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Pulverized and non-pulverized effect on *Chromolaena odorata* (L.) R.M.King & H.Rob. leaf extract—antimicrobial activity

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Abstract.

Aims. *Chromolaena odorata* R.M.King & H.Rob. leaves were studied for pulverized and non-pulverized extract effects. ***Methods***. Extraction was carried out through water maceration. Pulverized and non-pulverized dried leaves were extracted at 0.02, 0.04, 0.06, 0.08 and 0.10 g per 1 ml. The leave's phytochemical screening reflected availability of alkaloids, saponins, steroids, flavonoids, tannins with other secondary metabolites. Antimicrobial assay of the extracts was determined on *Staphylococcus aureus* and *Escherichia coli* by agar well diffusion procedure. ***Results.*** Extracts between 20 mg/mL and 100 mg/mL gave 13 mm to 22 mm zones of inhibition against *Staphylococcus aureus* for the pulverized leaves extract and 8 mm to 20 mm for non-pulverized while 12 mm to 23 mm and 8 mm to 19 mm zones of inhibitions against *Escherichia coli* were recorded for the pulverized and non-pulverized leaves extract respectively. ***Conclusions.*** Studies have shown that *C. odorata* leaf extract can be used as a potential natural source for drug development to treat some bacterial infections. The pulverized leaves demonstrated more inhibitory activity than non-pulverized against the microorganisms.

Key words: alkaloids, antibacterial activity, *Escherichia coli*, leaves phytochemical composition, *Staphylococcus aureus*.

Антимікробна дія екстрактів з подрібненого й неподрібненого листя *Chromolaena odorata* (L.) R.M.King & H.Rob.

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Реферат.

Мета. Визначити антибактеріальні ефекти екстрактів з подрібненого й неподрібненого листя *Chromolaena odorata* (L.) R.M.King & H.Rob. ***Матеріали і методи***. Екстракцію проводили способом водної мацерації. Подрібнене та неподрібнене висушене листя екстрагували з наважок 0,02, 0,04, 0,06, 0,08 та 0,10 г на 1 мл. У процесі фітохімічного скринінгу листя визначали наявність алкалоїдів, сапонінів, стероїдів, флавоноїдів, танінів з іншими вторинними метаболітами. Антимікробний аналіз екстрактів виконували на *Staphylococcus aureus* та *Escherichia coli* методом дифузії в агарі. ***Результати та обговорення***. Використані у концентраціях від 20 до 100 мг/мл екстракти з подрібненого листя забезпечили зони інгібування *Staphylococcus aureus* від 13 до 22 мм, а для нероздрібненого — від 8 до 20 мм, тоді як зони інгібування *Escherichia coli* були від 12 до 23 мм для подрібненого і від 8 до 19 мм для екстрактів з неподрібненого листя. ***Висновки.*** Дослідження показали, що екстракти з листя *C. odorata* можуть бути використані як потенційне природне джерело для розробки препаратів для лікування деяких бактеріальних інфекцій. Екстракти з подрібненого листя продемонстрували більшу інгібіторну дію проти мікроорганізмів, ніж з неподрібненого.

Ключові слова: алкалоїди, антибактеріальна дія, *Escherichia coli*, фітохімічний склад листя, *Staphylococcus aureus*.

Introduction. Research on healing or curing was developed on plants through instinctiveness, which led to the discovery of their medicinal activity as an emerging field of herbal preparation due to their effectiveness in combatting the activities of pathogenic microbes and the prevention, reduction and treatment of diseases since time immemorial (Petrovska, 2012). Medicinal plant exploration has been evolving for a long time (Sofowora et al., 2013). Various methods of plant medicinal preparation such as maceration, decoction, and others, have been reported (Abubakar & Haque, 2020). Plant medicinal use by the majority of the world population resulted from man's efforts against illnesses that brought about seeking medicine in roots, stems, barks, leaves, seeds, fruits, and flowers due to availability, low cost or a cheap price, affordability, administration ease, low alternative side effects, and drug

resistance (Asase et al., 2008). Accurate and safe dose evaluation has been a challenge for medicinal plants (Hosseini et al., 2018). However, medicinal plants are widely recognised and accepted due to their important roles in the treatment of many diseases (Prasathkumar et al., 2021). Medicines can be sourced through natural, synthetic, or biosynthetic means (Alamgir, 2017).

Medicinal sources such as plants, animals, microbes, chemicals, and genetically engineered organisms have been reported (Alamgir, 2017). Treatments with animal meat have been known. Plant-animal mixtures where seeds were a major part of the mixture recipes have been reported (Mussarat et al., 2021). Biological medicinal sources with plants that are more advantage of environmentally friendly have been reported (Ponarulselvam et al., 2021). Plant extract medicinal products uses have been widespread for health maintenance due to diagnoses based on theories, beliefs, and experiences for the avoidance or treatment of illness (Wachtel-Galor & Benzie, 2011). Plant-sourced material is cost-effective, affordable, toxic-free, and danger-free to life, and therefore preferred (Huang et al., 2007).

The plants of Siam weed, scientific name — *Chromolaena odorata* (L.) R.M.King & H.Rob., genus *Chromolaena* DC. in the tribe *Eupatorieae* Cass. of the family *Asteraceae* Bercht. & J.Presl (Asteraceae..., 2023; Okpashi et al., 2014; Vijayaraghavan et al., 2017), are efficacious in scientific pharmacological evaluation. A phytochemical study of *C. odorata* has reported the presence of chemical entities (Akinmoladun et al., 2007). The plant is one of the world's tropical weeds that has been used for herbal medicine due to its medicinal properties. It has been used for the treatment of external wounds and skin infections (Pandith et al., 2013), has cytoprotective effects, and has other significant medicinal properties. *C. odorata* according to the literature is an antioxidant and has activity against microorganisms (Harfiani et al., 2022). Its leaf is used for the treatment of cough (Yahya et al., 2014) and many other functions (Zahara, 2019). The earliest form of life involved microorganisms, which included bacteria, fungi, protists, and viruses that might be free-living or parasitic (Forterre, 2010). Microorganisms increase quickly because they always multiply inside other living things. Bacteria freely undergo reformation (Holmes & Jobling, 1996). The operation mode of genes enables bacteria to live and act in new places (Woods et al., 2020). Microorganisms are characterized by rapid evolution and resistance to treatment (Enright et al., 2002). Some microorganisms are beneficial, such as decomposers in ecosystems (Berg et al., 2020) for water and food production, while pathogens are harmful, causing diseases to plants and animals (Sweileh, 2017). The antimicrobial activity of *C. odorata* against widespread microorganisms is popular. However, the leaf extracts effects of pulverized *C. odorata* in comparison with non-pulverized *C. odorata* are not known. *C. odorata* has been reported for varieties of phytochemicals, except for comparison in extraction effectiveness due to pulverization and non-pulverization effects on the *C. odorata* leaves. This work therefore focused on the assessment of pulverization and non-pulverization of *C. odorata* leaf extract for growth inhibition effects due to pharmacological or medicinal activities against *Staphylococcus aureus* and *Escherichia coli*. It has been reported that pulverization decreases particle size, leading to a higher surface area and pore volume (Zhao et al., 2014).

Materials and Methodology. The plant materials used in the present study were obtained from the Alabata, Abeokuta, Odeda Local Governments, Ogun State and taxonomic identification was made by the Botanical Survey. Chemicals and reagents were obtained from Standard and Precision Scientific Co. The *C. odorata leaves* were cleaned and rinsed using aqueous and deionized water before being air dried. Some were pulverized, and some were not pulverized. The cold maceration method was employed for the plant extraction, in which 50 g pulverized and non-pulverized material was soaked for three days in 200 mL of distilled deionized aqueous, filtrate separated through filtration and distilled by a rotary evaporator. It was lyophilized, and powdery *C. odoraeta* was obtained (Eze & Jayeoye, 2021).

Phytochemical composition of both the pulverized and non-pulverized leaves of the *C. odorata* was analysed by special tests:

1. **Terpenoids test** — 1 ml separately of the pulverized and non-pulverized extract of the *C. odorata* was reacted with equal $CHCl_3$ and 0.5 ml acetic anhydride. The mixture was shaken and further mixed with a little quantity of concentrated H_2SO_4. Presence of terpenoids was reflected by green colour in a way similarly reported in literature (Theeba, & Kumar, 2015). Deep green was observed for the pulverized extract, while light green was shown by the non-pulverized extract.

2. Sterols determination — in each case of pulverized and non-pulverized *C. odorata* extract, 1 ml of equal volume $CHCl_3$ was added then mixed in the same volume concentrated H_2SO_4. Reddish and greenish yellow colours indicated presence of sterols (Geetha & Geetha, 2014).

3. Saponins evaluation — 2 ml of pulverized and 2 ml of non-pulverized leaves content were separately added with little quantity of sodium hydrogen carbonate and 5 ml deionized H_2O. Persisted honeycomb foaming nature formed in demonstration of saponins in the extract (Sandeep et al., 2014) after shaken thoroughly.

4. Tannins test — 1 ml of extract of the pulverized and non-pulverized *C. odorata* in 4 ml deionized H_2O was reacted with 0.2 ml of 10% ferric chloride. Presence of tannins was indicated by blue-black formation, as similarly reported in literature (Rufai et al., 2016).

5. Test for alkaloids — Alkaloids test was done in which 0.5 ml concentrated HCl plus 1 ml of each pulverized and 1 ml non-pulverized *C. odorata* was treated with some Wagner's reagent. Reddish brown appearance produced proof for alkaloids (Hassan et al., 2020) in pulverized *C. odorata* extract and reddish colour in non-pulverized *C. odorata* extract.

6. Flavonoids evaluation — was carried out on the pulverized and non-pulverized *C. odorata* for flavonoids determination as a chemical constituent of the extract. 1 ml each of the extract was treated by careful addition of 0.1 ml of concentrated hydrochloric acid, which instantly gave red colour due to presence of flavonoids (Rao et al., 2016).

7. Cardiac glycosides analysis — *C. odorata* extract both pulverized and not-pulverized 1 ml each was mixed with 1 ml glacial acetic acid further added with 0.1 ml $FeCl_3$ solution. Undiluted H_2SO_4 of 1 ml was slowly involved. Brownish red colour formed described positive test (Shukla et al., 2013).

8. Protein determination — some mercuric nitrate was reacted with same amount of pulverized *C. odorata* extract and non-pulverized *C. odorata* extract differently. There was formation of white precipitate that changed to red when heated, which indicated presence of protein (Yadav & Agarwala, 2011).

9. Carbohydrates analysis — *C. odorata* extract in pulverized and non-pulverized of 1 ml each was reacted with 1 ml of Benedict's solution and slightly heated. Precipitated red colour produced was attributed to presence of carbohydrate (Narasimhan & Sathiyavani, 2014).

10. Test for anthraquinones — pulverized and non-pulverized *C. odorata* leaves each of 0.2 ml filtrate with little quantity of $CHCl_3$ was shaken for some minutes. Few drops of 10% NH_3 was mixed after filtered, and then gently shook. Pink colour was identified for the anthraquinone presence (Ajayi et al., 2011).

11. Moisture determination — *C. odorata* leaves (5 g) were weighed repeatedly until constant weight (W_3) was obtained for moisture content determination treatment. It was carried out in triplicates and average (4.76 g) calculated. The moisture lost (0.24 g) was determined according to percentage moisture content calculation method (Park & Bell, 2002).

12. Ash content analysis — leaves (5 g) of the plant were weighed, them on hot plate and ignited in a furnace at 540° C which gave a whitish substance that showed removal of carbon (Pant et al., 2017). It was cooled in a desiccator, weighed and obtained 0.48 g after triplicates analysis.

13. Crude Fibre Determination — Soxhlet extractor used to defat 5g of *C. odorata* in n–hexane according to literature (Busuttil-Griffin et al., 2015) gave 4.40 g defatted sample digested in a mixture of some 2% H_2SO_4 with 2% NaOH. It was cooled in a desiccator after 105° C drying, 540° C ignition and weighed to obtain 2g. The analysis was carried out in triplicates and Crude fibre content which is loss in weight on ignition average (2.40g) determined in percentage.

Antimicrobial activity evaluation of *C. odorata* extracts was studied at different concentrations of 0.02, 0.04, 0.06, 0.08, and 0.1 gram per ml. Both pulverized and non-pulverized leaf extracts were examined against *Staphylococcus aureus* and *Escherichia coli* according to standard procedure (Khopade et al., 2012). The prepared agar mediums were then sterilized by autoclaving at a pressure of 18 psi and 120 °C for 30 min before being seeded for the *Staphylococcus aureus* and *Escherichia coli* assays. The extract was added to each well-prepared pulverized and non-pulverized sample and compared with distilled deionized water as a control to obtain ±SD.

Results and Discussion. An aqueous extract of *C. odorata* was analysed for phytochemical constituents. The constituents were examined in pulverized and non-pulverized leaves extracted through maceration method. Effectiveness of the maceration method has been attributed to the constituents' movement to low from higher concentration. The extraction process was void of heating, which in a way gave assurance for preservation of thermolabile active constituents (Warsi & Sholichah, 2017). Effect due to difference between pulverization and non-pulverization, demonstrated shift in phytochemical profile by occurrence of slight discrepancies in the evaluated constituents. Analysis of the *C. odorata* for

phytochemical agents in pulverized and non-pulverized leaves extract based on chemical properties indicated **positive** Flavonoid, protein**,** carbohydrate**,** others (Table 1).

Table 1. Phytochemical constituents of pulverized and non-pulverized *C. odorata* leaves extract

Phytochemical constituents	*Chromolaena odorata* leaves extract colour	
	pulverized	non-pulverize
Terpenoids	deep green	light green
Sterols	reddish, greenish yellow	light greenish yellow
Saponins	high honeycomb foaming	honeycomb foaming
Tannins	blue-black	blue
Alkaloids	reddish brown	red
Flavonoids	deep red	light red
Cardiac glycosides	brownish red	reddish
Protein	white precipitate-red	less precipitate-red
Carbohydrates	precipitate-red	less precipitate-red
Anthraquinones	pink	light pink

The difference in colour, honeycomb foaming and precipitate formation of the constituents in the pulverized involved small particle size and non-pulverized extracts was attributed to pulverization and non-pulverization effect which was the only uncommon factor to the *C. odorata leaves* extract analysis. Terpenoids showed deep green for the pulverized and light green for the non-pulverized, while Sterols indicated reddish, greenish yellow for the pulverized compared with light greenish yellow observed for the non-pulverized. The characteristic honeycomb foaming for Saponins (Sandeep et al., 2014) was high for the pulverized, but not as high for the non-pulverized. Tannins, alkaloids, flavonoids and cardiac glycosides produced blue-black, reddish-brown, deep-red and brown-red for the pulverized in comparison with blue, red, light red and reddish colours for the non-pulverized respectively. Protein and carbohydrate which were also tested positive reflected more precipitate for the pulverized than non-pulverized in both cases. The slight differences in the phytochemical profile suggested presence of the chemical composition in higher concentration in the pulverized extract than the non-pulverized due to decrease in particle size which increased surface area and pore volume (Zhao et al., 2014) of the pulverized *C. odorata* leaves compared with the non-pulverized leaves.

Terpenoids test — Presence of terpenoids was demonstrated by green colour after treatment with chloroform, acetic anhydride and concentrated sulphuric acid in agreement with literature (Theeba, & Kumar, 2015). Deep green was observed for the pulverized leaves extract, while light green was shown by the non-pulverized extract.

Sterols — Phytochemical screening of the *C. odorata* leaves extract was positive for the sterols test through addition of chloroform later mixed with concentrated sulphuric acid, as earlier reported (Geetha & Geetha, 2014). Pulverized

leaves extract gave reddish, greenish yellow, while non-pulverized gave light greenish yellow due to similar previous reasons.

Saponins — Persisted honeycomb bubbles emission in both the pulverized and non-pulverized *C. odorata* leaves extracts gave affirmation of saponins (Sandeep et al., 2014). The pulverized leaves foaming intensity was observed to be more than the non-pulverized leaves extract, which suggested higher concentration of saponins in the pulverized than non-pulverized. This could be attributed to particles size variation, which increased surface area and pore volume (Rao et al., 2016) in the pulverized leaves extract.

Tannins — Tannins proportion in the pulverized and non-pulverized leaves extracts was assessed by concentration difference exhibited by colour changes, where blue-black was obtained for higher concentration in the pulverized and blue colour for less availability in the non-pulverized when subjected to distilled deionized water addition before reaction with ferric chloride.

Alkaloids — Precipitate formed after addition of Wagner's reagent to the pulverized and non-pulverized *C. odorata* extracts indicated a reaction that occurred between the available electron nitrogen constituted alkaloids and the Wagner's reagent potassium ion similarly reported (Warsi, & Sholichah, 2017). Reddish brown precipitate was observed for the high alkaloids concentrated pulverized leaves extract while red precipitate was seen for the less alkaloids concentrated non-pulverized leaves extract which displayed effect between the pulverization and non-pulverization resulted from particle sizes that allowed for the more alkaloids in pulverized leaves extract than the non-pulverized.

Flavonoid — Red colour indicated presence of flavonoids in both pulverized and non-pulverized when treated with concentrated hydrochloric acid, in agreement with report in literature (Rao et al., 2016). However, variation in colour for pulverized leaves extract (deep red) against light red for non-pulverized leaves extract might be due to different flavonoid concentration effect between the pulverized and non-pulverized. The deep red colour which suggested higher concentration of flavonoid in the pulverized leaves extract could be attributed to decrease in particle size which increased surface area and pore volume (Zhao et al., 2014) of the pulverized leaves compared with the non-pulverized leaves.

Cardiac glycosides — Cardiac glycosides test was carried out and gave brownish red colour for pulverized leaves extract and reddish colour for non-pulverized leaves extracts, which acted as a pointer to variation in the cardiac glycosides' concentration.

Protein — Protein which was tested positive reflected more precipitate for the pulverized leaves extract than non-pulverized following reaction of the *C. odorata* leaves extract with mercuric nitrate. There was formation of white precipitate that changed to red when heated, which indicated the presence of protein (Yadav & Agarwala, 2011).

Carbohydrates — Benedict's solution when added to *C. odorata* leaves extract and slightly heated produced red precipitate that showed presence of carbohydrate (Narasimhan & Sathiyavani, 2014) observed to be more in the pulverized than non-

pulverized extract due to higher concentration of the carbohydrate in the pulverized as a result of pulverization effect.

Anthraquinones — *C. odorata* leaves extract mixed with chloroform later reacted with ammonia according to procedure in literature gave pink colour in demonstration of anthraquinone (Ajayi et al., 2011). The colour was lighter in the non-pulverized extract than pulverized. This suggested higher concentration of the anthraquinones in pulverized than the non-pulverized, which might be attributed to decrease in particle size that led to increased surface area and pore volume (Zhao et al., 2014).

Composition test — Moisture, ash content and crude fibre composition determined were expressed in percentage as shown in Figure 1.

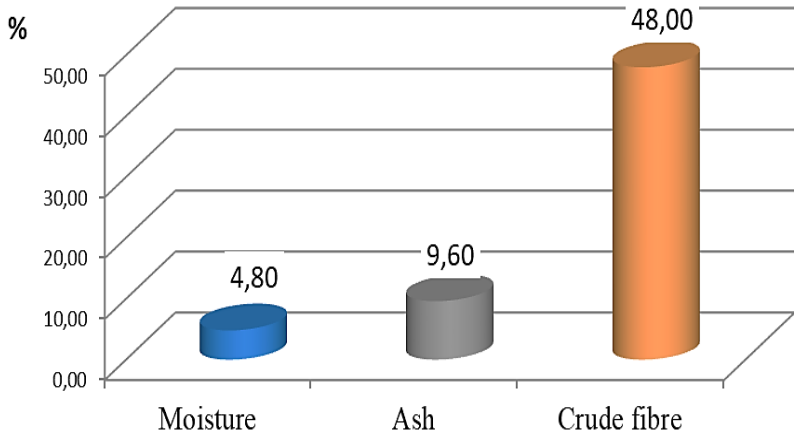

Figure 1. The percentage moisture, ash content and crude fibre in *Chromolaena odorata* leaves, %

Moisture content analysis of the *C. odorata* carried out according to literature method (Park & Bell, 2002) gave 4.80% when calculated. Similar loss on drying of plant has been reported (Sandeep et al., 2014). Ash value was obtained as 9.6% through calculation in agreement with literature (Pant et al., 2017). Determination of the *C. odorata* crude fibre yielded 48.00%.

Pulverized and non-pulverized *C. odorata* leaves of 0.02, 0.04, 0.06, 0.08 and 0.1 gram per ml displayed inhibitory effect against *S. aureus* from 13 to 22 mm while non-pulverized extract ranged from 8 to 20 mm. 12 mm–23 mm against *Escherichia coli* for the pulverized extract compared to non-pulverized that gave 8 to 19 mm (Table 2).

The inhibitory effect of the *C. odorata* leaf extract might be due to the properties of phytochemical constituents. Cardiac glucoside by literature has been reported for antimicrobial properties, antiparasitic, potential anticancer and treatment of diarrhea (Pongrakhananon, 2013). It has been reported that alkaloids have antiparasitic activity and have been used for the treatment of gout (Shinkafi, 2014). Tannin as an antidiarrheal has been known (Wakori et al., 1996). Treatment of prostate cancer using steroids has been reported (Marwat, 2005), which also act as hormone stimulators and regulators (Bruneton, 1999). Saponins have been reported as anti-inflammatory, hypocholesterolaemia and immune-stimulating (Tamura et al., 2012), while activity against ulcers has been attributed to flavonoids (Pal & Verma 2013). Higher inhibition effects observed for the pulverized leaf extract compared with the

non-pulverized suggested more active phytochemical constituents per equal ml of the former than the latter due to a higher particular boundary with a larger pore volume pulverized more than non-pulverized, which might have increased contact that led to higher inhibition. It agreed with literature where superfine pulverized plant powders performed better than fine pulverized powders (Riley et al., 2008). Different concentrations of the *C. odorata* leaf extract demonstrated different inhibitory zones.

Table 2. Antimicrobial activity of *C. odorata* extracts on *Staphylococcus aureus* and *Escherichia coli* (inhibition zone, mm)

Bacterium species and treatment source	Extract concentration (mg/mL)					Distilled deionized water (control)
	20	40	60	80	100	
Staphylococcus aureus pulverized leaves extract	13	15	18	21	22	0
Staphylococcus aureus non-pulverized leaves extract	8	12	14	17	20	0
Escherichia coli pulverized leaves extract	12	16	18	20	23	0
Escherichia coli non-pulverized leaves extract	8	14	15	18	19	0

Conclusions. Water was able to extract active ingredients from the *C. odorata* leaves. Constituents' composition screening displayed the presence of some phytochemical agents. The presence of phytochemical constituents was associated with the *C. odorata* leaves antimicrobial property. The study was able to compare the difference between the pulverized and non-pulverized *C. odorata* leaves. The research work showed that pulverized *C. odorata* leaf extract was more effective than non-pulverized extract against the selected microorganisms. The higher inhibition observed for the pulverized leaves than the non-pulverized leaves could be due to more ingredients in the former than the latter. The sterile water that was used as a control did not show any inhibition against the selected microorganisms.

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