposited in Department of Botany, University of Balochistan and identified by Plant taxonomist Dr. Rasool Baksh Tareen (Department of Botany, UOB). The plant samples were thoroughly washed and separated into stems, roots and leave which were dried in shade for 10 days to avoid enzymatic degradation. The dried material was powdered and stored in amber colored bottles till further analysis.

Extract preparations

The plant material (500 g) was soaked in methanol (80%) for three days, followed by filtration via whatmann filter paper. The extract was subjected to evaporation on rotary evaporator (RV10BS99 IKS., Germany) at 40°C, weighed and stored until further analysis (Lin *et al.*, 1999).

Test Animals

Seventy Wister rats (150 to 200 g) were purchased from Post Graduate Medical Institute (PGMI), Lahore, Pakistan fourteen days earlier to experimentation. Each animal was collected from the same breeding colony and batch. The rats were stored in controlled temperature (25±1°C) condition with balanced diet and free access to water according to light/dark cycle of 12:12 hours (Ahmad *et al.*, 1992).

Animal Groups

The rats were equally and randomly divided into fourteen groups, each group containing five rats. The groups were named as follow:

$N_{control}$: (Negative control) 1 mL of normal saline was given orally.
$P_{control}$: (Positive control) 50 mg/kg of Diclofenac sodium (used as standard drug)
DA_1: *Datura alba* extract (250 mg/kg)
DA_2: *Datura alba* extract (500 mg/kg)
MA_1: *Morus alba* extract (250 mg/kg)
MA_2: *Morus alba* extract (500 mg/kg)
MN_1: *Morus nigra* extract (250 mg/kg)
MN_2: *Morus nigra* extract (500 mg/kg)
SF_1: *Sophora flavescens* extract (250 mg/kg)
SF_2: *Sophora flavescens* extract (500 mg/kg)
VA_1: *Viscum album* extract (250 mg/kg)
VA_2: *Viscum album* extract (500 mg/kg)
WC_1: *Withania coagulans* extract (250 mg/kg)

WC_2: *Withania coagulans* extract (500 mg/kg)

Groups I and Group II were labeled as negative and positive controls respectively while remaining 12 groups were tagged as experimental.

Inflammation Measurement

A foot edema test was carried out in rats using Carrageenan solution (1% w/v). The edema was induced by injecting Carrageenan solution into the right hind foot of each rat under plantar aponeurosis. Each rat from the negative group (normal saline), positive group (Diclofenac sodium 50 mg/kg in 1 mL normal saline) and experimental group was treated orally (250 mg/kg or 500 mg/kg in 1 mL normal saline) 1 hour before induction of inflammation. Digital Plethysmometer (LE7500, Pan Lab, Harvard apparatus, Spain) was used to measure the foot edema (mm) before and after (0 h, 1 h, 2 h, 3 h and 4 h) the injection of Carrageenan solution. Inhibitory activity was calculated by the following formula:

$$Percentage\ Inhibition = 100\left(1 - \frac{a - x}{b - y}\right)$$

Where, a; mean paw size of treated rats after Carrageenan injection, x; mean paw volume of treated rats before Carrageenan injection, b; mean paw volume of control rats after Carrageenan injection, y; mean paw size of control rats before Carrageenan injection (Al-Ghamdi, 2001).

Antioxidant activity

Antioxidant properties (free radical-scavenging activity) of plant extracts were estimated by DPPH photometric assay analysis. Each sample stock solution (1 mg/ml) was diluted to concentrations of 500, 250, 125, 50, 25 and 10 µg/mL in methanol. A total of 1 mL of 0.1 mM DPPH methanol solution was added to 3 mL of sample solutions of different concentration and allowed to react at room temperature for 30 minutes. Similar method was adapted for the preparation of Vitamin C solution as a standard (positive control). The negative control was made by mixing DPPH solution plus methanol, while methanol plus plant extract solution (3 mL) was used as a blank (Mensor *et al.*, 2001). The absorbance values were recorded at 588 nm on UV-vis Spectrophotometer and converted to antioxidant activity using the following formula

$$Scavenging\ capacity\ (\%) = 100 - \left(\frac{Absorbance\ of\ sample - Absorbance\ of\ blank}{Absorbance\ of\ control}\right) \times 100$$

The average percent of scavenging capacity was estimated from three replicates (Choi *et al.*, 2002).

Statistical analysis

The statistical significance ($p<0.01$) of anti-inflammatory and antioxidant effect was evaluated with ANOVA using MiniTab software. P values less than 0.01 were considered to be statistically significant.

RESULTS

Anti-inflammatory activity of plant extracts

The result of paw size (mean ±S.D.) and Inhibition of paw edema (%) is depicted in Table 1 and graphically represented in Fig. **1** and Fig. **2**. Carrageenan-induced rat paw edema was markedly inhibited by VA_2 and WC_2 at 500 mg/kg as compared to $P_{control}$ (Diclofenac sodium 50 mg/kg). The least inhibition (%) was shown by MA_1 at 250 mg/kg dose and did not induce a significant effect ($p>0.10$), yet it was greater than $P_{control}$. There was a strong activity with 500 mg/kg of the extract where significant reduction was shown at 2nd hour of induction ($p<0.01$). Although the extracts at 250 mg/kg inhibited the inflammation more than $P_{control}$ but the result was not significant ($p>0.10$).

Antioxidant activity of plant extracts

The result for scavenging activity is given in Table 2 and shown in Fig. **3**. The maximum scavenging activity (%) was exhibited by SF extract, followed by MN extract with closer proximity. The least activity was shown by MA extract. Although the plant extracts showed potent antioxidant property but the DPPH radical reducing activity was comparably weaker than the control (Vitamin C). Within the groups, the scavenging activity (%) was not significantly different ($p>0.10$) but Vitamin C activity was significantly different ($p<0.01$) then plant extracts.

Table 1. Anti-inflammatory activity of Positive control, negative control and plant extracts after 2 hours of maximum activity.

Plant name	Treatment	Dose	Paw size (mm) after administration of plant extract ± S.D. (Percentage Inhibition of paw edema)
	$N_{control}$	10 ml	1.70±0.05
	$P_{control}$	50 mg/kg	0.76±0.06 (48.83%)
Datura alba	DA_1	250 mg/kg	0.51±0.025 (61.01%)
	DA_2	500 mg/kg	0.48±0.022 (69.62%)
Morus alba	MA_1	250 mg/kg	0.55±0.030 (50.94%)
	MA_2	500 mg/kg	0.51±0.047 (60.23%)
Morus nigra	MN_1	250 mg/kg	0.56±0.09 (52.80%)
	MN_2	500 mg/kg	0.55±0.074 (59.53%)
Sophora flavescens	SF_1	250 mg/kg	0.42±0.011 (67.79%)
	SF_2	500 mg/kg	0.40±0.019 (71.06%)
Viscum album	VA_1	250 mg/kg	0.47±0.015 (57.21%)
	VA_2	500 mg/kg	0.39±20.023 (73.69%)
Withania coagulans	WC_1	250 mg/kg	0.48±0.015 (59.81%)
	WC_2	500 mg/kg	0.33±0.051 (73.40%)

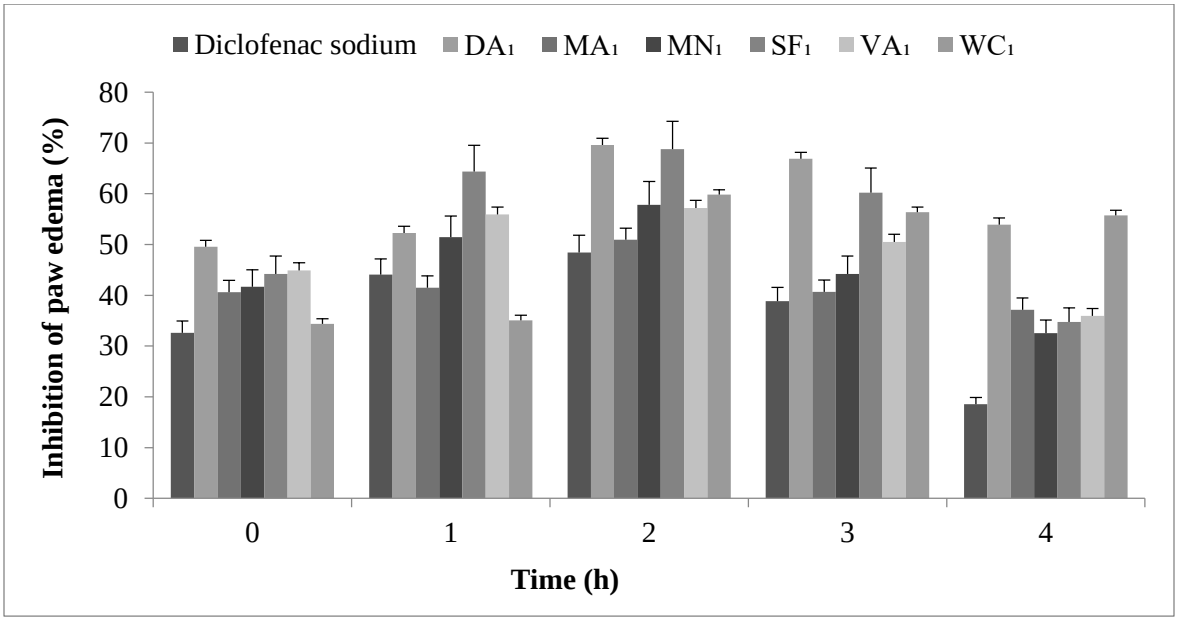

Fig. 1. Anti-inflammatory result based on 250 mg/kg dose of plant extract.

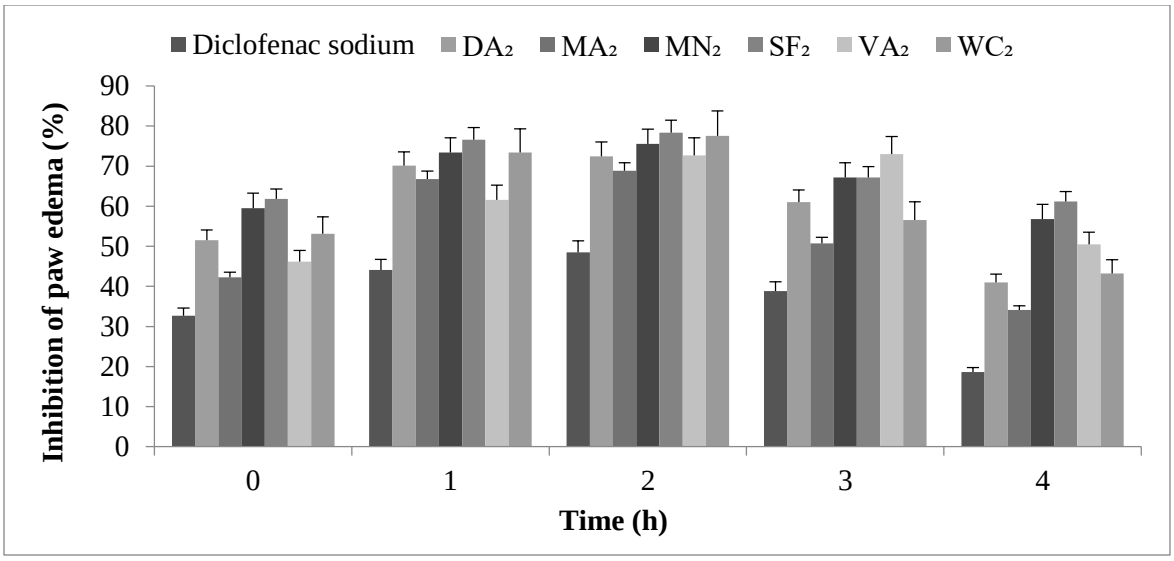

Fig. 2. Anti-inflammatory result based on 500 mg/kg dose of plant extract.

Table 2. Scavenging capacity of DPPH by Vitamin C (standard) and plant extract.

Plant name	Treatment	Scavenging Capacity (%) ± S.D.
	Vitamin C	86.66 ± 0.58
Datura alba extract	DA	70.33 ± 0.04
Morus alba extract	MA	68.67 ± 0.01
Morus nigra extract	MN	73.00 ± 0.00
Sophora flavescens extract	SF	74.33 ± 0.01
Viscum album extract	VA	72.12 ± 0.01
Withania coagulans extract	WC	71.33 ± 0.02

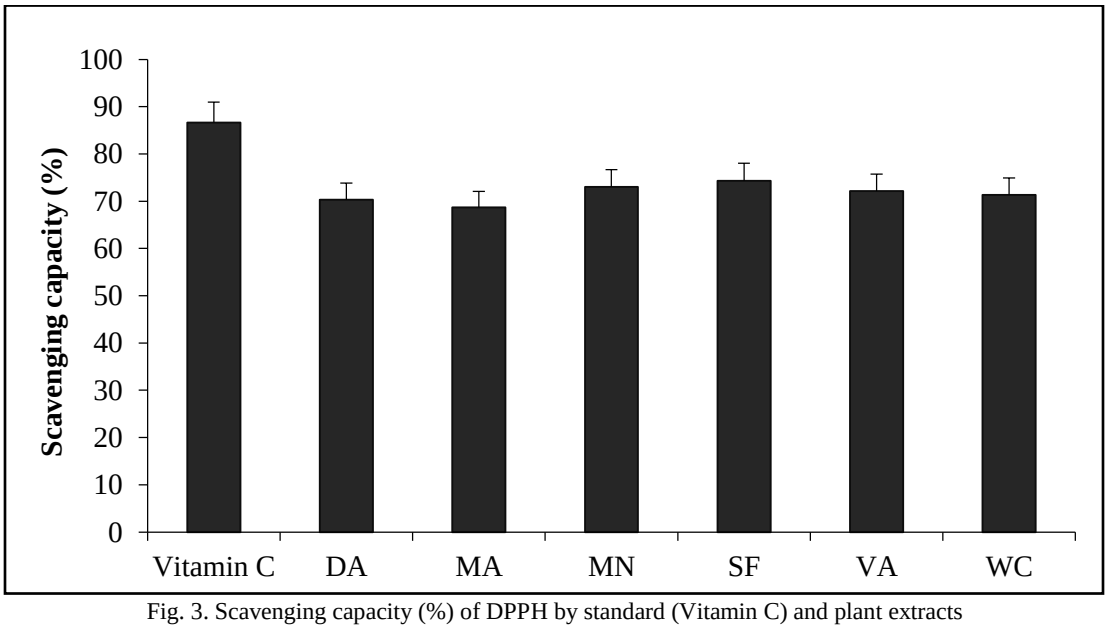

Fig. 3. Scavenging capacity (%) of DPPH by standard (Vitamin C) and plant extracts

DISCUSSION

It has long being recognized that plant extracts and naturally occurring substances have potential anti-inflammatory and antioxidant activity. In our present study, six native plants of Balochistan, Pakistan were selected for their possible activities with reference to the control synthetic components. The preliminary screening for anti-inflammatory and antioxidant activity indicated that the plant extracts were highly active which support their use in traditional medicine. Inflammation is clinically defined as a pathophysiological process which may be characterized by edema, fever, redness and pain. As the critical etiology is not completely understood, it is difficult to cure chronic inflammation as in the case of rheumatoid arthritis and atopic dermatitis. Therefore, there is a dire need of new and safe anti-inflammatory compound which makes research candidates interested in plant constituents and their extracts. Flavonoids are well known compounds to possess various biological and pharmacological activities, including anti-inflammatory potency. The anti-inflammatory compounds in the plant extract regulate the cellular activity of the inflammation-related cells: neutrophils, lymphocytes, mast cells and macrophages. They may also modulate the enzymatic activities of phospholipase $A_{2,}$ lipoxygenase, nitric oxide producing enzymes and cyclooxygenase. This leads to a reduction in production of arachidonic acid, nitric oxide, prostaglandins and leukotrienes that are the crucial mediators of inflammation (Mehmood *et al.,* 2016; Kim *et al.,* 2004). Significant anti-inflammatory activities were observed for the selected medicinal extract at 500 mg/kg dose.

DPPS is a stable free radical which gives violet color in ethanol. In the presence of an antioxidant this radical is reduced to a colorless or light yellow color. This results in the reduction of absorbance which is an indicative of the radical scavenging power of the extract (Okawa *et al.,* 2001). It an easy, rapid and sensitive method for the determination of antioxidant activity of plant extracts and medicinal compounds. Antioxidant compounds, through their scavenging power, are useful in the management of various diseases including cancer and neurogeneratve disease (Pourmorad *et al.,* 2006). The reported antioxidants present in the plant extracts include vitamins, polyphenols, flavonoids and carotenoids. The catechol group and position of hydroxyl group plays an important role in the mediation of potent DPPS reduction activity (Okawa *et al.,* 2001). The selected plant extracts possessed comparable antioxidant effect to Vitamin C and can effectively counteract the oxidative damage induced by various parasites and diseases (Ayoola *et al.,* 2008).

Conclusion

The result of the present study showed that the methanolic extract of *Viscum album* and *Withania coagulans* showed potent anti-inflammatory activity at 500 mg/kg dose whereas *Sophora flavescens* and *Morus nigra* had potential antioxidant property. Positive response of selected medicinal plant extracts in methanol as anti-inflammatory and antioxidant agent confirms the presence of bioactive compounds. Isolation and purification of such natural product can prove milestone in development of effective drug against inflammation and oxidation along with exposure of many hidden biological potential which are still to be discovered. The green synthesis of drugs also reduces chance of different side effects.

REFERENCES

Ahmad, F., R.A. Khan and S. Rasheed (1992). Study of analgesic and anti-inflammatory activity from plant extracts of Lactuca scariola and Artemisia absinthium. *Journal of Islamic Academy of Sciences,* 5(2): 111-114.

Al-Ghamdi, M. (2001). The anti-inflammatory, analgesic and antipyretic activity of Nigella sativa. *Journal of Ethnopharmacology,* 76(1): 45-48.

Asano, N., T. Yamashita., K. Yasuda, K. Ikeda, H. Kizu, Y. Kameda, A. Kato, R.J. Nash, H.S. Lee and K.S. Ryu (2001). Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). *Journal of Agricultural and Food Chemistry,* 49(9): 4208-4213.

Ayoola, G., H. Coker, S. Adesegun, A. Adepoju-Bello, K. Obaweya, E. Ezennia and T. Atangbayila (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research,* 7(3): 1019-1024.

Cha, J.-D., M.-R. Jeong, S.-I. Jeong and K.-Y. Lee (2007). Antibacterial activity of sophoraflavanone G isolated from the roots of Sophora flavescens. *Journal of microbiology and biotechnology,* 17(5): 858-864.

Choi, C.W., S.C. Kim, S.S. Hwang, B.K. Choi, H.J. Ahn, M.Y. Lee, S.H. Park and S.K. Kim (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant science,* 163(6): 1161-1168.

Choudhary, M.I., S. Maher, A. Begum, A. Abbaskhan, S. Ali and A. Khan (2010). Characterization and antiglycation activity of phenolic constituents from Viscum album (European Mistletoe). *Chemical and Pharmaceutical Bulletin,* 58(7): 980-982.

Cutler, R.G. (1984). Antioxidants, aging and longevity. *Free radicals in biology*, 6: 371-428.

Ewers, R. (1990). [Principles of anatomy, physiology, pathophysiology of the temporomandibular joint from the surgical viewpoint]. *Fortschr Kiefer Gesichtschir*, 35: 154-155.

Griffin, M.R., A. Yared and W.A. Ray (2000). Nonsteroidal antiinflammatory drugs and acute renal failure in elderly persons. *American Journal of Epidemiology*, 151(5): 488-496.

Khan, I., A. Qamar, S. Mehdi and M. Shahid (2011). Histopathological effects of Datura alba leaf extract on the midgut of Periplaneta americana. *Biol Med*, 3: 260-264.

Kim, H.P., K.H. Son, H. W. Chang and S.S. Kang (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. *J Pharmacol Sci*, 96(3): 229-245.

Leelaprakash, G. and S.M. Dass (2011). *In vitro* anti-inflammatory activity of methanol extract of Enicostemma axillare. *International Journal of Drug Development and Research*, 3 (3): 189-196.

Lin, J., A. Opoku, M. Geheeb-Keller, A. Hutchings, S. Terblanche, A.K. Jäger and J. Van Staden (1999). Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. *Journal of Ethnopharmacology*, 68(1): 267-274.

Mathur, D., R. Agrawal and V. Shrivastava (2011). Phytochemical screening and determination of antioxidant potential of fruits extracts of *Withania coagulans*. *Recent Research in Science and Technology*, 3(11): 26-29.

Maurya, R. (2010). Chemistry and pharmacology of *Withania coagulans*: an Ayurvedic remedy. *Journal of pharmacy and pharmacology*, 62(2): 153-160.

Mehmood, A., I. Hamid, A. Sharif, M.F. Akhtar, B. Akhtar, A. Saleem, J. Iqbal, M. Shabbir and S. Ali (2016). Evaluation of anti-Inflammatory, analgesic and antipyretic activities of aqueous and ethanolic extracts of seeds of *Buchanania lanzan spreng* in animal models. *Acta Poloniae Pharmaceutica*, 73(6): 1601-1608.

Mensor, L.L., F.S. Menezes, G.G. Leitão, A.S. Reis, T.C.D. Santos, C.S. Coube and S.G. Leitão (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research*, 15(2): 127-130.

Nasir, S., J. Ahmed and M. Asrar (2014). Medicinal Plants: a Promising Resource for Poverty Alleviation in the Milieu of Swat. *FUUAST Journal of Biology*, 4(2): 237.

Nomura, T. (1999). The chemistry and biosynthesis of isoprenylated flavonoids from moraceous plants. *Pure and applied chemistry*, 71(6): 1115-1118.

Ohiri, F., C. Esimone, S. Nwafor, C. Okoli and O. Ndu (2003). Hypoglycemic properties of Viscum album (mistletoe) in alloxan-induced diabetic animals. *Pharmaceutical Biology*, 41(3): 184-187.

Okawa, M., J. Kinjo, T. Nohara and M. Ono (2001). DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. *Biological and Pharmaceutical Bulletin*, 24(10): 1202-1205.

Orhan, D.D., M. Aslan, N. Sendogdu, F. Ergun and E. Yesilada (2005). Evaluation of the hypoglycemic effect and antioxidant activity of three *Viscum album* subspecies (*European mistletoe*) in streptozotocin-diabetic rats. *Journal of Ethnopharmacology*, 98(1): 95-102.

Pourmorad, F., S. Hosseinimehr and N. Shahabimajd (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African journal of biotechnology*, 5(11): 1142-1145.

Priya, K.S., A. Gnanamani, N. Radhakrishnan and M. Babu (2002). Healing potential of Datura alba on burn wounds in albino rats. *Journal of Ethnopharmacology*, 83(3): 193-199.

Ryu, S.Y., S.U. Choi, S.K. Kim, Z. No, C.O. Lee, J.W. Ahn and S.H.Kim (1997). In vitro antitumour activity of flavonoids from Sophora flavescens. *Phytotherapy research*, 11(1): 51-53.

Sandoval, M., N. Okuhama, X.-J. Zhang, L. Condezo, J. Lao, F. Angeles, R. Musah, P. Bobrowski and M. Miller (2002). Anti-inflammatory and antioxidant activities of cat's claw (*Uncaria tomentosa* and *Uncaria guianensis*) are independent of their alkaloid content. *Phytomedicine*, 9(4): 325-337.

Singh, G. (1998). Recent considerations in nonsteroidal anti-inflammatory drug gastropathy. *Am J Med*, 105(1B), 31S-38S.

Yong-gang, C., J. Shan, L. Lei, G. Jing-quan, S. Zhi-ying, L. Yan, X. Yan, W. Ming-li, W. Ye and X. Chang-qing (2010). Antiarrhythmic effects and ionic mechanisms of oxymatrine from Sophora flavescens. *Phytotherapy research*, 24(12): 1844-1849.

Youn, U.J., X. Chai, E.-J. Park, T.P. Kondratyuk, C.J. Simmons, R.P. Borris, B. Mirza, J.M. Pezzuto and L.C. Chang (2013). Biologically active withanolides from Withania coagulans. *Journal of natural products*, 76(1): 22-28.

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