INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization, medicinal plants are the best source to obtain a variety of drugs. In developed countries 80% of individuals use traditional medicine from which variety of compounds can be derived. Therefore, such plants should be investigated for better understanding of their properties, safety and efficiency.

Thousands of chemical compounds with different biological activities are produced from higher plants. Medicinal plants contain biologically active ingredients which have various effects. These active ingredients representing the value ‘in use’ are produced by biological synthesis in the plant in very small concentrations of the dry material content of the plant. Some of these active ingredients accumulate in certain parts of the plant. Hence, it is only those portions of these plants that contain active ingredient that are used in therapeutic purposes. The part that contains the active ingredient is taken in the form of extract, infusion and decoction. In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization.

On the other hand, since long time medicines of plant origin are used as vegetable by the tribals of Tripura. The leaves of the plant possess antimicrobial, antidiabetic, antipyretic, antiinflammatory, anti-spasmodic, cathartic and expectorant activities. It is informed that on fractionation of bioactive crude ethanolic extract led to the isolation of three pure fractions from which one was characterized as 4-hydroxy-3-methoxybenzaldehyde.

C. grandis is a dioecious perennial herbaceous vine. Its stems are mostly glabrous, produced annually from a tuberous rootstock, tendrils simple, axillarily and leaves are alternate, simple, blade broadly ovate, 4 lobed [(5-9) × (4-9)] cm, acute and mucronate at the apex, cordate with a broad sinus at the base. The native range of C. grandis extends from Africa to Asia including India, Philippines, China, Indonesia, Malaysia, Thailand, Vietnam, Eastern Papua, New Guinea and Northern Territories (Australia).

EXPERIMENTAL

Sample preparation: The leaf of Coccinia grandis were collected from Agartala in the month of May, 2010 and were shed dried for 7 days. Since certain compounds get denatured in sunlight, it is dried under shade to avoid decomposition. The dried leaves were then crushed to fine powder. The powdered plant materials (100 g) were defatted with petroleum ether. After washing with petroleum ether the residue were...
extracted exhaustively with 100 mL distilled methanol by using soxhlet apparatus. The extract was filtered through cotton followed by vacuum suction.

**Separation:** Components present in the extract of *Coccinia grandis* were separated by using a column chromatography. For separating the components of *Coccinia grandis* Petroleum ether (40-60 °C) was used as the mobile phase and silica gel for column chromatography as stationary phase. Separated parts were collected in individual beakers and solvents were allowed to evaporate at room temperature. The solids were collected and processed further. The size of each column was of 31 cm. The colour of the components and volume obtained is given in Table-1.

### Table 1: Components Separated of the Extract of *Coccinia grandis*

| Components separated (Eluted) | Colour          | Volume collected (mL) | Weight (mg) |
|------------------------------|-----------------|------------------------|------------|
| CG (1<sup>st</sup>)          | Yellow          | 180.0                  | 7.6        |
| CG (2<sup>nd</sup>)          | Deep green      | 15.0                   | 0.7        |
| CG (3<sup>rd</sup>)          | Brownish green  | 145                    | 6.3        |
| CG (4<sup>th</sup>)          | Brown           | 120                    | 6.0        |
| Absorbed                     | –               | 20                     | –          |

Melting point of all compounds were determined in open capillaries by melting point apparatus (Melting Point Apparatus, Indo, M-AB-92) and are uncorrected. The I.R. spectra were recorded (in Affinity-1 Fitr spectrophotometer IR solution Version 1.50SU Shimadzu Corporation) in KBr pellets. 13<sup>C</sup>NMR were run on Mercury- 400BB in CDCl<sub>3</sub>, while mass spectra was performed in Agilent 7890 GC coupled with 5975 MS.

**Screening of analgesic activity** (Acetic acid induced writhing in mice)<sup>11</sup>

Albino mice of either sex (weighing 25-30 g) were used as per experimental protocols approved by Institute of Bioresources and sustainable development (IBSD), Department of Biotechnology, Govt. of India, Imphal, Manipur, India. The animals were housed under standard environmental condition (25 ± 2 °C) and relative humidity (50 ± 5 %) and fed with standard diet and water *ad libitum*. The animals were acclimatized to laboratory environment for a period of 14 days before performing the experiments.

The mice were divided into four groups of five animals each. The first group comprised the control. The remaining four groups were administered test doses. The test doses were prepared in distilled water to get the desired concentration of the extract and the separated compounds.

Acetic acid (1 % v/v, 10 mL/kg) was injected into the peritoneal cavities of mice, which were placed in a large plastic tray and the intensity of nociceptive behaviour was quantified by the number of writhes was counted for 10 min beginning from 5 min after the acetic acid injection. Test drugs and control vehicle were administered 0.5 h before the acetic acid injection. The writhing response consists of a contraction of the abdominal muscle turning of trunk (twist) together with a stretching of the hind limbs. The antinociceptive activity was expressed as the writhing scores over 20 min. Per cent inhibition of writhing was calculated using the relation:

Inhibition of writhing (%) = \(100 \left[ 1 - \frac{\text{Mean writhing number of treated mice}}{\text{Mean writhing number of control mice}} \right]\)

Results are given in Table-2.

**Table 2: Number of Writhing Response and % of Inhibition Shown by Methanolic Extract of Leaf of *Coccinia grandis* CG, and Its Separated Component (CG<sub>n</sub>)**

| S. No. | Animal No. | Treatment | Dose | Number of writhing (in 10 min duration) | Responders (n/n) | Mean of writhing ± standard error mean (SEM) | Inhibition (%) |
|--------|------------|-----------|------|-----------------------------------------|------------------|--------------------------------------------|---------------|
| 1      | 1          | Control water + (acetic acid) | 10 mL/kg + 0.1 mL/kg | 41 | 5/5 | 45.4 ± 1.86 | 0 |
|        | 2          |           |      | 41 | 41/45 | 41 | 47 | 50 |
|        | 3          |           |      | 48 | 48/50 | 48 | 79.73 | 79.73 |
|        | 4          |           |      | 12 | 47 | 12 | 9.2 ± 1.02 | 79.73 |
|        | 5          |           |      | 07 | 47 | 07 | 9.2 ± 1.02 | 79.73 |
| 2      | 1          | Aspirin Std. + (acetic acid) | 100 mg/kg + 10 mL/kg | 11 | 5/5 | 9.2 ± 1.02 | 79.73 |
|        | 2          |           |      | 09 | 5/5 | 9.2 ± 1.02 | 79.73 |
|        | 3          |           |      | 07 | 5/5 | 9.2 ± 1.02 | 79.73 |
|        | 4          |           |      | 12 | 5/5 | 9.2 ± 1.02 | 79.73 |
|        | 5          |           |      | 07 | 5/5 | 9.2 ± 1.02 | 79.73 |
| 3      | 1          | Sample CG<sub>1</sub> + (acetic acid) | 100 mg/kg + 10 mL/kg | 14 | 5/5 | 13.2 ± 1.02 | 70.92 |
|        | 2          |           |      | 16 | 5/5 | 13.2 ± 1.02 | 70.92 |
|        | 3          |           |      | 10 | 5/5 | 13.2 ± 1.02 | 70.92 |
|        | 4          |           |      | 12 | 5/5 | 13.2 ± 1.02 | 70.92 |
|        | 5          |           |      | 14 | 5/5 | 13.2 ± 1.02 | 70.92 |
| 4      | 1          | Sample CG<sub>2</sub> + (acetic acid) | 100 mg/kg + 10 mL/kg | 12 | 5/5 | 14.20 ± 0.97 | 68.72 |
|        | 2          |           |      | 13 | 5/5 | 14.20 ± 0.97 | 68.72 |
|        | 3          |           |      | 17 | 5/5 | 14.20 ± 0.97 | 68.72 |
|        | 4          |           |      | 13 | 5/5 | 14.20 ± 0.97 | 68.72 |
|        | 5          |           |      | 16 | 5/5 | 14.20 ± 0.97 | 68.72 |
groups 1-3 and 4 received 200 mg/Kg of compound \( Y_1 \) and extract \( Y_2 \) by oral route. The positive control received (2nd group) standard diclofenac sodium (8 mg/Kg P.O.) by oral route. All the suspensions were administered 1 h before the injection of carrageenan (0.1 mL of 1 % w/v). The hind paw volume was measured plethysmometrically before and after the carrageenan injection at hourly intervals for 5 h and percentage inhibition of inflammation was calculated using the relation:

\[
\text{Inhibition of oedema (\%)} = 100 \left[ 1 - \frac{\text{Mean paw volume of treated rats}}{\text{Mean paw volume of control rats}} \right]
\]

Results are given in Tables 3 and 4.

### RESULTS AND DISCUSSION

Sample \( CG_1 \) showed average writhing of 13.20 ± 1.02, while its extract, \( CG_1 \) had little more than that of \( CG_1 \), of 14.20 ± 0.97. Aspirin, which has average writhing of 9.2 ± 1.02. On calculating the percentage inhibition of the separated component, \( CG_1 \), the extract, \( CG_1 \), it was found to be 70.92 % for \( CG_1 \) and 68.72 % for the extract \( CG_1 \). The percentage of inhibition for \( CG_1 \) was found to be near to that of aspirin which has percentage of inhibition as 79.73 %.

It was observed that the paw volume started decrease after 3 h in case of the standard diclofenac sodium, but this activity was observed only 4 h in case of the component \( CG_1 \) and the extract \( CG_1 \). The paw volume reduction continued even after 4 h just as it was found for diclofenac sodium. The percentage of protection of the component \( CG_1 \) was little more than the extract \( CG_1 \). The percentage of protection found for component \( CG_1 \) was 35.84 % and that of the standard 45.28 %, but considering that \( CG_1 \), a natural product and the standard, diclofenac sodium, a synthetic compound, the percentage of protection of \( CG_1 \), can be considered to be better than that of the standard diclofenac sodium. Therefore the structure of \( CG_1 \) compound were elucidated as under.

The activities not found significant of the fractionated components were not reported here. The most active compound \( CG_1 \) was subjected for structural elucidation. The melting point of the \( CG_1 \) compound was found to be 87 ºC. This was also showing positive alkaloidal qualitative test. In the IR spectral studies in KBr pellets, the peak at 1635 cm\(^{-1}\) can be of aromatic conjugated system. The peak at 1384 cm\(^{-1}\) is due to C-C=C-C stretching. The peak at 1298 cm\(^{-1}\) is due to C-N stretching of 3º aromatic amine in ring. The peak 1159 cm\(^{-1}\) is due to C=O stretching. The peak at 1483 cm\(^{-1}\) is due to C=O stretching in arylic conjugated system. =C=C=C-C stretching.

### TABLE 3

| Treatment | 0 h | 1 h | 2 h | 3 h | 4 h | 5 h |
|-----------|-----|-----|-----|-----|-----|-----|
| Normal control (1 mL dist. Water P.O.) | 0.30 | 0.40 | 0.60 | 0.65 | 0.65 | 0.55 |
| | 0.20 | 0.30 | 0.45 | 0.55 | 0.40 | 0.40 |
| | 0.30 | 0.35 | 0.55 | 0.60 | 0.60 | 0.55 |
| Standard diclofenac sodium (8 mg/kg P.O.) | 0.20 | 0.30 | 0.45 | 0.50 | 0.50 | 0.35 |
| | 0.20 | 0.35 | 0.35 | 0.30 | 0.30 | 0.30 |
| Sample CG1 + (acetic acid) | 0.10 | 0.30 | 0.30 | 0.40 | 0.60 | 0.50 |
| | 0.20 | 0.30 | 0.40 | 0.60 | 0.50 | 0.30 |
| Sample CG1 + (acetic acid) | 0.10 | 0.30 | 0.50 | 0.70 | 0.60 | 0.40 |
| | 0.20 | 0.40 | 0.60 | 0.60 | 0.40 | 0.30 |
| | 0.10 | 0.30 | 0.50 | 0.70 | 0.60 | 0.50 |
| | 0.20 | 0.40 | 0.60 | 0.60 | 0.40 | 0.30 |

### TABLE 4

| Treatment | 0 h | 1 h | 2 h | 3 h | 4 h | 5 h | Protection (%) |
|-----------|-----|-----|-----|-----|-----|-----|----------------|
| Normal control (1 mL dist. Water P.O.) | 0.26±0.024 | 0.38±0.025 | 0.55±0.027 | 0.64±0.037 | 0.56±0.043 | 0.53±0.034 | 0 |
| Standard diclofenac sodium (8 mg/kg P.O.) | 0.24±0.024 | 0.38±0.054 | 0.42±0.025 | 0.40±0.041 | 0.32±0.033 | 0.28±0.024 | 45.28 |
| Sample CG1 + acetic acid | 0.16±0.24 | 0.34±0.024 | 0.46±0.031 | 0.58±0.049 | 0.48±0.037 | 0.34±0.024 | 35.84 |
| Sample CG1 + acetic acid | 0.16±0.024 | 0.36±0.024 | 0.54±0.032 | 0.60±0.032 | 0.48±0.037 | 0.36±0.039 | 32.75 |
In the $^1$H NMR spectral analysis signal near 8.491 (singlet) is for $^1\text{CH}$ proton, peaks at 7.996, 7.963, 7.931 (multiplet) is for $^{11}\text{CH}$, $^{12}\text{CH}$, $^{13}\text{CH}$ protons, peaks at 3.486, 3.194, 3.161 (multiplet) is for $^{15}\text{CH}$, $^{16}\text{CH}$, $^{17}\text{CH}$ protons. Peak at 3.152 (singlet) is for $^8\text{CH}$ and peak at 2.500 (singlet) is for $^4\text{CH}$ proton.

In the $^{13}$C NMR spectra, the peak around 78.754 is due to $^{11}\text{C}$, $^{12}\text{C}$, $^{13}\text{C}$, the peak around 78.624 is due to $^1\text{C}$, $^2\text{C}$, $^3\text{C}$. The peak around 78.426 is due to $^5\text{C}$ and the peak around 78.098 is due to $^7\text{C}$. The peak around 39.919 is due to $^9\text{C}$ and $^{10}\text{C}$, the peak around 39.714 is due to $^8\text{C}$, at 39.500 is due to $^4\text{C}$. The peak at 39.294 is due to $^{15}\text{C}$, $^{16}\text{C}$, $^{17}\text{C}$, the peak at 39.081 is due to $^{14}\text{C}$.

From the above spectral analysis, the proposed structure is

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Further it was confirmed by mass spectral analysis. In mass spectral analysis the compound showed a molecular ion peak (M + 1)$^+$ at m/z 276. The base peak (M + 2)$^+$ was recorded as m/z 137 for C$_{9}$H$_{13}$N$^+$, hence the possibility of N in 4º- form.

The bioactive alkaloidal compound isolated from methanolic extract of *Coccinia grandis* was found to be 1-tert-butyl-5,6,7-trimethoxyisoquinolene.

**Conclusion**

1-tert-Butyl-5,6,7-trimethoxyisoquinolene, a natural alkaloid isolated newly from methanolic extract of *Coccinia grandis*, shows a better analgesic activity and antiinflammatory activity. The antimicrobial activity, analgesic activity and anti-inflammatory activity as shown by the plant extract may be due to the isolated alkaloid and further work is necessary to draw a clear cut conclusion.

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