

# Evaluation of anti-inflammatory and anti-oxidant potential of seed extracts of *Vernonia anthelmintica*

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**Abstract:** Seeds of *Vernonia anthelmintica* in the form of Ethanol seed extract of *Vernonia anthelmintica* (EEVA), Hexane extract of *Vernonia anthelmintica* (HEVA) and water decoction of *Vernonia anthelmintica* (WDVA) were evaluated for their *in-vivo* anti-inflammatory potential in carrageenan induced rat paw model. The results were compared to anti-inflammatory activity of standard drug (ibuprofen) and untreated groups. *In-vitro* evaluation of antioxidant potential of EEVA and HEVA were also conducted by “DPPH scavenging assay”. The results of present study depicts that HEVA and EEVA in higher dose possess a strong anti-inflammatory potential as compared to standard anti-inflammatory drugs, whereas WDVA showed milder anti-inflammatory potential. DPPH assay has revealed strong anti-oxidant potential of EEVA with the percentage Radical Scavenging activity (%RSA) of 89.709 at concentrations of 500  $\mu$ l as compared to standard drugs gallic acid (23.436 $\pm$ 0.43) and acetyl salicylic acid (111.44 $\pm$ 0.7) at concentrations of 95.95  $\mu$ M. The other extract HEVA has shown to have insignificant %RSA at the concentration of 500 $\mu$ l. Hence the present study revealed that selected extracts of *Vernonia anthelmintica* exhibited significant *in-vitro* antioxidant and *in-vivo* anti-inflammatory potential.

**Keywords:** Anti-inflammatory activity, Carrageenan induced rat paw edema, DPPH assay, Oxidants, *Vernonia anthelmintica*.

## INTRODUCTION

*Vernonia anthelmintica* is one of the most popular herbal medicine and known to be bitter, sharp in taste also known as kali jiri in subcontinent. The herb has established role as anthelmintic and astringent. Some literature also reported it to have anti-inflammatory potential. Leaf extracts have shown to have antioxidant and cytotoxic potential (Patnaik and Bhatnagar, 2015).

Antioxidants are needed by human body to nullify the effects of reactive oxygen species (ROS) in times of inflammation due to activation of immune cells. Patients with ongoing inflammation present with low levels of antioxidants either due to less intake or high consumption in situations of overwhelming ROS generation by activated immune cells. Thus antioxidants are known to impede the disease related oxidative stress (Mangge, Becker *et al.*, 2014).

It is therefore logical to support the patient with exogenous antioxidants in situations where endogenous antioxidants are overwhelmed. Thus the need of finding new antioxidants from dietary and herbal origin is justified in order to prevent from chronic illnesses like cancer, cardiac heart disease and varying neurodegenerative diseases. In certain concentration free radicals are needed by the body as a part of human host defense mechanism. ROS (reactive oxygen species) and

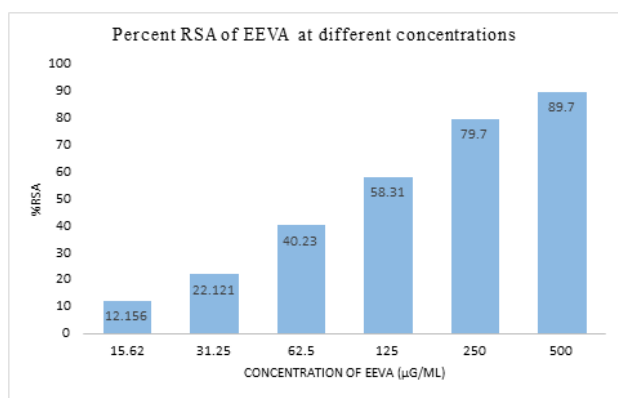
RNS (reactive nitrogen species) for example are two free radicals required by human body for the integrity and maturation of cell and cellular structures. The human defense system for example phagocytes; neutrophils and monocytes etc., all need free radicals in sufficient amount to eradicate the invading pathogen (Dröge, 2002).

It can be summarized that these free radicals at certain concentration are vital to normal human physiology. However when free radicals and oxidants generated in excess they may cause a condition termed as oxidative stress. Oxidative stress refers to the condition of imbalance between generation and neutralization of free radicals and oxidants and may lead to the deleterious effects on cell membranes, proteins, lipoproteins and even DNA. These deleterious effects on cellular structures are the key factors in the pathogenesis of varying diseases (Young and Woodside, 2001).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay is used for the evaluation of antioxidant potential of crude and raw samples. It has advantage over expensive methods as it is quick, simple and cheap *in-vitro* assay employed for the evaluation of compounds having ability to scavenge free radical scavenging or act as a hydrogen donor (Chen, Bertin *et al.*, 2013). Previous studies revealed the antioxidant potential of ethanol extract of *Vernonia anthelmintica* seeds. However further studies were recommended for other fractions and extracts of seeds (Santosh, Attitalla *et al.*, 2013).

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Inflammation is a general phenomenon that becomes active in the cases of infections, injuries or exposure to pathogens and contaminants that can be collectively or individually a sign of homeostatic dysfunction. Inflammation is mediated by elicitation of innate immune receptors against pathogens or injured cells and coordinated by immune signaling molecules and cytokines. Unfortunate is the condition where inflammation persists for longer times without getting its beneficial objective and hence inflammatory cells overwhelm an organ system resulting in damage to the host cells. This damage to host cells appears in the form of various chronic illnesses such as colitis, pancreatitis, ulceration, diabetes, cardiovascular diseases, arthritis and hepatitis (Ashley, Weil *et al.*, 2012).



**Fig. 1:** Percentage radical scavenging activity of ethanol extract of *Vernonia anthelmintica* seed

The Carrageenan test is a sensitive tool to elucidate the anti-inflammatory potential of oral anti-inflammatory agents. It provides an excellent acute phase inflammation model (Di Rosa, Giroud *et al.*, 1971). Carrageenan is a mucopolysaccharide extract derived from alga chondrus crispus discovered by British pharmacist Stanford in 1862. It is made up of complex group of polysaccharides constituted of galactose related repeated monomers and are of three main types: lambda, kappa and iota. All have their own thermally reversible gelling characteristics. Lambda type is unique in the way as it does not gel at room temperature and can be used for induction of inflammatory response (Morris, 2003).

Carrageenan induced paw edema is a method used for measuring edema caused by 0.1ml of 1% solution of carrageenan into the plantar tissues of hind paw of the rat. Within first 3-4 hours, peak edema develops. Drugs to be evaluated for anti-inflammatory potential can be analyzed by checking for inhibition of edema by pre-treatment of the animals by unit oral dose (Winter, Risley *et al.*, 1962). Carrageenan induces acute, non-immune, well studied and easily reproducible inflammation with immediate appearance of basic signs such as edema, redness and hyperalgesia following injection. These inflammatory responses are the result of release of pro-inflammatory

mediators e.g. histamine, bradykinin, tachykinins, complement and reactive nitrogen and oxygen species (Morris, 2003).

Previous studies on petroleum ether and methanol extracts of *Vernonia anthelmintica* revealed their anti-inflammatory potential (Ashok, Koti *et al.*, 2010a). However data for hexane extract and water decoction of *Vernonia anthelmintica* is lacking in previous literature. Thus present study is aimed to evaluate the in-vitro antioxidant activity and in-vivo anti-inflammatory potential of different extracts of *Vernonia anthelmintica* seeds.

## MATERIALS AND METHODS

### Collection and identification of plant material

The dried seeds of *Vernonia anthelmintica* were obtained from commercial market of Karachi, Pakistan. The identification of the seed was carried out by center of plant conservation, Karachi University Herbarium and Botanic Garden, university of Karachi through Herbarium No. G.H 92629.

### Extraction

The seeds were cleaned manually for removal of impurities like straws and dirt. The seeds were ground to coarse powder in a mechanical grinder and soaked in n-hexane for 10 days with occasional shaking. On 11th day the solvent containing hexane soluble constituents was filtered through muslin cloth. The filtrate was loaded in rotary evaporator (model Rotavapor R-3 BUCHI) for evaporation of solvent at 50°C at high pressure to obtain yellowish green oily hexane extract of *Vernonia anthelmintica* referred to as HEVA (hexane extract of *Vernonia anthelmintica*). The residual after filtration was soaked again in ethanol to obtain brownish black gummy ethanol extract of *Vernonia anthelmintica* referred to as EEVA (ethanol extract of *Vernonia anthelmintica*) by the same methodology described for hexane extract. To prepare water decoction of *Vernonia anthelmintica* seeds (WDVA), its coarse powder was boiled in distill water each time before dosing.

### DPPH ASSAY

DPPH solution is prepared by dissolving it in 100% ethanol to get 300 µM solution. HEVA and EEVA were dissolved in DMSO (dimethyl sulfoxide) and 5µl of these samples were added to 96 well plate and absorbance is noted at 515 nm. 95µl of DPPH was added to each well. The plate was then incubated for 30 minutes at 37°C after covering the plate in order to avoid any evaporation of solvent. Plate was subjected to microplate reader (microtitre plate spectrophotometer, Spectra Max, Molecular Devices, CA, USA) at 515 nm to get final absorbance. Control contained only DMSO while Gallic acid and N-acetyl cysteine served as the standard in this assay. (Uddin, Siddiqui *et al.*, 2011)

**Table 1:** Anti-inflammatory activity of *Vernonia anthelmintica* in rat paw

| Group (dose/kg) | Edema volume (ml) (% inhibition of inflammation) |                       |                       |                        |                       |                        |
|-----------------|--------------------------------------------------|-----------------------|-----------------------|------------------------|-----------------------|------------------------|
|                 | 1 h                                              | 2h                    | 3h                    | 4h                     | 5h                    | 24h                    |
| Control         | 0.78±0.08                                        | 1.47±0.20             | 2.20±0.11             | 2.45±0.27              | 2.55±0.20             | 1.05±0.11              |
| Ibuprofen (100) | 0.64±0.11*<br>(18.7)                             | 0.35±0.04**<br>(82.3) | 0.51±0.11**<br>(76.8) | 0.66±0.11**<br>(73.06) | 0.54±0.13**<br>(78.8) | 0.35±0.07**<br>(71.60) |
| HEVA (150)      | 0.76±0.06<br>(3.5)                               | 1.12±0.06<br>(8.55)   | 2.19±.11<br>(0.4)     | 2.40±0.26<br>(2)       | 2.52±0.34<br>(1.1)    | 0.89±0.15<br>(15.2)    |
| HEVA (300)      | 0.73±0.08<br>(14.4)                              | 0.79±0.19*<br>(58.2)  | 0.64±0.11**<br>(70.9) | 1.14±0.29**<br>(53.4)  | 1.13±0.28**<br>(55.6) | 0.20±0.09**<br>(80.9)  |
| EEVA (150)      | 0.72±0.07<br>(8.6)                               | 1.27±0.05<br>(21.3)   | 2.18±0.11<br>(0.9)    | 2.31±0.25<br>(5.71)    | 2.47±0.13<br>(3.1)    | 0.59±0.11**<br>(43.8)  |
| EEVA (300)      | 0.44±0.08<br>(44.1)                              | 0.72±0.18*<br>(58.2)  | 1.10±0.11**<br>(50)   | 0.98±0.15**<br>(60)    | 1.14±0.15**<br>(55.2) | 0.38±0.09**<br>(68.8)  |
| WDVA (150)      | 0.66±0.12<br>(16.2)                              | 1.16±0.14<br>(25.8)   | 1.45±0.53<br>(34.0)   | 1.55±0.2<br>(36.7)     | 1.19±0.16**<br>(53.3) | 0.44±0.03**<br>(58.09) |
| WDVA (300)      | 0.56±0.13<br>(28.9)                              | 0.72±0.16*<br>(42.1)  | 2.18±0.1<br>(0.9)     | 1.93±0.19<br>(21.22)   | 2.15±0.21<br>(15.6)   | 0.14±0.02**<br>(86.1)  |

n=8

Values are expressed as mean ±S.E.M

\*P ≤ 0.05 significant; \*\*P ≤ 0.005 highly significant difference as compared to control

HEVA= Hexane extract of *Vernonia anthelmintica*EEVA= Ethanol extract of *Vernonia anthelmintica*.WDVA= Water decoction of *Vernonia anthelmintica***Table 2:** Antioxidant bioassay of HEVA and EEVA

| Sample            | IC <sub>50</sub> ±SEM | %RSA (radical Scavenging activity) |
|-------------------|-----------------------|------------------------------------|
| HEVA              | Inactive              | 40.03                              |
| EEVA              | 95.10±2.01 mg/ml      | 89.70                              |
| Gallic acid       | 23.43±0.43 uM         | 93.93                              |
| N-Acetyl cysteine | 111.44±0.7 uM         | 95.95                              |

n=3

HEVA = Hexane extract of *Vernonia anthelmintica*EEVA= Ethanol extract of *Vernonia anthelmintica*

Following mathematical equation is used to calculate % radical scavenging activity (%RSA)

$$\% \text{ RSA} = 100 - \frac{(\text{O.D of sample/O.D of control} \times 100)}{(\text{O.D designates optical density})}$$

### Experimental animals

Healthy Sprague dawley rats (200-250 g) of either sex not used before for any behavioural or biochemical study were procured from H.E.J, University of Karachi, Pakistan. One week acclimatization to laboratory conditions was allowed to all animals before experimentation. Standard rat feed and free access to water was given to each animal. However all experimental animals were starved to feed 12 h prior to experimentation but not water. All experimental protocols were approved by BASR (board of advanced studies and research), University of Karachi. Copy of the letter is provided to editor of the journal.

### Carrageenan induced Inflammation

Carrageenan induced rat paw model was used to assess the anti-inflammatory potential of *Vernonia*

*anthelmintica*. Sixty four Sprague Dawley rats were divided into eight groups, of eight rats each. 0.1ml of 1% carrageenan was injected to induce oedema as the sign of inflammation into the sub-plantar area of the right hind paw. Control group of rats was given normal saline in the equivalent volume as that of test groups and standard group of rats received ibuprofen in the dose of 100 mg/kg through oral route. The treatment groups of rats were subjected to the oral administration of 150 and 300 mg/kg of the hexane and ethanol extracts and water decoction of *Vernonia anthelmintica* seeds 1 h before carrageenan administration in hind paw. The paw volume was thus measured using digital plethysmometer (Ugo Basile) before administration of carrageenan referred to as V<sub>0</sub> and 1, 2, 3, 4, 5, 6 and 24 h thereafter referred to as V<sub>t</sub>. Paw edema is the marker of inflammation and it was calculated as increase in paw volume after treatment subtracted from baseline paw volume of respective animals. Percentage reduction in volume was also calculated to quantify and compare the inhibition of edema with respect to control. Following formula is used and results were expressed as percentage of inhibition of edema (Winter, Risley *et al.*, 1962).

Percent reduction in volume =  $[(V_t - V_0)_{\text{control}} - (V_t - V_0)_{\text{treated}}] / (V_t - V_0)_{\text{control}} \times 100$

## STATISTICAL ANALYSIS

All the results were expressed as mean  $\pm$  S.E.M results obtained were fed in SPSS (statistical package for social sciences) and analysed by one way analysis of variance (ANOVA) followed by tukey's posthoc multiple comparison test. Differences between the groups were considered significant at  $P < 0.05$ .

## RESULTS

### Anti-inflammatory activity

Table 1 shows results of anti-inflammatory potential of three different extracts of *Vernonia anthelmintica* (HEVA, EEVA and WDVA) in two different doses (150mg/kg and 300mg/kg) as compared to control and standard anti-inflammatory drug ibuprofen in the dose of 100mg/kg in carrageenan induced rat paw model. There was a significant decrease in paw edema of most of the treatment groups and standard group as compared to control at 1h, 2h, 3h and 4 h, 5h and 24h.

### Antioxidant bioassay

Table 2 shows DPPH assay of hexane and ethanol extracts of *Vernonia anthelmintica* seed. Results depict that EEVA has potent antioxidant activity with  $IC_{50}$  value of 95.10% RSA of 89.70 against gallic acid ( $IC_{50}$  value:  $23.43 \pm 0.43$ ) and N- acetyl cysteine ( $IC_{50}$  value:  $111.44 \pm 0.7$ ), whereas HEVA was found to be inactive with % RSA of 40.03.

### Radical Scavenging activity of EEVA:

Fig. 1 reveals the dose dependent effect of active antioxidant EEVA. 500 $\mu$ g/ml of EEVA shows the maximal inhibition of 89.70%

## DISCUSSION

In the present study, the effects of control, treatment and standard groups were assessed for volume of paw edema and percent inhibition as compared to control in carrageenan-induced paw edema rat model. The result (table 1) revealed the strong anti-inflammatory activity of Ibuprofen in the dose of 100mg/kg with the maximum inhibition (82.3%) at 2 h which remained decrease till 24 hour. HEVA in the dose of 150mg/kg did not show any significant anti-inflammatory activity but 300mg/kg dose revealed strong anti-inflammatory potential started at 2 h, maximum at 3 h (70.9%) and remain significant till 24 hour of treatment. EEVA in the dose of 150mg/kg showed significant decreased paw edema only at 24 hour that would be more appropriately considered as reversal of inflammation. However 300mg/kg dose of EEVA revealed strong anti-inflammatory potential that was

started at 1h, achieve significance at 2 h, became maximum at 4 h and remained significant till 24 hours. WDVA showed a little aberrant result as 150 mg/kg dose produces its anti-inflammatory effect at 5 h and 300mg/kg dose only showed significant decrease in paw volume at 2<sup>nd</sup> hour of treatment. Carrageenan model was chosen because of its sensitivity towards orally administered anti-inflammatory agents against acute inflammatory conditions (Di Rosa, Giroud *et al.*, 1971)

The results of present study clearly depicts that HEVA and EEVA in higher dose possess a strong anti-inflammatory potential that is comparable to standard anti-inflammatory drugs, whereas WDVA showed milder potential against acute inflammation. Towards the mechanistic approach HEVA and EEVA in the doses of 300 mg/kg might showed this potential due to potent effects on second phase of inflammation that is caused by increased production of prostaglandins, free radicals and cyclooxygenase pathway products and weak effects on first phase that is merely due to release of mediators (histamine, serotonin and kinins) and accounts for first hour of inflammatory changes after carrageenan injection (Vinegar, Schreiber *et al.*, 1969).

Present study also revealed the strong antioxidant potential of EEVA and hence strong potential to scavenge free radicals and it is the added advantage of ethanol extract in producing sustained anti-inflammatory effect. WDVA on contrary showed a different pattern as 150 mg/kg dose showed more pronounced effects on second phase of inflammation whereas 300mg/kg dose showed activity in first phase only. These results are in accordance with a previous study which showed that petroleum ether and alcohol extracts of *Vernonia anthelmintica* on oral intake suppressed the edema after 2 hour and sustained till 5 hour having strong prostaglandin inhibition effect (Ashok, Koti *et al.*, 2010b).

Oxygen is supposed to be a necessary element required by all living cells for their survival. In normal physiology and metabolism, certain percentage of oxygen becomes univalent reduced to generate free radicals such as nitric oxides, hydroxyl, super oxides and hydrogen peroxides. These free radicals termed as ROS (reactive oxygen species) exert oxidative stress to human cells and their extent is so much so that each cell faces thousands of oxidative hits per second (Mondal, Chakraborty *et al.*, 2006).

Medicinal plants and their purified extracts have shown to exhibit antioxidant potential. This antioxidant capacity of plants is due to presence of varying secondary metabolites namely flavones, flavonoid, isoflavones, coumarins, anthocyanins, lignans, catechins and isocatechins (Aqil, Ahmad *et al.*, 2006). Drugs with antioxidant principal are required and being in use for the prevention and cure of

various complex diseases of chronic nature like stroke, diabetes, atherosclerosis, alzheimer's and cancer (Devasagayam, Tilak *et al.*, 2004).

General mechanism of antioxidant to combat with free radicals is to break up the propagation of their chains by simply providing an electron or hydrogen atom and thus receiving their excessive energy (Lachman, Lieberman *et al.*, 1986). It is documented need to find natural antioxidants in order to replace synthetic antioxidants because later cannot be used freely because of their potential side effects like carcinogenicity (Kumaran and Karunakaran, 2007). The natural antioxidants work by scavenging free radicals, complexing metals with oxidant potential, exhausting single oxygen and as reducing agents (Ebadi, 2006).

Present study has undertaken DPPH assay in order to estimate the free radical scavenging capacity of *Vernonia anthelmintica* extracts. The results (table 2) revealed that HEVA showed non-significant radical scavenging activity (% RSA 40.03). Whereas EEVA has shown to have strong dose dependent DPPH radical scavenging activity with an IC<sub>50</sub> value 95.10± 2.01 and maximal inhibition of 89.70% (Graph-1). Present work further strengthen the claims made for chloroform fraction of *Vernonia anthelmintica* as an antioxidant in a previous study (Arya, *et al.*, 2012).

Present study with reference to anti-inflammatory activity lack *in-vivo* biochemical analysis of antioxidant parameters and is not backed up by histopathological analysis of inflamed rat paw as compared to normal rat paw.

## CONCLUSION

It is thus concluded that selected extracts of *Vernonia anthelmintica* possess strong anti-inflammatory and antioxidant potential and can be used in the preparation of medicines for the treatment of inflammation and diseases that are attributable to oxidative stress.

## REFERENCES

- Aqil F, Ahmad I and Mehmood Z (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turkish J. Biol.*, **30**(3): 177-183.
- Arya A, Achoui M, Cheah SC, Abdelwahab SI, Narrima P, Mohan S, Mustafa MR and Mohd MA (2012). Chloroform fraction of *Centrathurum anthelminticum* (L.) seed inhibits tumor necrosis factor alpha and exhibits pleotropic bioactivities: Inhibitory role in human tumor cells. *Evid. Based Complementary Altern. Med.*, 627256. doi: 10.1155/2012/627256. Epub 2012 Feb 2.
- Ashley NT, Weil ZM and Nelson RJ (2012). Inflammation: Mechanisms, costs and natural variation. *Annu. Rev. Ecol. Evol. Syst.*, **43**: 385-406.
- Ashok P, Koti B, Thippeswamy A, Tikare V and Dabadi P Viswanathaswamy A (2010a). Evaluation of antiinflammatory activity of *Centrathurum anthelminticum* (L) kuntze seed. *Indian J. Pharm. Sci.*, **72**(6): 697.
- Ashok P, Koti B, Thippeswamy A, Tikare V and Dabadi P Viswanathaswamy A (2010b). Evaluation of antiinflammatory activity of centrathurum anthelminticum (L) kuntze seed. *Indian J. Pharm. Sci.*, **72**(6): 697-703.
- Chen Z, Bertin R and Frolidi G (2013). EC 50 estimation of antioxidant activity in DPPH assay using several statistical programs. *Food Chemistry*, **138**(1): 414-420.
- Devasagayam T, Tilak J, Boloor K, Sane KS, Ghaskadbi SS and Lele R (2004). Free radicals and antioxidants in human health: Current status and future prospects. *Japi.*, **52**(794804) 4.
- Di Rosa M, Giroud J and Willoughby D (1971). Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. *J. Pathol.*, **104**(1): 15-29.
- Dröge W (2002). Free radicals in the physiological control of cell function. *Physiological Reviews*, **82**(1): 47-95.
- Ebadi M (2006). *Pharmacodynamic basis of herbal medicine*: CRC press.
- Kumaran A and Karunakaran RJ (2007). *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *Food Sci. Technol.*, **40**(2) 344-352.
- Lachman L, Lieberman HA and Kanig JL (1986). The theory and Practice of Industrial Pharmacy: Lea & Febiger.
- Mangge H, Becker K, Fuchs D and Gostner JM (2014). Antioxidants, inflammation and cardiovascular disease. *World J. Cardiol.*, **6**(6): 462.
- Mondal SK, Chakraborty G, Gupta M. Mazumder U (2006). *In vitro* antioxidant activity of *Diospyros malabarica* Kostel bark. *Indian Journal of Experimental Biology*, **44**(1): 39.
- Morris CJ (2003). Carrageenan-induced paw edema in the rat and mouse. *Inflammation Protocols*, pp.115-121.
- Patnaik S and Bhatnagar S (2015). Evaluation of cytotoxic and antioxidant properties and phytochemical analysis of *Vernonia anthelmintica*. Willid. leaf extracts. *Int. J. Biosci. Technol.*, **8**(1): 1.
- Santosh C, Attitalla IH and Mohan MM (2013). Phytochemical Analysis, Antimicrobial And Antioxidant Activity of Ethanolic Extract of *Vernonia Anthelmintica*. *Int. J. Pharm. Bio. Sci.*, **4**(1): 960-966.
- Uddin N, Siddiqui BS, Begum S, Bhatti HA, Khan A, Parveen S and Choudhary MI (2011). Bioactive flavonoids from the leaves of *Lawsonia alba* (Henna). *Phytochemistry Letters*, **4**(4): 454-458.

- Vinegar R, Schreiber W and Hugo R (1969). Biphasic development of carrageenin edema in rats. *J. Pharm. Exp. Ther.*, **166**(1): 96-103.
- Winter CA, Risley EA and Nuss GW (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Exp. Biol. Med.*, **111**(3): 544-547.
- Young I and Woodside J (2001). Antioxidants in health and disease. *J. Clin. Pathol.*, **54**(3): 176-186.