Antibacterial effect of *Chromolaena odorata* (Awolowo Leaf) aqueous leaf extract on *Pseudomonas aeruginosa* induced gastrointestinal tract infection in adult Wistar rat

Wisdom Nzubechukwu Obiefu *, Nnaemeka Jireh Okolie, Chizaram Winners Ndubueze and Joy Nkeiruka Dike-Ndudim

Department of Medical Laboratory Science, Faculty of Health Sciences, Imo State University, Owerri, Nigeria.

GSC Biological and Pharmaceutical Sciences, 2021, 14(01), 055–064

Publication history: Received on 02 January 2021; revised on 09 January 2021; accepted on 10 January 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.14.1.0006

Abstract

The effect of *Chromolaena odorata* aqueous leaf extract on *Pseudomonas aeruginosa* induced gastrointestinal tract infection in adult rats was studied using thirty six (36) adult wistar rats, whose weights range from 72-100g. The rats were allowed to acclimatize for two weeks and checked for fitness through weight check and urinalysis. Twelve rats were used for toxicological study (LD<sub>50</sub>) while 24 rats were used to determine the antibacterial effects of *Chromolaena odorata* leaf extract. The 12 rats for LD<sub>50</sub> were divided into three groups of four rats each while the other 24 rats were divided into four groups of six rats each. The antibacterial effect of *Chromolaena odorata* extract was significantly high in group Q after treatment (4.5±2.12 Cfu/ml) when compared with the mean value before treatment (20.0±4.24 Cfu/ml). The antibiotic effect of ciprofloxacin was higher in group R rats after treatment (0.00±0.00 Cfu/ml) when compared with the mean value before treatment (24.0±1.41 Cfu/ml). But in group S, the mean value of count after treatment was significantly higher (26.5±13.44 Cfu/ml) when compared with the mean value before treatment (14.5±4.95 Cfu/ml) and this is because this group was not treated with either leave extract or antibiotics. Conclusively, the leave extract of *Chromolaena odorata* could be adopted as an alternative treatment against gastrointestinal tract infections especially those due to *Pseudomonas aeruginosa*.

Keywords: Antibiotic effect; Medicinal plants; Bioactive compounds; Phytochemical compound; Antimicrobial substances

1. Introduction

Many works have been done which aimed at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections (both topical and systemic applications) as possible alternative to chemically synthetic drugs to which many infectious microorganisms have become resistant. Literature reports and ethno-botanical records suggest that plants are the sleeping giant of pharmaceutical industry [1]. They may provide natural source of antimicrobial drugs that will provide novel or lead compounds that may be employed in controlling some infections globally.

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Several scientific studies carried out on plant species such as garlic (*Allium sativum*), Mango (*Mangifera indica*), guava (*Psidium guajava*), bitter leaf (*Vernonia amygdalina*) and ginger (*Zingiber officinale*) have confirmed the traditional claims of their effectiveness in treating diarrhoea related infection [2].
Chromolaena odorata, a flowering shrub is native to North and Central America, and was later introduced to parts of Asia, Africa and Australia. C. odorata is also known by various other names such as Armstrong’s weed, baby tea, bitter bush, butterfly weed, Christmas bush, devil weed, eupatorium, Jack in the bush, king weed, paraffin bush, paraffin weed, Siam weed, turpentine weed and triflid weed [3]. The fresh leaves of C. odorata or the decoction has been used by practitioners of traditional medicine for the treatment of human burns, soft tissue wounds, ulcerated wounds, burn wounds, postnatal wounds and also for the treatment of leech bites, indigestion and skin infection [4]. Other pharmacological properties of this plant include anti-helminthic [5], analgesic [6], anti-inflammatory, antipyretic, anti-spasmodic [7], antimicobacterial, insecticidal, antioxidant [8], anti-gonorrhoeal [9], fungicidal, diuretic [10], blood coagulating [11], and antimicrobial effects. However, at present there is only limited information available pertaining to the wound healing properties of the plant [12].

Pseudomonas aeruginosa is an increasingly prevalent opportunistic human pathogen and is the most common Gram-negative bacterium found in nosocomial and life-threatening infections of immunocompromised patients [13]. Patients with cystic fibrosis are especially disposed to P. aeruginosa infections, and for these persons the bacterium is responsible for high rates of morbidity and mortality [14]. Gastrointestinal infections are viral, bacterial or parasitic infections that cause gastroenteritis, an inflammation of the gastrointestinal tract involving both the stomach and the small intestine. Symptoms include diarrhea, vomiting and abdominal pain. Dehydration is the main danger of gastrointestinal infections. So rehydration is important, but most gastrointestinal infection are self-limited and resolved within few days [15].

Assessment of the antibacterial property of aqueous leaf extract of Chromolaena odorata which has been used for centuries as remedies for human diseases because of its therapeutic value was carried out. Hence, this study was designed to determine the phytochemical composition as well as the in vivo antimicrobial properties of aqueous extracts of C. odorata using Pseudomonas aeruginosa induced wister rats. Therefore, this study aimed at assaying the antibiotic effect of aqueous Chromolaena odorata extract on Pseudomonas aeruginosa induced gastrointestinal tract infection in adult wistar rats.

2. Material and methods

2.1. Experimental Design

A total of 36 wistar rats whose weights range from 72-100g were used for this study, twelve (12) rats for toxicological study and 24 rats for the experiment. Those for toxicological study were divided into 3 groups of 4 rats each, while those for the experiment were divided into 4 groups of 6 rats each.

Group Q: test (Rats inoculated with Pseudomonas aeruginosa and treated with extract)
Group R: test control (Rats inoculated with Pseudomonas aeruginosa and treated with antibiotics)
Group S: positive control (Rats inoculated with Pseudomonas aeruginosa and not treated)
Group T: negative control (rats not inoculated with Pseudomonas aeruginosa and not treated)

2.2. Acclimatization

Two weeks acclimatization period was observed which helped the rats to adapt to their new environment and also enabled the researcher got acclimatized. The following parameters were checked: age, weight, agility and temperature. Urinalysis using combi 9 test strip was carried out to ascertain the health and physical fitness of the animals before the experiment proper. The rats were housed in a well ventilated metallic cages and fed with formulated rat feed (vital feed) which constituted the following nutrients: 16.5% crude protein, 2.5% fat, 3.50% calcium, 10% crude fibre, 0.4% phosphorus, and 2700Kcal/kg metabolisable energy and then distilled water.

2.3. Toxicological Study

Twelve (12) wistar rats whose weights range from 80 – 100g and whose age ranges from 8 – 12 weeks were used for the study. The aim was to determine the lethal dose for fifty percent (50%) of the rat (LD50) and curative dose of aqueous extract of Chromolaena odorata leaf extract that will have curative and lethal effects on the wistar rats. The rats were divided into three groups of four albino rats each. Each group of rats was given 3000mg/kg, 6750mg/kg, 15000mg/kg, 6000mg/kg, 6375mg/kg, 13500mg/kg, and 33000mg/kg doses of extract orally using intragastric catheter. The extract was given three times daily after the animals had fasted for 18 hours for LD50 determination. The number of death in each group within 48 hours was recorded. Besides, delayed mortality up to 24 hours was considered as lethal dose. This was done by observing the rats for toxicity signs. The increment was limited to 33000mg/kg because all the rats almost died at this dose.
2.4. Collection of Isolate (Test Microorganism)

*Pseudomonas aeruginosa* was gotten from stool of patient attending Emergency clinic at Federal Medical Center (FMC), Owerri and was confirmed at the Medical Microbiology Laboratory of Medical Laboratory Science Department, Imo State University, Owerri. It was cultured in Nutrient broth for 24 hours at 37°C prior to oral inoculation.

2.5. Preparation and Sterilization of Materials

The working bench was first disinfected by swabbing with cotton wool soaked in 70% alcohol. The glass wares were washed, air dried, wrapped with aluminum foil and sterilized in the hot air oven at 170°C for 2 hours. Aseptic techniques were applied in the working environment as stipulated by Ochei and Kolhatkar [16]. The media namely; Nutrient agar, Blood agar, Chocolate agar and MacConkey were prepared and used according to the manufacturer's instruction.

2.6. Sample Collection and Processing of animal faeces

After the inoculation of *Pseudomonas aureginosa* isolate, stool sample were collected from groups Q, R and S after 7 days using a sterile universal container and it was transported immediately to the laboratory for stool microscopy, culture, viable count and sensitivity testing.

2.7. Enumeration of Total Viable Bacteria Counts:

The total viable counts of bacteria were estimated using "Pour plate method". For samples with more bacteria, 1ml of undiluted sample (dilution factor = 10) was used to estimate the bacterial load. This means adding the sample without diluting it to the petri dish containing the molten agar (Nutrient agar, Blood agar, Chocolate agar and MacConkey agar) using sterile pipette. 1ml of the 100x dilution was added to the petri dishes containing Nutrient agar, Blood agar, Chocolate agar and MacConkey agar respectively. The plates were gently mixed well and allowed to solidify. The plates were then inverted and incubated at 37°C for 24-48 hours.

The number of colonies that developed in the plates for each location were counted and the average recorded. Discrete colonies were aseptically sub cultured on nutrient agar plate for purification and incubated at 37°C overnight. Pure colonies were later sub cultured in bijou bottles containing nutrient agar slants and stored at refrigerator temperature as stock cultures for further biochemical tests.

2.8. Viable Count

For each media in duplicate form for Nutrient agar, Blood agar, Chocolate agar and MacConkey, numbers of colonies each for the two plates and added together and divided by two to obtain the viable count.

The (Colony Forming Unit) CFU/ml can be calculated using the formula:

\[ CFU/ml = \frac{(\text{number of colonies} \times \text{dilution factor})}{\text{volume of culture}} \]

2.9. Isolation and Identification of Pseudomonas aeruginosa from the faecal samples

Before being used, the isolates were re-identified from the slope culture by inoculating it on a solid media such as Nutrient agar, Blood agar, Chocolate agar and MacConkey. The pure isolates were identified using their morphological and microscopic features. The identity of the organism was confirmed by employing some biochemical tests.

2.10. Inoculation of Bacteria

Broth culture of *Pseudomonas aureginosa* was subcultured using nutrient agar and blood agar and incubated overnight at 37°C overnight for 24 hours to obtain a pure isolate of *Pseudomonas aeruginosa* colony, it was then adjusted according to Macfarlane standard of 0.5 CFU/ml, *Pseudomonas* saline suspension was prepared using 0.5 CFU of *Pseudomonas aeruginosa* 0.5 of 0.85% of saline solution prior to inoculation, it was done via oral administration once daily for three days until 1 ml calibrated insulin syringe. The same colony forming unit was given to group Q, R and Y.

2.11. Urinalysis

Test strip for the rapid determination of blood, urobilinogen, bilirubin, protein, nitrite, ketone, ascorbic acid, glucose and pH value in the urine produced by Macherey - nagel was used.

2.12. Antimicrobial Susceptibility Testing

Pure culture of *P. aeruginosa* isolate was subjected to antimicrobial susceptibility test using the disc diffusion technique, to determine the sensitivity of the organism for R (test control) with the aid of an antibiotic disc. They had all the
concentration of various antibiotics. They were evenly distributed on the inoculated media plates. The plates were incubated at 37°C to obtain zone of inhibition.

2.13. Preparation of *Chromolaena Odorata* Aqueous Leaf Extract Using Infusion Method

Leaves of *Chromolaena odorata* was gotten within the premises of Imo State University, Owerri and was identified by the department of plant science and biotechnology, Imo State University Owerri. The *Chromolaena odorata* leaves were washed in several changes of clean water and sundried for three days. 400g of the powdered *Chromolaena odorata* leaves were dissolved in 1000ml of boiled water, stirred for 30 seconds and allowed to stand for 15 minutes. The mixture was sieved twice and the filtrate was collected under strict aseptic conditions in fresh sterilized bottles and stored at 4°C until use.

2.14. Extract Administration

From the stock solution the *Chromolaena odorata* extract was administered to the rats with the aid of a sterile intragastric catheter. On the first day, group one received 3750mg/kg, group two received 7500mg/kg, and group three received 15000mg/kg for 100g rat. On day two, group one received 3750mg/kg, group two received 7500mg/kg and group three received 30000mg/kg for 100g rat. Rats below or above 100g were extrapolated.

2.15. Statistical Method

Data were digitally analyzed using statistical software package SPSS version 20. All values were expressed in mean ±SEM. Treatment effect over time were compared between control and treated groups by analysis of covariance. The results were analyzed statistically using student T-test to determine LD50 and the mean bacterial count before and after treatment analysis of variance using one way ANOVA to identify possible differences of body weights. P values less than 0.05 were considered statistically significant.

3. Results and discussion

The animals were acclimatized to laboratory condition for two weeks after which they were labeled from R1 – R36

Table 1 shows that all the rats used in the experiment were all fit and healthy before the commencement of the experiment reducing the chances of other factors that could interfere with the result outcome.

Table 1 Bio data of the wistar rats used in the study

| S/N | Initial weight (g) | Age  | Sex | Agility | Final Weight (g) |
|-----|-------------------|------|-----|---------|------------------|
| R1  | 70                | 5Weeks | M   | V.agl   | 90               |
| R2  | 60                | 4 weeks | F   | Agl.    | 80               |
| R3  | 85                | 8 weeks | F   | Agl.    | 100              |
| R4  | 70                | 5 weeks | F   | V.agl   | 90               |
| R5  | 85                | 9 weeks | M   | V.agl   | 100              |
| R6  | 70                | 5 weeks | F   | V.agl   | 90               |
| R7  | 75                | 6 weeks | M   | V.agl   | 95               |
| R8  | 80                | 8 weeks | F   | V.agl   | 100              |
| R9  | 80                | 8 weeks | M   | V.agl   | 100              |
| R10 | 60                | 6 weeks | M   | V.agl   | 85               |
| R11 | 65                | 5 weeks | F   | Agl.    | 70               |
| R12 | 82                | 8 weeks | M   | V.agl   | 92               |
| R13 | 65                | 5 weeks | M   | V.agl   | 80               |
| R14 | 72                | 5 weeks | M   | V.agl   | 95               |
| R15 | 70                | 5 weeks | M   | V.agl   | 90               |
| R16 | 80                | 10 weeks | F  | Agl.    | 100              |
Similarly, all the rats were also screened for possible injury/infection which could interfere with the result outcome using Combinine (9) test strips before the experiment as represented in Table 2. This showed that all parameters in this study were negative.

Table 2 Urinalysis test result of all the rats before the experiment.

| S/N | PH  | Glucose | Ascorbic Acid | Ketone | Nitrite | Protein | Bilirubinogen | Urobilinogen | Blood |
|-----|-----|---------|---------------|--------|---------|---------|---------------|--------------|-------|
| R1  | 5   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R2  | 5   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R3  | 5   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R4  | 6   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R5  | 5   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R6  | 5   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R7  | 6   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R8  | 6   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R9  | 5   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R10 | 6   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R11 | 5   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R12 | 5   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R13 | 5   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |
| R14 | 5   | Negative| Negative      | Negative| Negative| Negative| Normal        | Negative      |       |

Keys: V.agl = Very agile; Agl = Ag
Therefore the lethal dose (LD50) of *Chromolaena odorata* aqueous leaf extract = \( \sqrt{\text{Last non} \times \text{Lethal dose}} \).
Therefore the last non lethal dose = 13,500mg/kg while the lethal dose = 33000mg/kg hence, \( \sqrt{13500 \times 33000} = 21106.9 \text{mg/kg body weight.} \)

**Table 4** The LD50 (50% lethal dose) result obtained at the dose of 33000mg/kg in group 3 and on the second day.

| DAY | DOSE mg/kg | MORTALITY |
|-----|------------|-----------|
| One | 13500      | NO        |
| Two | 33000      | YES       |

Table 5 Shows that the antibacterial effect of *Chromolaena odorata* was significantly high in group S rats after treatment (4.5±2.12 Cfu/ml) when compared with the mean value before treatment (20.0±4.42 Cfu/ml). The antibiotics effect of ciprofloxacin was higher in group R rat after treatment (0.00±0.00 Cfu/ml) when compared with the mean value before treatment (24.0±1.41 Cfu/ml) at P<0.05. But in group T rats that were infected but not treated with either aqueous extract of *Chromolaena odorata* or ciprofloxacin the mean value of count after treatment (26.5±13.44 Cfu/ml) was significantly higher after infection than the count before infection (14.5±4.95 Cfu/ml).

**Table 5** Mean±SD of the antibacterial effect of *Chromolaena odorata* aqueous leaf extract on *Pseudomonas aeruginosa* induced gastrointestinal tract infection in wistar rat during infection and after treatment with both extract and ciprofloxacin.

| Group | During infection (Cfu/ml) | After treatment (Cfu/ml) |
|-------|--------------------------|--------------------------|
| Q (*Chromolaena odorata*) | 20.0±4.42 | 4.5±2.12 |
| R (Ciprofloxacin) | 24.0±1.41 | 0.0±0.0 |
| S (Untreated) | 14.5±4.95 | 26.5±13.44 |

Table 6 The mean weight value of final of group 3 (116.5±1.73) at the dose of 15000mg/kg was significantly higher (P<0.05) when compared with the mean weight value of the final weight of group 2 (104.0±0.04) at the dose of 6750mg/kg and group 1 (93.0±1.15) at the dosage of 3000mg/kg at P < 0.05.

**Table 6** The Mean±SD values of the effect of *Chromolaena odorata* aqueous leaf extract on the body weight of group 1, 2 and 3 rats when treated at the doses of 3000, 67500 and 15000mg/kg.

| Group | Mean initial weight | Dose (mg/kg) | Mean final weight | Mean weight change |
|-------|---------------------|--------------|------------------|-------------------|
| 1     | 83.75±2.5           | 3000         | 93.0±1.15        | 9.25±2.06         |
| 2     | 95.0±0.03           | 6750         | 104.0±0.04       | 9.0±0.02          |
| 3     | 107.5±2.87          | 15000        | 116.5±1.73       | 9.0±1.15          |

The mean ±SD value of final weight of group 3 (108.75±4.78) at the dose of 33000mg/kg was significantly higher at P < 0.05 when compared with the mean value of final weight of group 2 (103.5±0.57) at the dose of 13500mg/kg and group 1 rats (94.0±4.0) at the dose of 6375mg/kg.

**Table 7** Shows the mean ±SD values of the effect of *Chromolaena odorata* aqueous leaf extract on the body weight of group 1, 2 and 3 rats when treated at the doses of 6375, 13500 and 33000mg/kg.

| Group | Mean initial weight | Dose (mg/kg) | Mean final weight | Mean weight change |
|-------|---------------------|--------------|------------------|-------------------|
| 1     | 93.05±1.0           | 6375         | 94.0±4.0         | 0.5±5.0           |
| 2     | 104.0±0.02          | 13500        | 103.5±0.57       | -0.5±0.57         |
| 3     | 116.50±1.73         | 33000        | 108.75±4.78      | -7.75±6.39        |
4. Discussion

The clinical success of medicinal plants extracts, have rekindled the interest in medicinal plants as potential sources of novel drugs. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into the African continent. Herbal medicine has been widely used and forms an integral part of primary health care in China, Ethiopia and Argentina. A significant proportion of pharmaceutical products in current use are designed from plants. *Chromolaena odorata* is primarily indigenous to Nigeria/Africa and used extensively in the management and treatment of a number of ailments like diabetes mellitus [17]. *Pseudomonas aeruginosa* is a gram negative, non sporing motile rod that is capable of inducing gastrointestinal tract infection in humans, especially immunocompromised individuals. The result of this work showed that administering 3000mg/kg, 6750mg/kg, and 15000mg/kg of *Chromolaena odorata* extract, the weight of the rats increased and further increment reflected on their weight. At toxic level of 33000mg/kg and 31500mg/kg in group three on day two, rats decreased in weight. This shows that *Chromolaena odorata* has effect on increasing the weight of the rat due to metabolic activities, but the effect decreased later at the dose of 33000mg/kg and 31500mg/kg. It was found that *Chromolaena odorata* extract has a high antibacterial effect against *Pseudomonas aeruginosa* which may be due to its phytochemical constituent such as alkaloids, tannins, flavonoids, and other phenolic compounds, which produce a definite physiological action on the body weight according to the report of [18]. Ciprofloxacin which was the antibiotics used was able to clear the organism after infection. Comparing the antibacterial property of antibiotics and extract, it was found out that the antibiotics has a higher antibacterial activity (0.00±0.00) as shown in table 5 though the extract has a significant antibacterial effect (4.5±2.12) against *Pseudomonas aeruginosa* induced gastrointestinal tract infection though will have high inhibitory activity against *Pseudomonas aeruginosa* when administration is prolonged. The most important aspect bioactivity of this plant extract is its potency on both gram negative and gram positive bacteria which have over the years defied therapeutic functions of of chemosynthetic agents. However, Okigbo and Ajalie [19] and Naidoo et al. [20] reported less potency of this extract on enteric pathogens. It has been shown in 2015 that leaf extract of this plant species was inhibitory against four human pathogens; *Bacillus cereus, Staphylococcus aureus, E. coli* and *Salmonella typhi* of which two of these are enteric pathogens as in this present study [21]. The potency of both solvent extracts of *C. odorata* leaf against *P. aeruginosa* in this study was commendable and this is in contrast with result obtained by Hanphakphoom and Krajangsang [22], using the same solvent (methanol) and plant extract. Difference in strains used might have accounted for such variation. Also plant's geographical location and differential power of technique might have contributed to the disparity. There are many factors that could influence the potency of medicinal plants; these include the age of plant, extracting solvent, method of extraction, and even the time of harvesting the plant materials as reported by Okigbo and Ajalie [19]. These and other factors could have accounted for the aforementioned disparities.

5. Conclusion

*Chromolaena odorata* aqueous leave extract possesses antibacterial effect on gastrointestinal tract infection induced by *Pseudomonas aeruginosa* and thus, presents a promising source for medicines of which when carefully tapped and explored has numerous therapeutic potential. It is also important to note that while herbal-derived medicine may be an alternative for combating microbial infections, care should be taken to minimize the risk associated with them. *Chromolaena odorata* aqueous leave extract can be used as an alternative treatment to *Pseudomonas aeruginosa* induced gastrointestinal tract infection within the doses of 21106.9mg/kg body weight of which the curative ability is time dependent though on combination with other plant extract might the longtime of cure.

Compliance with ethical standards

Acknowledgments

Our unalloyed gratitude goes to the Head of the Department, Staff and Students of the Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria for their immense support and assistance throughout the period of this work.

Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval
Ethical approval for the use of adult wistar rats in this research work was obtained from Imo state Ministry of Agriculture and Natural Resources.

References

[1] Akinpelu DA, Onakoya TM. Antimicrobial activities of medicinal plants used in folklore remedies in South-Western States of Nigeria. African Journal of Biotechnology. 2006; 5(11): 1078 – 1081.

[2] Okorondu SI, Braide W, Ogbulie TE, Akujobi CO. Antimicrobial and Phytochemical properties of some traditional species. Nigeria Journal of Microbiology. 2006; 20(3): 1301 – 1308.

[3] McFadyen R. Chromolaena in East Timor: history, extent and control. In: Day, M.D., McFadyen, R.E. (eds): Chromolaena in the Asia-Pacific Region. Proceedings of the 6th International Workshop on Biological Control and Management of Chromolaena odorata. ACIAR Technical Reports. 2004; 55: 8 – 10.

[4] Panyaphu K, Sirisa-Ard P, Sirisa-Nga P, ChansaKaow S, Nathakarnkitkul S. Medicinal plants of the Mien (Yao) in Northern Thailand and their potential value in the primary healthcare of postpartum women. Journal of Ethnopharmacology. 2011; 135: 226–237.

[5] Patel J, Kumar GS, Qureshi MS, Jena PK. Anthelmintic activity of ethanolic extract of whole plant of Eupatorium odoratum. International Journal of Phytomedicine. 2010; 2: 127–132.

[6] Chakraborty AK, Sujit R, Umesh KP. Chromolaena odorata (L.): An overview. Journal of Pharmacy Research. 2011; 43: 573–576.

[7] Oludare TB, Olumayokun OA, Olufunmilola SO, Modupe MJ. Anti-inflammatory, antipyretic and antispasmodic properties of Chromolaena odorata. Pharmaceutical Biology. 2000; 38: 367–370.

[8] Phan TT, Wang L, See P, Grayer RJ, Chan SY, Lee ST. Phenolic compounds of Chromolaena odorata protect cultured skin cells from oxidative damage: Implication for cutaneous wound healing. Biological and Pharmaceutical Bulletin. 2011; 24: 1373–1379.

[9] Gopinath R, Sunilson JA, Radhamani S, Das A, Nilugal K. Diuretic activity of Eupatorium odoratum (L). Journal of Pharmaceutical Research. 2009; 2: 844–846.

[10] Triratana T, Suwannuraks R, Naengchomnong W. Effect of Eupatorium odoratum on blood coagulation, Journal of Medical Association Thailand. 1991; 74(5): 283–287.

[11] Biswal PR, Sardar KK, Parija SC, Mishra PR, Mishra, SN. Wound healing effect of Eupatorium odoratum Linn, and Himax in rabbits. Indian Journal of Indigenous Medicine. 1997; 19: 71–74.

[12] Van Delden C, Iglowski BH. Cell-to-cell signaling and Pseudomonas aeruginosa infections. Emergent of Infectious Distance. 1998; 4: 551–560.

[13] Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. Clinical Microbiology Reviews. 2002; 15: 194–222.

[14] Schlossberg D. Clinical infectious diseases. 2nd edition. Cambridge: Cambridge University Press. 2015; 334.

[15] Ochei JO, Kolhatkar AA. Medical laboratory science: Theory and Practice. New York: Tata McGraw-Hill Publishing Company Limited. 2008; 637–745.

[16] Akah PA. Mechanism of hemostatic activity of Eupatorium odoratum, International Journal of Crude Drug Research. 2001; 28(4): 253–256.

[17] Hill AF. Economic botany. A textbook of useful plants and plant products. (2nd edition). McGraw-Hill Book Company Inc, New York. 2003.

[18] Okigbo RN, Ajalie AN. Inhibition of some human pathogens with tropical plants extracts (Chromolaena odorata and Citrus aurantifolia) and some antibiotic. International Journal of Molecular Medicine and Advance Sciences. 2005; 1(1): 34–40.

[19] Naidoo KK, Cooposamy RM, Naidoo G. Screening of Chromolaena odorata (L) King and Robinson for antibacterial and antifungal properties. Journal of Medicinal Plants Research. 2011; 5(19): 4859–4862.
[21] Ijato JY, Tedela PO. Phytotoxic potentials of cold and hot aqueous extracts of *Chromolaena odorata* against fungal deteriorating agents of yam tubers (*Dioscorea rotundata*, Poir) after harvest. American Journal of Experimental Agriculture. 2015; 5(3): 262-266.

[22] Hanphakphoom S, Krajangsang S. Antimicrobial activity of *Chromolaena odorata* extracts against bacterial human skin infections. Modern Applied Science. 2016; 10(2): 1913-1844.