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# Preliminary Phytochemical Screenings and Antipyretic, Analgesic and Anti-inflammatory Activities of Methanol Extract of *Vernonia cinerea* Less. (Fam: Asteraceae)

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors MI, IS, MIH designed the study, performed the statistical analysis. Authors MRK, MKB wrote the protocol and wrote the first draft of the manuscript. Authors MKB and MI managed the analyses of the study. Authors RBR, ZT managed the literature searches. Author MAR revised manuscript. All authors read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

**Aims:** The aim of the current study was to undertake phytochemical screenings and evaluate antipyretic, analgesic and anti-inflammatory activities of the methanol extract of whole plant of *Vernonia cinerea* Less. (VCME).
**Place and Duration of Study:** The study was carried out for one year in 2012 in the Department of Pharmacy, Southern University Bangladesh, Chittagong, Bangladesh.
**Methodology:** For preliminary phytochemical screenings, the crude methanol extract of *V. cinerea* was subjected to various tests to determine the chemical nature of the extract. Antipyretic activity was assessed by the yeast-induced hyperthermia in mice. The analgesic property was evaluated by formalin-induced writhing test. Acetyl salicylic acid

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(ASA) was used as standard in *in-vitro* anti-inflammatory activity test.
**Results:** Results of the preliminary phytochemical screening revealed the presence of alkaloids, flavonoids and triterpenoids in the extract. In yeast-induced pyrexia, the crude extract demonstrated a significant (p=0.05) reduction of mices' body temperature after elevation by the administration of yeast. These effects were pronounced at the 2nd and 3rd h post-treatment with the extract. VCME exhibited a dose dependent activity in analgesic activity test with 32.61% and 52.17% protection at the dose of 200 and 400mg/kg, respectively as compared to that 76.09% exhibited by standard diclofenac sodium. In the anti-inflammatory test, the crude extract at the dose of 400µg/ml showed 65.12% inhibition of protein denaturation whereas standard acetyl salicylic acid (ASA) revealed 76.74% inhibition.
**Conclusion:** These results revealed that *V. cinerea* may be used in pharmaceutical applications because of its effective pharmacological properties.

*Keywords: Vernonia cinerea; phytochemical; antipyretic; analgesic; anti-inflammatory.*

## 1. INTRODUCTION

In recent years, the interest in the plant-based medicine has noticeably increased worldwide. One of such plants belonging to genus *Vernonia* and known to have healing potential is *Vernonia cinerea* (Family: Asteraceae). This is an annual plant widely distributed in Bangladesh, India, Sri Lanka and Malay island [1]. This plant is extensively used in indigenous medicine as stomachic and for cold, asthma and bronchitis [2]. The roots of the plant are traditionally used for the treatment of eruptive boils, accidental wounds, and viral fevers. The seeds are used in dysuria [3]. The young leaves are used for the treatment of tonsillitis [4], skin diseases and dysentery in children [5]. Besides, the plant is used in smoking cessation, cough, fever, malaria, urinary calculi, arthritis [6] and leprosy [7]. The plant also possesses antimicrobial [8], antibacterial, anti-inflammatory, analgesic, antipyretic [9], anti-flatulent, antispasmodic [3] and anti-diuretic properties [10].

As part of our ongoing studies with medicinal plants of Bangladesh [11-13] the present study was undertaken to further evaluate the chemical nature of secondary metabolites and analgesic, antipyretic and anti-inflammatory activities of the crude methanol extract of *V. cinerea* as well as to find evidences for its folk uses.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Materials

The whole plant of *V. cinerea* was collected from the Bhatiyari and Pahartoli, Chittagong, Bangladesh in June, 2012. The plant was identified by the experts of Bangladesh Forest Research Institute Herbarium, Chittagong where voucher specimen has been deposited.

#### 2.1.1 Drying and grinding

After collection, the whole plant was washed with running tap water and dried at room temperature not exceeding 50ºC. The dry materials were ground into a coarse powder with the help of a grinder and kept in airtight container in a cool and dark place until extraction commenced.

### 2.1.2 Hot extraction by soxhlet extractor

About 130gm of powder was subjected to hot extraction with 700ml of methanol (99.98%) with a Soxhlet apparatus (Quickfit, England). A gummy residue (yield 16.70%) was obtained after the evaporation of the plant extract with a rotary evaporator (Heidolph, Germany) under reduced temperature and pressure.

### 2.1.3 Chemicals

Standard drugs such as paracetamol, diclofenac sodium, acetyl salicylic acid were obtained from Square Pharmaceuticals Ltd as gift samples. Solvents used in this experiments were of analytical grade and purchased from Merck, Germany.

### 2.1.4 Experimental animals

For the experiment *Swiss albino* mice of either sex, 6-7 weeks of age, weighing between 25-30g, were collected from the Animal Resources Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR,B). The mice were maintained under standard environmental conditions of temperature: (27.0±1.0ºC), relative humidity: 55-65% and 12h light/12hr dark cycle and had free access to ICDDR,B formulated diet and water ad libitum. Appropriate measures were taken to minimize the pain or discomfort of animals and the mice were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee [14].

## 2.2 Preliminary Phytochemical Investigation

For preliminary phytochemical investigation, the crude methanol extract of *V. cinerea* was subjected to various tests to determine the chemical nature of the extract. The presence of alkaloid content was determined by performing Mayer's test; white precipitate (ppt) indicated the presence of alkaloids. The formation of intense yellow coloration upon the addition of few drops of sodium hydroxide and the subsequent loss in color upon the addition of dilute acetic acid indicated the presence of flavonoids. The existence of glycoside in the sample was identified by performing Salkowski's test as well as Libermann-burchard's test; Orange-reddish color at the junction of 2 layers confirms the presence of glycosides. The presence of both the reducing sugar and gums were confirmed by Fehling's test and Molisch's reagent, respectively [15,16].

## 2.3 Test for Antipyretic Activity

The crude extract of *V. cinerea* was subjected to test for the antipyretic activity using Swiss albino mice (25–30g) of either sex. Before the start of experiment the selected healthy animals were acclimatized to laboratory conditions. The animals were randomized into three groups, each group containing seven mice. The normal body temperature of each mouse was recorded using digital clinical thermometer and then by injecting 20% aqueous suspension of Brewer's yeast (10ml/kg, s.c.) pyrexia was induced in all mice [17]. All groups were fast overnight but free access to drinking water was provided. After 24h rectal temperature of each mouse was recorded again. The induction of pyrexia was confirmed by rise in temperature of more than 32.9ºF, while animals showing less than 32.9ºF rise of temperature were excluded from experiment. Group-I received saline (10ml/kg) as a

negative control, group-II received paracetamol (150mg/kg) as a standard drug while the remaining group-III received 500mg/kg body weight of the plant extract, respectively. Rectal temperature was recorded periodically at 1, 2 and 3hr after drugs administration.

## 2.4 Test for Analgesic Activity

The analgesic activity of the crude extract was evaluated using formalin-induced writhing method in mice. Experimental animals (Swiss albino mice) were randomly selected and divided into three groups denoted as group-I, group-II, and group-III consisting of 7 mice in each group. Each group received a particular treatment *i.e.* control, standard and the two doses of the extract. Test samples (about 200 and 400mg/kg body weight of the plant extract), control and diclofenac sodium were given orally by means of a feeding needle. An interval of thirty minutes was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, formalin solution (5%) was administered intraperitoneally to each of the animals of all groups. After an interval of 10mins, which was given for absorption of formalin, number of squirms (writhing) was counted for 5mins [18].

## 2.5 Test for Anti-inflammatory Activity

To determine the anti-inflammatory activity of the methanol extract of *V. cinerea*, 15 clean centrifuge tubes (three for positive control, acetyl salicylic acid, three for negative control, 99.8% ethanol and nine for crude extract) were used. 1.0ml of 5% egg albumin solution was added to all test tubes. Later on 1ml of acetyl salicylic acid (0.1mg), 1ml of methanol and 1ml of crude extract (1000mg/kg) were added to the positive, negative control and test groups, respectively. The pH (5.6±0.2) of all the reaction mixtures was adjusted by 1NHCl. These were heated, cooled and after filtration, the absorbance was measured spectrophotometrically at 660nm [19].

## 3. STATISTICAL ANALYSIS

Results are expressed as the mean±SEM. Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group; p=0.05 was considered as statistically significant.

## 4. RESULTS

## 4.1 Phytochemical Test

The crude extract of *V. cinerea* was found to contain alkaloids, glycosides, flavonoids, reducing sugars and gums (Table 1).

## 4.2 Antipyretic Activity

The experimental mice showed at least an increase of about 34.27ºF in rectal temperature, 18 hrs after Brewer's yeast injection. The crude methanol extract (VCME) exhibited a significant (p=0.05) lowering of mices' body temperature which was elevated by the administration of yeast. These effects were pronounced at the $2^{nd}$ and $3^{rd}$ hr post-treatment with extract. The antipyretic effects of the extract were comparable to that of the standard paracetamol. Results have been summarized in (Table 2).

## 4.3 Analgesic Activity

The results showed that the pain relief was achieved in a dose dependent manner, at all test doses (200 and 400mg/kg, i.p.) as shown in (Table 3). The pain relieving dose 400mg/kg was found to be significantly active in comparison to the standard, diclofenac sodium. Total writhing were 31, 22 at the dose 200 and 400mg/kg, respectively while the standard diclofenac sodium produced 11. The crude extract exhibited 32.61% and 52.17% protection at the dose of 200 and 400mg/kg, respectively as compared to 76.09% exhibited by standard diclofenac Na.

## 4.4 Anti-inflammatory Activity

In the present study for *In-vitro* anti-inflammatory test, the crude extract at the dose of 400µg/ml showed 65.12% inhibition of protein denaturation whereas standard acetyl salicylic acid (ASA) exhibited 76.74% (Table 4). The ability of this extract was found to be significant in inhibiting heat-induced protein denaturation.

**Table 1. Chemical analysis for phytoconstituents in the crude extract of *V. cinerea***

| Examination | Test performed | Results |
|---|---|---|
| | Mayer's test | + |
| | Dragendorff's reagent | + |
| Alkaloids | Wagner's reagent | + |
| | Hager's reagent | + |
| | Tannic acid | - |
| Glycosides | Salkowski test | + |
| | Libermann-Burchared test | + |
| Steroids | Salkowski test | - |
| | Libermann-Burchared test | - |
| Tannins | Ferric chlorides | - |
| | Potassium dichromate | - |
| Flavonoids | Conc. HCl and alcoholic test | + |
| Saponins | Shake test (aq. solution) | - |
| Reducing sugars | Fehling's solution test | + |
| | Benedict's test | + |
| Gums | Molisch's reagent | + |
| (+) = present; (-) absent | | |

**Table 2. Effect of crude extract at 500mg/kg i.p. in yeast-induced pyrexia**

| Drug | Dose (mg/kg) | Rectal temperature in ºF at time (hr) | | | |
|---|---|---|---|---|---|
| | | After administration of drug | | | |
| | | Basal temperature | 1 hr | 2 hr | 3hr |
| Control | 150 | 98.77±1.22 | 98.73±1.19 | 98.77±1.22 | 98.63±1.19 |
| VCME | 500 | 99.00±0.14 | 96.5±0.28 | 95.93±0.11* | 94.5±0.24* |
| Paracetamol | 150 | 98.57±0.88 | 92.5±0.61* | 91.2±0.86* | 92.87±0.57* |

*Seven animals in each group; Values are mean±SEM, * p=0.05 when compared to control, temperature after 18hr of yeast injection and just after sample administration*

**Table 3. Analgesic activity of crude extract 200,400mg/kg in formalin-induced test**

| Animal group | Total writhing | % Writhing | % Protection |
|---|---|---|---|
| Control | 46 | 100 | 0 |
| Diclofenac sodium (25mg/kg) | 11 | 23.91 | 76.09 |
| VCME (200mg/kg) | 31 | 67.39 | 32.61 |
| VCME (400mg/kg) | 22 | 47.83 | 52.17 |

*Here, n=Number of animals= 07*

**Table 4. *In-vitro* anti-inflammatory activity of crude extract and control**

| Test groups | Total inhibition of protein denaturation |
|---|---|
| Control | 00.00±0.0141 |
| Standard ASA (100µg/ml) | 76.74±1.141* |
| VCME (100µg/ml) | 37.98±0.88 |
| VCME (200µg/ml) | 50.00±1.187 |
| VCME (400µg/ml) | 65.12±0.56 |

*SD=Standard deviation, SEM=Standard error of mean, n=7; Total inhibition of protein denaturation=% MIPD ±SEM, *p=0.05*

## 5. DISCUSSION

During the preliminary phytochemical screenings, important therapeutic principles like alkaloids, flavonoids, glycosides, reducing sugar, gums were detected in the crude extract of *V. cinerea*. Therefore, the current findings can be attributed to all or some of these groups of chemical compounds. The results of the present research work also suggested that the crude extract has considerable antipyretic, analgesic and anti-inflammatory effects with a reasonable safety profile. Subcutaneous injection of Brewer's yeast increases the synthesis of prostaglandin in the cell and thereby induces pyrexia. It is a useful test for the screening of plant materials as well as synthetic drugs for their antipyretic effect [20,21]. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators for pyrexia and the inhibition of these mediators are responsible for the antipyretic effect [22]. The subcutaneous administration of the plant extract significantly lowered the rectal temperature of yeast induced febrile mice. Thus it can be postulated that the plant contained pharmacologically active principle(s) that interfere with the release of prostaglandins.

The formalin-induced pain as an experimental model of analgesia is useful for elucidating mechanism of pain and analgesia. Subcutaneous injection of dilute formalin into mice hind-paw produces biphasic nociceptive response namely: the first transient phase is caused by the direct effect of formalin on sensory C-fibers, and the second prolonged phase is associated with the development of the injury induced spinal sensitization, responsible for facilitated pain processing, a central sensitization of the dorsal horn neuron occurs during inflammatory pain [23]. Drugs that act centrally, such as the narcotics, inhibit both phases of formalin-induced pain, while peripherally acting drugs such as aspirin only inhibit the late phases [24]. Results of the present study showed that the crude extract of *V. cinerea* inhibit both the early and late phases of formalin-induced pain, thus suggesting its central and peripheral anti-nociceptive actions. During the *In-vitro* anti-inflammatory test, the crude

extract exhibited a dose dependent activity in anti-inflammatory activity test with 50.0% and 65.12% inhibition of egg albumin induced inflammation at the dose of 200 and 400mg/kg, respectively. The anti-inflammatory activity may be due to the inhibition of release of histamine, serotonin and kinins, prostaglandin-like substance. The anti-inflammatory effects of the plant can be attributed to the phytochemical constituents in the extract of *V. cinerea* such as tannins, saponins and steroids. Tannins function mainly as an astringent. Further study is needed with *V. cinerea* to find the exact mechanism of action for its antipyretic, analgesic and anti-inflammatory effects and to isolate the active molecules.

## 6. CONCLUSION

It can be concluded that the methanol extract of *V. cinerea* has moderate antipyretic, analgesic and anti-inflammatory activities in mice model and this strongly supports the ethno-pharmacological uses of this plant as antipyretic, analgesic and anti-inflammatory agent. The role of alkaloids, flavonoids and triterpenoids needs to be evaluated in future studies.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

Not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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