Research Article

Preliminary pharmacological screening of *Bryonia laciniosa* L. for ulcerative colitis in rats

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Abstract

Objective: Objective of present study was to investigate protective effect of ethanolic extract of *Bryonia laciniosa* seeds for ulcerative colitis in rats. **Material and methods**: Ethanolic extract of *Bryonia laciniosa* seeds was tested for protective effect in Dextran Sulphate Sodium (DSS) induced colitis in rats. The disease activity index and wet colon weight for different groups were observed. Treatment groups also observed for MPO activity and antioxidant substance level. **Results and conclusion**: DSS induced colitis significantly elevated MPO activity, whereas administration of EEBL strongly inhibited MPO activity in rats with 200mg/kg as well as near to the standard drug. The results of MDA level in tissue also indicated that colonic content of MDA decreased significantly and similar to the standard drug when compared to the DSS model group. The effect of EEBL on the various antioxidant level (SOD, CAT and GSH) were observed. The ethanolic extract of *Bryonia laciniosa* (EEBL) seeds restored up to the normal level of antioxidant parameters, that was confirmed the potent antioxidant effect of ethanolic extract. Phytochemical study revealed that flavonoids and phenolic compounds are present in ethanolic extract of *Bryonia laciniosa* seeds. The free radical scavenging property of these flavonoids plays a significant role in ulcer healing. Significant increase in GSH level and reduction in MDA level has also been revealed in extracts treated groups while investigating *in vivo* antioxidant activity. Hence, the probable mechanism of healing of ulcerative colitis by ethanolic extract of *Bryonia laciniosa* (EEBL) seeds may be attributed to antioxidant and free radical scavenging property.

Keywords: *Bryonia laciniosa,* Dextran Sulphate Sodium, ulcerative colitis, antioxidant

Introduction

Ulcerative colitis (UC) is a rectal and colonic mucosal chronic, idiopathic, inflammatory bowel disease (IBD). It is characterized by colonic inflammation, most likely due to the infiltration of polymorphonuclear cells, lymphocytes, monocytes and plasma cells, accompanied by oxygen-free radicals, which ultimately leads to mucosal alteration and ulceration (Cho *et al*., 2007).

Treatment in UC is directed towards inducing and maintaining remission of symptoms and mucosal inflammation. The choice of the therapeutic method depends on both the extent of colonic involvement and the severity of the disease at presentation, which are the key parameters to be assessed for the most appropriate treatment during the whole disease course. For years the therapeutic repertoire for UC included aminosalicylates, corticosteroids and immunomodulators.

Bryonia laciniosa Linn. is commonly referred to as 'Shivlingi' and is distributed across the India. *Bryonia laciniosa* is an annual climber with bright red fruits and has been reported to have higher medicinal potency against various disease alignment (Singh et al., 2009). The seeds of *B. Laciniosa* are also commonly known as ' Shivlingi ' due to some specific texture of upper seed surface has a marking structure similar to ' Shivling, ' an icon of Lord Shiva, a popularly worshiped Hindus deity (Sivakumar et al., 2004; Kirtikar and Basu, 1987). It is an important constituent of 'Striravallabhpugpak' an Ayurvedic formulation to improve sexual behaviour and as a general tonic. The seeds are reported to be useful in curing cases of sterility (Panda, 2004).

The main active constituents of the plants are Bryonin, a

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bitter principle, punicic acid, and source of seed oil, non-ionic glucomannon and goniothalamin was studied (Sivakumar et al., 2005; Gowrikumar et al., 1981). The seeds of *Diplocyclos palmatus* contains 23% oil and 15% protein. The seeds are used for increasing sperm count also as an aphrodisiac (Bhogankar, 2006). It is also taken in impotency as a tonic. Whole plant is used to treat adenopathy, ague, asthma, bronchitis, carbuncles, cough, delirium, fertility, headache, megalosplenyb, paralysis, phthisis, snake bite. Leaves of *Bryonia laciniosa* as chloroform extract exhibited significant anti-inflammatory activity (Gupta, 2003). This plant also has been reported to have positive activity as Antimicrobial, larvicidal, anti-inflammatory, cytotoxic, analgesic, anti-pyretic and anti-diabetic (Chaudhari and Avlaskar, 2013; Patel et al., 2012).

Literature available revealed that *Bryonia laciniosa* L. has been widely used as a folklore medicine in tropical and subtropical areas with beneficial effects in numerous diseases, including infection, inflammation, infertility and it has also been identified as an antioxidant (Wealth of India, 1988; Moghe et al., 2011). Most of the remedies were taken from plants and proved to be useful in the indigenous system of medicine. However, the literature review revealed that *Bryonia laciniosa* has been used traditionally for cuts, injuries and inflammatory disorders but not reported any systematic study by any researcher. Therefore, the present studies aim to open new avenues for the improvement of medicinal uses of this indigenous plant for the inflammatory diseases. Hence, in the present study, *Bryonia laciniosa* seeds have selected for phytochemical investigation and evaluation of healing effect in experimentally induced ulcerative colitis in rats.

Materials and methods

Collection and identification of plant material

The seeds of *Bryonia laciniosa* Linn. were collected in the month of August to September around the locality of Bhopal (M.P.). A herbarium sheet was prepared to authenticate the plant species and deposited in Department of Botany, Barkatullah Vishwavidyalaya, Bhopal (M.P.). Plant material was dried under shed at room temperature and coarsely powdered stored for further use.

Preparation of extract and phytochemical analysis

The plant extract were subjected for different qualitative chemical tests to detect the plant constituents of the plant extracts (Kokate et al., 1996; Jain t al., 2016). The ethanolic extract of *Bryonia laciniosa* seeds was concentrated under reduced pressure to dryness and then suspended in 0.5% CMC-Na solution for pharmacological evaluation afterwards.

Animal protocol

Wistar albino rats (150-200g) of either sex were selected for the experiment. They were housed individually in well-ventilated, temperature controlled (26 ±2°C) animal room for seven days of period prior experiment. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and they were kept under standard environmental conditions of laboratory temperature and water *ad libitum*. The animals were maintain alternate cycle of darkness and light at 12 hours. The animals were fasted for at least 12 hours before the onset of experiment. The experimental protocols were approved by Institutional Animal Ethics Committee.

Acute toxicity study

Before exploring any new drug moiety, be a natural or synthetic, its safety studies have to be performed in order to find out the therapeutic window, minimum effective concentration and toxic dose level. This is done to assess that till which concentration, the drug under investigation is safe to be further explored for its therapeutic usefulness. Previously, the Lethal Dose Studies used to be conducted, known as LD50 determinations, in which the dose at which 50% of the cattle die was calculated to estimate the drug's toxicity rate and was a determining factor in the calculation of the therapeutic dose. The acute oral toxicity study of crude extract of *Bryonia laciniosa* seeds was carried out as per the guidelines set by Organization for Economic Co–operation and Development (OECD) guideline 423. As the second alternative to the conventional acute toxicity test, described in Test Guideline 401, the original Guideline 423 was adopted in March 1996.

Dextran Sulphate Sodium (DSS) induced colitis

The administration of DSS contained in water causes haematochezia, body weight loss, shortening of the intestine, mucosal ulcers and neutrophil infiltration. Acute colitis is regarded to be induced but not obtained by innate immunity. On the other side, the chronic stage is reported to be caused by lymphocytes activated by the cytokines secreted from the activated macrophages (Jurjus et al., 2004).

In rats, ulcerative colitis was caused by adding DSS (Dextran Sulfate Sodium) to water bottles, resulting in a 3% (w / v) solution (Hirata et al., 2001). Free access to water comprising 3 percent oral DSS for 7 days was provided to the cattle. For 7 consecutive days, all treatment regimens were continued. Drugs were administered once daily by oral gavage and suspended in Sodium CMC. Clinical activity results were evaluated on the 8th day and the animals were anesthetized with ether and blood was gathered for biochemical assessment through retro orbital puncture. Daily recorded body weight, consistency of stools and gross bleeding.

The animals were divided into 5 groups consisting 6 animals in each: Group I normal or untreated animals; Group II is control received Dextran sodium sulfate (3%w/v in drinking water) + 0.9% saline at a dose of 50 ml/kg; Group III received Dextran sodium sulfate (3%w/v in drinking water) + ethanolic extract of *Bryonia laciniosa* seeds, suspension 150 mg/kg; Group IV received Dextran sodium sulfate (3%w/v in drinking water) + ethanolic extract of *Bryonia laciniosa* seeds, suspension 200 mg/kg; Group V received Dextran sodium sulfate (3%w/v in drinking water) + Sulfasalazine in a dose of 500 mg/kg suspension.

Assessment of colon damage by macroscopic scoring

A clinical score assessing weight loss, stool consistency, and colon bleeding as described by Cooper, divided by 3, quantified the score for disease activity (Niu et al., 2013).

Assessment of biochemical parameters

Myeloperoxidase (MPO) assay

MPO activity was identified using an MPO detection kit using the O-dianisidine technique (Liu and Wang, 2011; Yang et al., 2012). Blood was gathered and centrifuged from the eyes. The MPO activity was evaluated at 460 nm by absorbance using a spectrophotometer (Shimadzu). MPO activity was described as the enzyme degrading 1μmol per minute at 37μC and expressed in units per liter of serum.

Determination of malondialdehyde (MDA) content

By Mihara and Uchiyama (1978), lipid peroxidation was evaluated as the colon's MDA content. In short, MDA's colorimetric determination is based on the response of one reactive aldehyde molecule with two thiobarbituric acid molecules at low pH (2–3) and 45 min at a temperature of 95°C. By treatment with N-butanol obtained the resulting purple color and spectrophotometrically determined the absorbance at 532 and 520 nm. As a measure of colonic MDA content, the distinction in optical density between the two wavelengths was used. MDA's final value was depicted as protein nmol/mg.

Determination of antioxidants level

For antioxidant assay, catalase was estimated following the breakdown of hydrogen peroxide according to the method of Beers and Sizer (1952). Superoxide dismutase (SOD) was assayed according to Misra and Fridovich (1972) based on the inhibition of epinephrine autoxidation by the enzyme. Reduced glutathione (GSH) content was determined in granuloma tissue by the method of Moron et al, (1979).

Statistical analysis

The data were expressed as mean standard deviation (SD). The statistical significance of the difference in each parameter among the groups was evaluated using one-way analysis of variance (ANOVA) followed by the followed by the multiple comparison test of Tukey–Kramer tests. Criterion for statistically significant difference was chosen to be at $P<0.01$.

Results and discussion

Phytochemical screening

The powdered plant material was successively extracted with different solvents in increasing polarity order such as petroleum ether, chloroform, ethyl acetate, ethyl alcohol and aqueous with chloroform. Then all extracts were concentrated under vacuum desiccator and weighed. The yield were found to be 5.25, 1.52, 1.25, 3.64 and 0.84 respectively, for petroleum ether, chloroform, ethyl acetate, ethyl alcohol and aqueous with chloroform and then subjected to phytochemical screening. Petroleum ether and ethanol extract showed higher percentage of yield. The qualitative chemical analysis was confirmed the presence of sterols in petroleum ether extract and carbohydrates, proteins, flavonoids and amino acid were present in ethanol extract. Due to presence of number of chemical constituents in ethanol extract, it was selected for further studies.

Acute toxicity study

Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours. In all cases no one death was observed within first 24 hours. Additional observations like changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and motor activity and behavioral pattern. Attention was also given to observation of tremors and convulsions. We have selected one tenth dose of highest toxic dose level.

Effect of ethanolic extract on Dextran Sulphate Sodium (DSS) induced colitis

DSS produces severe macroscopic edematous inflammation in the colon. The disease activity index and wet colon weight for different groups were observed. The disease activity index and weight of colon for colitis control group were found as 4.62±0.28, 197.2±7.81, respectively. The disease activity index and colon weight for EETO treated group of animals were observed significantly decreased in dose dependent manner. EEBL 150 mg/kg dose decreases disease activity index and weight of colon significantly as 2.15±0.52 (53.46) and 141.2±7.24, respectively (Table 1, Figure 1 and Figure 2). However, the EEBL200 mg/kg showed better results as 1.75±0.69 (62.12) and 131.84±8.22 for disease

Table 1. Effect of ethanolic extract of *Bryonia laciniosa* (EEBL) seeds on macroscopic observations in rats

Groups	Disease activity Index (% protection)	Weight of colon (mg/cm)
Normal control (0.9% saline)	0	128.5±8.46
Control (0.9% saline+ DSS)	4.62±0.28	197.2±7.81
EEBL 150 mg/kg+ DSS	2.15±0.52 (53.46)*	141.2±7.24*
EEBL200 mg/kg+ DSS	1.75±0.69 (62.12)*	131.84±8.22*
Sulfasalazine (500mg/kg) + DSS	1.72±0.61(62.77)*	130.51±7.61*

n = 6 albino rats per group, value represents Mean S.D. *P< 0.01, when compared each treated group with control group

Table 2. Effect of ethanolic extract of *Bryonia laciniosa* (EEBL) seeds on MPO and MDA level of colonic tissues of DSS induced colitis in rats

Treatment groups	MPO (OD/g tissue)	MDA (OD/g tissue)
Normal control (0.9% saline)	21.63±1.57	35.62±2.18
Control (0.9% saline+ DSS)	45.42±2.61	68.38±4.88
EEBL 150 mg/kg+ DSS	31.57±2.71*	48.73±3.42*
EEBL200 mg/kg+ DSS	24.81±2.08*	37.64±2.83*
Sulfasalazine (500mg/kg) + DSS	22.76±1.98*	36.72±2.16*

Values are presented as mean of optical density (OD) ± SD, *P < 0.05, represent significant value compared with control group

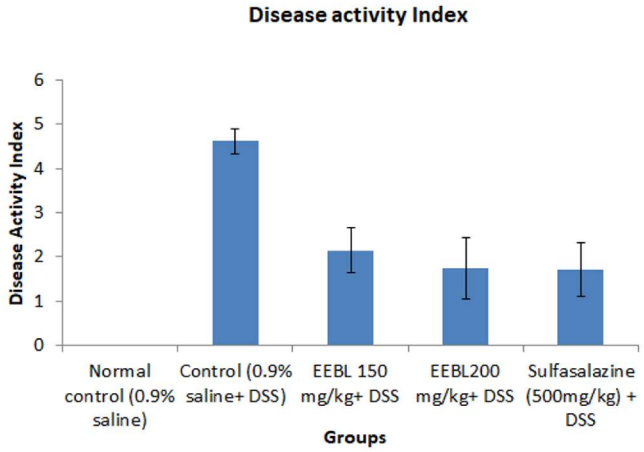

Figure 1. Effect of ethanolic extract of *Bryonia laciniosa* (EEBL) seeds on disease activity in rats

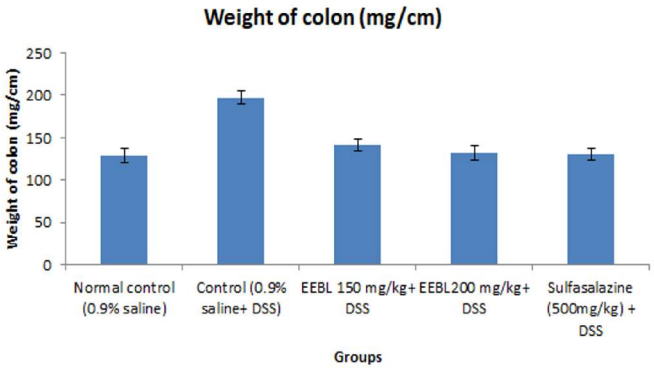

Figure 2. Effect of ethanolic extract of *Bryonia laciniosa* (EEBL) seeds on weight of rat's colon

activity index and weight of colon, respectively, indicating its potent activity at the dose tested. These data were also comparable to the standard drug treatment group.

The effect of ethanolic extract of *Bryonia laciniosa* (EEBL) seeds on different biochemical parameters were also observed in dose dependent manner. In the experiment, we found that MPO activity was correlated with the development of colonic inflammation. DSS induced colitis significantly elevated MPO activity, whereas administration of EEBL strongly inhibited MPO activity in rats with 200mg/kg as well as near to the standard drug (Table 2 and Figure 3). The results of MDA level in tissue also indicated that colonic content of MDA decreased significantly and similar to the standard drug when compared to the DSS model group. Treatment with EEBL exerted, to some extent, effects on reducing the colonic MDA level compared to animals that received DSS alone.

The effect of EEBL on the various antioxidant level (SOD, CAT and GSH) were mentioned in table 3. The ethanolic

Table 3. Effect of ethanolic extract of *Bryonia laciniosa* (EEBL) seeds on antioxidants level of colonic tissues in rats

Groups	Antioxidants level		
	SOD(µg/50 mg tissue)	CAT(µmol/50 mg tissue)	GSH(µmol/50 mg tissue)
Normal control (0.9% saline)	34.25±2.05	25.76±1.82	29.53±1.75
Control (0.9% saline+ DSS)	13.52±0.82	11.37±0.92	14.28±0.96
EEBL 150 mg/kg+ DSS	17.26±1.27	19.43±1.43	22.94±1.29
EEBL200 mg/kg+ DSS	30.82±2.63	24.29±1.67	27.13±1.48
Sulfasalazine (500mg/kg) + DSS	31.75±2.77	25.13±1.63	28.08±2.04

n = 6 albino rats per group, value represents Mean S.D. *P< 0.01, when compared each treated group with control group

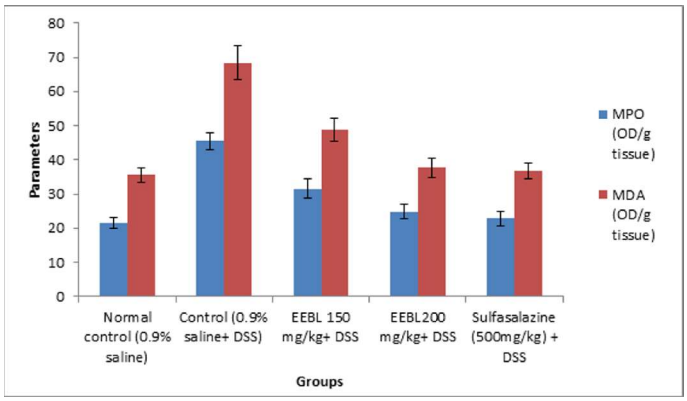

Figure 3. Effect of ethanolic extract of *Bryonia laciniosa* (EEBL) seeds on MPO and MDA level of colonic tissues in rats

extract of *Bryonia laciniosa* (EEBL) seeds restored up to the normal level of antioxidant parameters, that was confirmed the potent antioxidant effect of ethanolic extract.

The level of antioxidants in colon tissues were observed significant decrease in colitis control group, may be due increasing free radicles generation. This decreasing level of SOD, CAT and GSH was slightly increased in treatment group with 150mg/kg dose of EEBL. But a significant improvement in level of SOD, CAT and GSH was found in treatment group of 200mg/kg dose of EEBL as well as standard drug treated group, when compared to colitis control group (Figure 4).

Phytochemical study revealed that flavonoids and phenolic compounds are present in ethanolic extract of *Bryonia laciniosa* seeds. The free radical scavenging property of these flavonoids plays a significant role in ulcer healing. Significant increase in GSH level and reduction in MDA level has also been revealed in extracts treated groups while investigating *in vivo* antioxidant activity. Hence, the probable mechanism of healing of ulcerative colitis by ethanolic extract of *Bryonia laciniosa* (EEBL) seeds may be attributed to antioxidant and free radical scavenging property, while free radical scavenging activity may be attributed to flavonoids and phenolic compounds.

Conclusion

Phytochemical study revealed that flavonoids and phenolic compounds are present in ethanolic extract of *Bryonia laciniosa* seeds. The free radical scavenging property of these flavonoids plays a significant role in ulcer healing. Significant increase in GSH level and reduction in MDA level has also been revealed in extracts treated groups while investigating *in vivo* antioxidant activity. Hence, the probable mechanism of healing of ulcerative colitis by ethanolic extract of *Bryonia laciniosa* (EEBL) seeds may be attributed to antioxidant and free radical scavenging property, while free radical scavenging activity may be attributed to flavonoids and phenolic compounds.

Conflict of interest

None

References

Beers RF, Sizer IW, 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Journal of Biological Chemistry, 195: 133–140.

Bodhankar SL, Vyawahare NS. 2004. A text book of physiopathology. Nirali prakashan, Pune.

Chaudhari VM, Avlaskar AD. 2013. Role of Shivlingi in Infertility. Journal of Homeopathy & Ayurvedic Medicine, 2: 5.

Cho CH, Pfeiffer CJ. 1981. Gastrointestinal ulceration in the guinea pig in response to dimaprit, histamine, and H1- and H2-blocking agents. Digestive Diseases and Sciences, 26 (4): 306–311.

Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi, India: Council of Scientific and Industrial Research, 1956.

Gowrikumar G, Mani VVS, Chandrasekhararao T, Kaimal TNB, Lakshminarayana G. 1981. Diplocyclos palmatus L: a new seed source of punicic acid. Lipids, 16: 558–559.

Gupta M, Mazumdar UK, Sivakumar T, Vamsi ML, Karki SS,

Sambathkumar R. 2003. Evaluation of anti-inflammatory activity of chloroform extract of *Bryonia laciniosa* in experimental animal models. Biological and Pharmaceutical Bulletin, 26: 1342–1344.

Harborne JB. Phytochemical methods – a guide to modern techniques of plant analysis. New York: 2nd ed. 1984.

Hirata I, Murano M, Nitta M, Sasaki S, Toshina K, Maemura K, Katsu K. 2001. Estimation of mucosal inflammatory mediators in rat DSS-induced colitis. Digestion, 63, 73–80.

Jain AP, Bhandarkar S, Rai G, Yadav AK, Lodhi S. 2016. Evaluation of Parmotrema reticulatum Taylor for Antibacterial and Antiinflammatory Activities. Indian Journal of Pharmaceutical Sciences, 78(1):94-102. doi:10.4103/0250-474x.180241

Jain AP, Pawar RS, Lodhi S, Singhai AK. 2012. Immunomodulatory and anti-oxidant potential of Alpinia galanga Linn. rhizomes. Pharmacognosy Communications, 2(3):30-7.

Jurjus AR, Khoury NN, Reimund JM. 2004. Animal models of inflammatory bowel disease. Journal of Pharmacological and Toxicological Methods, 50(2): 81-92.

Khadelwal KR. Practical Pharmacognosy Technique and Experiments. Nirali Prakashan, Pune, 2002; pp 149-56.

Kirtikar KR, Basu BD. Indian Medicinal Plants, 2nd.ed., Lalit Mohan Basu Allahabad, India , 1961; pp 314-315.

Kokate CK, Purohit AP, Gokhale SB. Text book of pharmacognosy, Nirali Prakashan, Pune, 1996; pp A.1-A.4.

Liu X, Wang JM. 2011. Anti-inflammatory effects of iridoid glycosides fraction of Folium syringae leaves on TNBS-induced colitis in rats. Journal of Ethnopharmacology, 133: 780–787.

Misra HP, Fridovich I, 1972. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry, 247: 3170–3175.

Moghe AS, Gangal SG, Priya R. 2011. Shilkar. In vitro cytotoxicity of Bryonia laciniosa (Linn.) Naud. On Human cancer cell lines. Indian Journal of Natural Products and Resources, 2(3): 322-9.

Moron MA, Depierre JW, Mannervick B. 1979. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica Biophysica Acta 582(1), 67–78.

Mourya P, Shukla A, Rai G, Lodhi S. 2016. Hypoglycemic and hypolipidemic effects of ethanolic and aqueous extracts from Ziziphus oenoplia (L) Mill on alloxan-induced diabetic rats. Beni-Suef University Journal of Basic and Applied Sciences, 6 (1). doi: 10.1016/j.bjbas.2016.12.002

Niu X, Fan T, Li W, Huang H, Zhang Y, Xing W. 2013. Protective effect of sanguinarine against acetic acid-induced ulcerative colitis in mice. Toxicology and Applied Pharmacology, 267:256–265.

OECD, 2001. Guidelines for Acute Toxicity of Chemicals; Organization for Economic Co-operation and Development: Paris, France, 2001; No. 423.

Patel S, Santani D, Shah M, Patel V. 2012. Anti-hyperglycemic and Anti-hyperlipidemic Effects of Bryonia Laciniosa Seed Extract and its Saponin Fraction in Streptozotocin-induced Diabetes in Rats. Journal of Young Pharmacists, 4(3):171-6.

Sivakumar T, Sambath Kumar R, Perumal P, Vamsi MLM, Sivakumar P, Kanagasabai R, Baskaran MV, Karki Subhas S, Mazumder UK, Gupta M. 2005. Antitumor and antioxidant activities of *Bryonia laciniosa* against Ehrlich's Ascites Carcinoma bearing Swiss albino mice. Oriental Pharmacy and Experimental Medicine 5(4):322-330.

Sivakumar T, Perumal P, Kumar RS, Vamsi ML, Gomathi P, Mazumder UK, Gupta M. 2004. Evaluation of analgesic, antipyretic activity and toxicity study of Bryonia laciniosa in mice and rats. American Journal of Chinese Medicine, 32(4):531-9.

The Wealth of India: A Dictionary of Indian Raw Materials and Industrial products, 1988, Publication and Information Directorate, CSIR, New Delhi, 10: 87-88.

Yang T, Zou K, Qian W. 2005. Effects of intestinal trefoil, factor on colonic mucosa in experimental colitis of rats. Journal of Huazhong University of Science and Technology, 25(3): 300-302.