PHYTOCHEMICAL SCREENING AND ANALGESIC ACTIVITY OF SYRUP FORMULATED FROM *Ageratum conyzoides*, *Curcuma longa*, AND *Chromolaena odorata* ETHANOLIC CRUDE EXTRACT

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Abstract

The study aimed to determine the phytochemicals present in the ethanolic crude extracts of *Ageratum conyzoides*, *Curcuma longa*, and *Chromolaena odorata* and to evaluate the analgesic activity of the formulated syrup in Swiss mice using the hot plate method. These plants are known for local folks for its wound-healing effect and used in local traditional medicine for the management of pain and inflammation. The syrup was formulated from each extract and administered intraperitoneally at a dose of 15 mg/kg using paracetamol as a standard. Phytochemical screening of each extract revealed the presence of terpenoids, flavonoids, saponins, tannins, alkaloids, cardiac glycosides, glycosides, steroids, resins, coumarins, and phenols. Analgesic activity of the three formulated syrup differs significantly (p<0.05) with the positive and negative control. Each formulated syrup attains its peak activity at 150 minutes. *C. longa* syrup produced maximum possible analgesia (MPA) of 15.77% while paracetamol achieved a peak MPA of 31.07%. The presence of biologically active compounds, particularly alkaloids, flavonoids, tannins, and saponins can be the contributory factors in the induction of analgesia.

**Key Words:** analgesic activity, *Ageratum conyzoides*, *Curcuma longa*, *Chromolaena odorata*, and syrup
Introduction:

Pain is a feeling activated in the nervous system that can range from mild to intense, may come and go or constant (Ashburn, 2013; Fusee & Vargas-Schaffer, 2012; Morriss & Goucke, n.d.). People can experience pain in many different ways obtained from various causes, and likewise, it can help diagnose a particular disease. Without pain, the body might be seriously hurt without knowing it or might not realize that a medical problem needs treatment. Treatment of pain varies depending on its cause. From massage therapy and meditation to pain relievers, acupuncture, and sometimes, surgery can help in the management of pain (MedlinePlus, 2018). Agents like analgesic can relieve pain by acting to the central nervous system. The pain medicine available in the market has its benefits and risks. Some types of pain respond better to a different kind of medication (Ashburn, 2013; Morriss & Goucke, n.d.) Pain, as well as inflammation, are common non-specific manifestations of diseases. Non-steroidal anti-inflammatory drugs and opiates have been used to manage the conditions, but there are some adverse reactions occur with these drugs such as renal damage, gastrointestinal disturbances and possible dependence on these drugs (Morriss & Goucke, n.d.). Today, there has been an increasing interest to find a novel anti-inflammatory and analgesic drugs with possibly fewer side effects from the natural sources.

The utilization of plant extracts with known anti-inflammatory and analgesic properties have an enormous implication in treatments. Folkloric uses of herbal plants take place in the modern treatment of infections. Leaves, barks, and roots were used as a decoction for washing of wounds, cuts, and bruises (Dhal, Panda, & Muduli, 2015; Namukobe et al., 2011). Active constituents were being determined to ascertain the object constituents that exert anti-inflammatory and analgesic properties. Identification and isolation of bioactive components were also employed in research of plants to rationalize the claims of old practices. Various studies had been conducted to prove the efficiency of the plant extracts. These plant extracts play a vital role in developing new alternative drugs in pharmaceutical industries to overcome the drawbacks possessed by the synthetic drugs. Nowadays, much attention is focused on natural products as a strategy in combating antibiotic resistance due to its high therapeutic effect and low adverse side effect. The mission of the Philippine Board of Pain Medicine is to professionalize the practice of pain medicine in the country, and this involves scientific evidence and professionals who are advocates in pain medicine (Philippine Board of Pain Medicine, 2013). In today's world, many are embracing the use of alternative drugs and the incorporation of folkloric practices.

_Ageratum conyzoides, Curcuma longa, and Chromolaena odorata_ were locally and readily available plants which have been used traditionally by faith healers to manage the painful and inflammatory condition. These plants are essential due to their potential sources of effective remedies for managing pain. The present study was done to determine the analgesics activity of the syrup formulated from the ethanolic extracts of the said plants in Swiss mice using the hot plate method. The utilization of these plants extract would provide cheaper alternative medicine for managing pain and inflammation. The syrup formulated from these plants would lessen the drawback of using synthetic analgesic syrup with adverse side effects to humans and the environment.

Material and methods

Preparation and Extraction of Plant Sample

The preparation and extraction of plant sample were carried out as described by Claustro et al., 2005; Tiwari, Kumar, Kaur, Kaur, & Kaur, 2001; Zakaria et al., 2014. _A. conyzoides, C. longa, and C. odorata_ were obtained from the town of Manapla, Negros
Occidental. The leaves of *A. conyzoides* and *C. odorata* and *C. longa* rhizomes were utilized; washed with running water to remove the unwanted debris and put it a screen bag for air drying. The ethanolic extract was prepared by cold extraction technique. About 400 g of each sample were macerated with 95% ethanol for 48 hours at room temperature. The mixture was then filtered, and the collected filtrates were concentrated under reduced pressure and temperature using the rotary evaporator. A flame test was conducted to ensure that the solvent was evaporated from the extract. The extract was further dried to semi-solid form using the same evaporator for syrup formulation.

**Phytochemical Screening of the Ethanolic Crude Extract**

Phytochemical screening of the ethanolic crude extracts of the sample was carried out using the test tube method as described (Claustro et al., 2005; Kumar Bargah, 2015; Suman Kumar, Venkateshwar, Samuel, & Rao, 2013; Tiwari et al., 2001; Zakaria et al., 2014). Test reaction was interpreted as positive (+) for the presence of the compound and negative (-) for the absence or undetected.

**Detection of Terpenoids** – extracts were dissolved in 2 mL of chloroform and evaporated to dryness over the water bath. The dried residue was added with two mL of sulfuric acid and heated for about two minutes. The reddish color formation indicates the presence of terpenoids.

**Detection of Flavonoids** - Detection of flavonoids was carried out using the lead acetate test. The extract of each sample was treated with a few drops of lead acetate solution. Formation of a yellow-colored precipitate indicates the presence of flavonoids.

**Detection of Saponins** - Detection of saponins was carried out using froth test. Each extract was diluted with deionized water to 20 mL and shaken in a test tube for 15 minutes. Formation of a one cm layer of foam indicates the presence of saponins.

**Detection of Tannins** - Detection of tannins was carried out using the gelatin test. The extract was added with 1% gelatin solution containing sodium chloride. Formation of a white precipitate indicates the presence of tannins.

**Detection of Alkaloids** - each plant extract was dissolved in dilute hydrochloric acid and filtered. The filtrate was mixed with potassium mercuric iodide (Mayer's reagent). Formation of a yellow-colored precipitate indicates the presence of alkaloid.

**Detection of Cardiac glycosides** – about ten mL of each extract was mixed with a glacial acetic acid, and a drop of FeCl$_3$ (2.0% solution) added with one mL concentrated sulfuric acid. A brown ring formed between the layers shows a positive result.

**Detection of Glycosides** - Extracts were hydrolyzed with dilute hydrochloric acid and subjected to test for the presence of glycosides using modified Borntrager’s test. Each extract was treated with ferric chloride solution and immersed in boiling water for about five minutes. The mixture was cooled and extracted with an equal volume of benzene, and the benzene layer was treated with an ammonia solution. Rose-pink color formation in the ammonical layer indicates the presence of anthranol glycosides.

**Detection of Steroids** – about two mL of each plant extract was dissolved in 2 mL of acetic anhydride and treated with concentrated sulfuric acid and acetic acid. The formation of a greenish color indicates the presence of steroids.
Detection of Resins – about two mL of the plant extract were treated with 2-3 drops of acetic anhydride solution and two mL sulfuric acid. Formation of orange to yellow color gives a positive result for resins.

Detection of Coumarins – about two mL of each plant extracts were taken in a test tube covered with filter paper which was previously treated with 1 N sodium hydroxide. The test tube was placed in boiling water for a few minutes. The filter paper was removed and examined in UV light. The presence of yellow fluorescence indicates the positive result for coumarins.

Detection of Phenols - Detection of phenols was carried out using the ferric chloride test. Each extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish-black color indicates the presence of phenols.

Formulation of Syrup

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant extract</td>
<td>40 g</td>
</tr>
<tr>
<td>Citric acid (hydrous)</td>
<td>2.1 g</td>
</tr>
<tr>
<td>Peppermint spirit</td>
<td>2 mL</td>
</tr>
<tr>
<td>Sucrose</td>
<td>825 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 mL</td>
</tr>
</tbody>
</table>

Forty grams of the plant extract was mixed with 2.1 g citric acid, 2 mL of peppermint oil, ½ part of sucrose, and ½ part of the purified water in a bottle shaking machine. Filter the solution. The remaining half of sucrose was dissolved in the clear filtrate using the bottle shaking machine. The solution was transferred to the graduated cylinder and add enough distilled water to the desired volume. Mixed and filtered the solution and stored in a sterile bottle and attached the label.

Test Animals

The experiment utilized Swiss albino mice weighing 25–30g and 8–10 weeks’ old of both sexes were obtained from the Animal Pet Shop, Kalibo, Aklan and were kept under room temperature (27 ± 2°C; 12 h light/darkness cycle) in the Animal Holding Unit of St. Gabriel College, Kalibo, Aklan. The mice were given with food and water freely in 7 days for proper acclimatization. The mice were handled and followed the current guidelines for laboratory animal care and the ethical guidelines for investigations of experimental pain in conscious animals (Institutional Animal Care and Use Committee). All experiments were performed between 9:30 AM to 12:00NN.

Analgesic Activity (Hot Plate Test)

The evaluation of the analgesic activity of each formulated syrup was carried out using the hot plate method (Claustro et al., 2005; Fan, Ali, & Basri, 2014a). The temperature of the metal surface was set at 50°C +/- 2°C. The mice were pretreated with distilled water (negative control), 15mg/kg paracetamol (positive control), or syrup formulated from the extracts of *A. conyzoides*, *C. longa*, and *C. odorata* (15 mg/kg). Sixty minutes after the respective test solution administration, the mice were placed on the heated metal surface, and the latency to a discomfort reaction was recorded. The reaction time or latency period was determined as the time taken for the mice to react with heat by licking their paws or jumping. The cut-off time of 20 seconds was chosen to avoid tissue injury of the paws. Latency was recorded before (0min) and 60min,
90 min, 120 min, 150 min, 180 min, and 210 min following oral administration of the treatments. The prolongation of the latency times compared with the values of the controls was used for statistical comparison (Zakaria et al., 2014). The maximum possible analgesia (MPA) was calculated using the formula (Fan, Ali, & Basri, 2014b):

\[
MPA = \frac{\text{Reaction time for treatment} - \text{reaction time for distilled water}}{20 \text{ seconds} - \text{reaction time for distilled water}}
\]

**Statistical Analysis**

Data were presented as mean ± standard error mean (SEM) using the software SPSS20.0 version. A statistically significant difference between groups was calculated using the Analysis of variance (ANOVA) followed by LSD. Level of significance was at \(p<0.05\).

**Results and discussion**

Table 1 shows the result of the phytochemical screening of ethanolic crude extracts of *A. conyzoides*, *C. longa*, and *C. odorata* using the test tube method. Bioactive constituents with positive remarks are present in each ethanolic crude extracts of *A. conyzoides*, *C. longa*, and *C. odorata*. The value of medicinal plants lies on the bioactive or phytochemical components that cause a definite pharmacological action in humans (Naz & Bano, 2013; Shojaii, Motevalian, & Rahnama, 2015). Alkaloids, as one of the bioactive components common to both ethanolic crude extracts of *A. conyzoides*, *C. longa*, and *C. odorata*, are known for its pharmacological applications as anesthetics, and central nervous system stimulants, it possessed an antibacterial property, and anticancer agent. Conversely, flavonoids derived from plants possess several health effects, including anti-inflammatory, antioxidant, antiallergic, anticarcinogenic, antibacterial, and hepatoprotective, among others. The flavonoids has protective effects in biological systems which include apoptosis, detoxification of enzymes, protection of genomic vitality and among others which may be useful as dietary compounds for the prevention of cancer and degenerative disease (Al Shaikh Hamed & Al Omari, n.d.; Cushnie & Lamb, 2005; Gul, Jan, Faridullah, Sherani, & Jahan, 2017; Kamboh et al., 2015; Naz & Bano, 2013; Tapas, Sakarkar, & Kakde, 2008).

**Table 1. Phytochemical Screening of Ethanolic Crude Extracts A. conyzoides, C. longa, and C. odorata**

<table>
<thead>
<tr>
<th>Secondary Metabolites</th>
<th>Reagents</th>
<th>Positive Results</th>
<th>Experimental Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>A. conyzoides</em></td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Sulfuric Acid</td>
<td>Reddish-brown</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lead acetate</td>
<td>Yellow</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Distilled water</td>
<td>Frothing with water and emulsion with oil Brown</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>1% gelatin solution</td>
<td>Green to Blue-black</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Mayer’s reagent</td>
<td>Cream with Mayer’s reagent Brown ring</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Sulfuric</td>
<td>Brown ring</td>
<td>+</td>
</tr>
</tbody>
</table>
The analgesic activity of syrup formulated from *A. conyzoides*, *C. longa*, and *C. odorata* extracts using the hot plate method are shown in Table 2. The results showed that there was no significant difference in the thermal response of Swiss mice treated with distilled water (control) from 0 to 210 minutes. A significant difference exists in the thermal stimulus of Swiss mice treated with paracetamol, *A. conyzoides* syrup, *C. longa* syrup, and *C. odorata* syrup. Increase in reaction time was observed from each formulated syrup and paracetamol. The mean latency time for the response of Swiss mice is higher in paracetamol, followed by *C. longa* syrup, *C. odorata* syrup, and *A. conyzoides* syrup. Paracetamol, *C. longa*, and *C. odorata* syrup have a greater reaction time from 150 to 180 minutes, while *A. conyzoides* syrup has a higher reaction time between 120 to 150 minutes. Each formulated syrup possessed an analgesic activity.

### Table 2: Reaction time in seconds (mean ± SEM)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
<th>210 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water)</td>
<td>7.20±0.034</td>
<td>7.61±0.045</td>
<td>7.91±0.075</td>
<td>7.87±0.042</td>
<td>7.51±0.032</td>
<td>7.43±0.030</td>
<td>7.17±0.047</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>7.39±0.032</td>
<td>8.60±0.071</td>
<td>9.35±0.072</td>
<td>10.14±0.026</td>
<td>11.39±0.011</td>
<td>12.43±0.008</td>
<td>11.76±0.011</td>
</tr>
<tr>
<td><em>A. conyzoides</em> syrup</td>
<td>7.00±0.041</td>
<td>7.63±0.112</td>
<td>8.15±0.173</td>
<td>8.63±0.085</td>
<td>9.20±0.040</td>
<td>8.43±0.072</td>
<td>7.60±0.081</td>
</tr>
<tr>
<td><em>C. longa</em> syrup</td>
<td>7.23±0.031</td>
<td>8.00±0.054</td>
<td>8.59±0.152</td>
<td>9.49±0.110</td>
<td>10.38±0.014</td>
<td>11.79±0.018</td>
<td>10.94±0.012</td>
</tr>
<tr>
<td><em>C. odorata</em> syrup</td>
<td>7.09±0.036</td>
<td>7.63±0.091</td>
<td>8.54±0.081</td>
<td>8.68±0.089</td>
<td>9.48±0.223</td>
<td>9.62±0.194</td>
<td>8.53±0.064</td>
</tr>
</tbody>
</table>

All t-test values are significant at p < 0.05, and SEM = standard error mean.

Figure 1 illustrates the analgesic effect of paracetamol, syrup formulated from the ethanolic crude extracts of *A. conyzoides*, *C. longa*, and *C. odorata* using the maximum...
possible analgesia. Paracetamol (positive control) elicited a significant analgesic effect 60 minutes after the administration with a gradual increase up to 180 minutes and started to decline at 210 minutes. The same trend was observed in *C. longa* and *C. odorata* syrups except that *C. odorata* syrup elicited analgesic effect at 90 minutes. Each formulated syrup attains its peak activity at 150 minutes. *C. longa* syrup produced maximum possible analgesia (MPA) of 15.77% while paracetamol achieved a peak MPA of 31.07%. The MPA value for each formulated syrup.

**Figure 1.** The maximum possible analgesia (MPA) (%) representing the effect of each formulated syrup compared to paracetamol (positive control) administered into Swiss mice using the hot plate method.

*A. conyzoides* are utilized in traditional medicine by different cultures in the world and valuable agricultural resource as a natural biocide or agent for pest management and contain potential analgesic and anti-inflammatory activities (Chau Ming, 1999). The water-soluble fraction of *A. conyzoides* was able to decrease the paw elevation time significantly with doses of 30 and 50 mg/kg i.p. or 90 and 150 mg/kg p.o. (Magalhães et al., 1997). Alcoholic extracts of *A. conyzoides* and *Emilia sonchifolia* were compared in their analgesic effects in an acetic acid-induced writhing model and formalin-induced licking model of Swiss albino mice. Results showed that extracts inhibited 49.85 and 39.47% of acetic acid-induced pain at the highest dose 2.0 g/kg body weight and statistically significant as compared to morphine (0.5 mg/kg). The analgesic effect concerning writing response was significant at all doses of the extracts, but the activity was less compared to diclofenac sodium, and *A. conyzoides* extract was found more effective than *E. sonchifolia* (Bin Emran et al., 2012; Rahman et al., 2012). Anti-inflammatory activity of *A. conyzoides* methanolic extract depends on the flavonoid fraction that produces a protective action against free radical-mediated damage in cells and tissue (Galati et al., 1994)

Curcumin found in *C. longa* has been demonstrated to be safe in six human trials for its anti-inflammatory activity (Chainani-Wu, 2003). Analgesic activity of ethanolic crude extract of *C. longa* was administered to Winster rats at a dose of 400 mg/kg found to be comparable with aspirin using tail immersion test and hot plate test (Jogdand & Bhattacharjee, 2017; Neha, Ranvir, & Jangade, 2009); the same method was used in administering the hydroalcoholic extract of *C. longa* rhizomes in albino rats and showed significant activity and comparable with pentazocine (Bin Emran et al., 2012; Chowdhury, Swain, Dey, & Rao, 2017).

Considered as tropical weed, *C. odorata* exhibits an analgesic, anti-inflammatory, hemostatic, and other numerous relevant medicinal properties which are used to treat various ailments including the wound healing effect (Pandith et al., 2013; Vijayaraghavan, Rajkumar, Bukhari, Al-Sayed, & Seyed, 2017). Analgesic effect *C. odorata* aqueous extract is more effective than paracetamol with a difference of inhibition of 24.15% at the dose of 400 mg/kg and 27.56% at the dose of 800 mg/kg of
the aqueous extract in acetic acid-acid-induced writhing pain. Likewise, its aqueous extract (800 mg/kg) is comparable with tramadol against neurogenic and inflammatory pain in 2.5% formaldehyde induced pain (Itou, Ossibi, Ntandou, & Ngouabi, 2017).

Phytochemicals like alkaloids, flavonoids, steroids, and tannin have been isolated from several medicinal plants which possess a significant analgesic activity (Sengupta, Sheorey, & Hinge, 2012). The phytochemical screening of the ethanolic crude extract of *A. conyzoides*, *C. longa*, and *C. odorata* was positive for the said bioactive components. The presence of these compounds might have attributed to the analgesic activity of the formulated syrup.

This preliminary study did not adequately demonstrate the dose-dependent analgesic effect of the three formulated syrups since they were not tested on different formulated concentrations, which is considered as one of the limitations of this study. Future studies will be done on the formulation of different levels of concentrations of the extract and the use of other animal models and compare with different methods other than the hot plate to confirm the effectiveness of the analgesic activity.

**Conclusions**

It can be concluded that the syrup formulated from the ethanolic crude extracts of *A. conyzoides*, *C. longa*, and *C. odorata* displayed analgesic activity and supported the traditional use of these plant extracts in managing pain and inflammation. It is recommended that isolation of the bioactive components present in each extract and elucidation of the mechanisms involved for the better analgesic activity of syrup. Further study on fractional extraction using solvents of different polarities, use of instrumental methods for confirmatory detection of phytochemical components obtained from the test tube methods, utilization of different parts of *A. conyzoides*, *C. longa*, and *C. odorata* plant for testing the analgesic activity and in the different administration of concentration in test animals were also recommended.

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**References**


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