Rovedar

Research in Biotechnology and Environmental Science. 2024; 3(1): 9-17.

DOI: 10.58803/rbes.v3i1.29

http://rbes.rovedar.com/

Original Article

Quantification of Phytochemical Constituents, and Non-Enzymatic Antioxidants of Polyherbal-Formulated Tea on Antitussive, Expectorant, and Analgesic Activity in Rodent

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ARTICLE INFO

Article History:
Received: 03/12/2023
Revised: 19/12/2023
Accepted: 11/03/2024
Published: 30/03/2024

Keywords:
Analgesic
Antioxidant
Antitussive
Expectorant
Polyherbal-formulated tea

ABSTRACT

Introduction: Polyherbal formulations maximize therapeutic effects and reduce toxicity by combining effective herbs in specific ratios. The present study aimed to quantify some phytochemical constituents, and some non-enzymatic antioxidants and to estimate the analgesic, expectorant, and antitussive properties of polyherbal-formulated tea (*Curcuma longa, Citrus limon, Zingiber officinale, Allium sativum,* and *Moringa oleifera*).

Materials and Methods: Some phytochemical constituents and some non-enzymatic antioxidants of the polyherbal tea were evaluated using colorimetric methods. The antitussive efficacy was assessed by examining the cough induced by citric acid in 20 healthy guinea pigs and ammonium in 20 mice. The expectorant activity was evaluated using phenol dye secretion in mice. The analgesic properties were analyzed using pain caused by a hot plate and writhing test caused by acetic acid. Four groups were formed by randomly dividing 20 healthy adult experimental animals (mice and guinea pig), with 5 of both sexes' animals in each group. Group 1 was given distilled water (10 ml/kg), group 2 was given 5 mg/kg of the polyherbal-formulated tea, group 3 was given 10 mg/kg of the polyherbal-formulated tea, and group 4 was given standard drugs depending on the model of animals used. The tea and standard drugs were administered orally.

Results: The result showed that the polyherbal-formulated tea contains phenolic compounds (53.57±1.96 mg/g), alkaloids (40.93±5.96 mg/g), flavonoids (99.44±1.96mg/g), Vitamin C (862±18.76mg/g), carotenoid (5200±6.93 mg/g) and Lycopene (19.50±1.35mg/g). The polyherbal-formulated tea decreased the number of cough bouts and raised the percentage of cough suppression caused by citric acid when *compared to* the control group. Tea decreased the number of cough bouts caused by ammonium in mice compared to the control group and it raised phenol dye secretion in the expectorant experiment. In hot plate-induced pain, tea increased the latency of the pain threshold in mice and reduced the number of writhing the percentage of pain inhibition increased compared to the control group in acetic acid-induced pain.

Conclusion: The polyherbal-formulated tea contains phenolic compounds, alkaloids, flavonoids, Vitamin C, carotenoid, and lycopene and has antitussive, expectorant, and analgesic activity.

1. Introduction

Herbal medicine has been always utilized to address different medical conditions, including malaria, warts, digestive issues, heart conditions, and persistent pain[1]. The knowledge about herbs has passed down from one generation of pharmacists and doctors to the next. Herbal medicine, was known as herbalism or botanical medicine, which focuses on the utilization of plants and their components for consumption or application to promote good health[1]. Schulz *et al.*[2] stated herbs as sources of traditional medicines, that many modern medicines indirectly derived from plants. The popularity of herbal tea is attributed to their health advantages and delightful

► *Cite this paper as:* Uwaya DO, Effiong ON. Quantification of Phytochemical Constituents, and Non- Enzymatic Antioxidants of Polyherbal-Formulated Tea on Antitussive, Expectorant, and Analgesic Activity in Rodent. Research in Biotechnology and Environmental Science. 2024; 3(1): 9-17. DOI: 10.58803/rbes.v3i1.29

sensory characteristics, making them a widely cherished choice. Frequently, people combine plant medicines to benefit from their optimal advantages, which is widely known as polyherbal formulation.

Curcuma longa (turmeric) belongs to the family Zingiberaceae[3]. This herb thrives in tropical and subtropical regions and is cultivated extensively in Asian countries, particularly in India and China. Traditionally, it is used to manage medical conditions, such as tumor, coughs, diabetes, stiffness, diarrhea, swelling, psoriasis, hepatobiliary illnesses, skin conditions, stomach ulcers, and peptic ulcers[3]. *Curcuma longa* enhances the circulation of blood, eliminates blockages, relieves depression, and flavors the food[4].

Citrus limon (lemon) is a popular fruit, which belongs to the Citrus genus and grows on a small tree. It is widely cultivated in areas, such as Sicily, Italy, and Spain. Lemon juice, derived from the *Citrus limon* (*C. limon)* was traditionally used to treat irregular menstruation, the common cold, high blood pressure, and scurvy prior to the identification of Vitamin C[5]. Additionally, the essential oil derived from *C. limon* is an effective remedy for cough[6-8].

Moringa oleifera (moringa), belongs to the family Moringaceae[9], *is found in India, Pakistan, Asia Minor, Africa, and Arabia[3].* This plant treats various conditions, including skin disorders, epilepsy, asthma, fever, eye disease, and hemorrhoids[10,11].

Allium sativum (garlic) is a small bulbous crop that grows beneath the ground. It belongs to the Amaryllidaceae family[12]. Taking this remedy boosts the treatment of a range of illnesses, such as pertussis, respiratory conditions, digestive problems, postpartum disorders, respiratory infections, eye discomfort, and ear pain. Furthermore, it prevents cardiovascular diseases[12].

Zingiber officinale (ginger), which is in the Zingiberaceae family[13] is originally found in Southeast Asia and is the most widely utilized spice across the globe[14]. It helps to relieve headaches, alleviate rheumatism, and address symptoms associated with cold and cough[14].

The goal of the current study was to quantify phytochemical constituents, non-enzymatic antioxidants and to assess the antitussive, expectorant, and analgesic activity of polyherbal-formulated tea comprising of *Curcuma longa, Citrus limon, Zingiber officinale, Allium sativum,* and *Moringa oleifera.*

2. Materials and Methods

2.1. Chemical materials and equipment

The list of Chemicals/Solvent used in this study were: sodium nitrate (Lobachem, India), aluminum chloride (JHD, China), aluminum chloride (Lobachem, India), folin-ciocalteu's (Lobachem, India), sodium carbonate (Lobachem, India), TCA (Lobachem, India), DNPH (Lobachem, India), H_2SO_4 (Lobachem, India), thiourea (Kermel ,China), potassium hydroxide (Lobachem, India), petroleum ether (Lobachem, India), ammonium chloride (JHD, China), acetic acid (JHD, China), ammonium, citric acid (Kermel, China) and distilled water,. The drugs used were: bromohexane, sodium cromoglycate, dihydrocodeine (Accord, UK), pentazocine, aspirin. The equipment used were: UV spectrophotometer (Model 501, UK), food dehydrator (Model No.: SF-4006), water bath, hot plate, nebulizer glass chamber (24 x 12 x 24 cm), and nebulizer.

2.2. Composition of polyherbal tea

The polyherbal tea comprises *Curcuma longa, Citrus limon, Zingiber officinale, Allium sativum, and Moringa oleifera*.

2.3. Location of the study

This study was carried out in the department of science laboratory technology, faculty of life sciences, university of Benin, Benin City, Edo State, Nigeria. During the months of June through September (Wet Season).

2.4. Plant collection

Lemons bought from the New Benin Market in Oredo Local Government Area. Turmeric, ginger, garlic, and cloves were purchased at Oregbeni Market in Ikpoba Okha Local Government Area. *Moringa oleifera* leaves were gotten from farm land at the Faculty of Agriculture, University of Benin, in Ovia North East Local Government Area, all in Edo State, Nigeria.

2.5. Preparation of plant material/ polyherbal tea formulation

Lemon, ginger, garlic, and turmeric were properly washed and copped into smaller pieces. The moringa leaf was separated from its stalk and properly washed. The chopped lemon, ginger, garlic, turmeric, and moringa leaf were dehydrated using a food dehydrator (Model No.: SF-4006) at 50 degrees Celsius. Then, the dehydrated lemon, ginger, garlic, turmeric, and moringa were separately ground to a fine powder using an impact mill. The powdered lemon, ginger, garlic, turmeric, and moringa were weighed and mixed in an equal proportion (1:1:1:1:1) to formulate the herbal tea. The herbal tea was formulated in such a way that 1g in a tea bag contained 200 mg of each material.

2.6. Polyherbal tea extraction

Formulated polyherbal tea (1g) was weighed into 50 ml of warm distilled water in a 250 ml beaker. The mixture was stirred using a stirrer and left undisturbed for 10 minutes. The solution was filtered using a cosmonice filter into a 100ml sample bottle with a lid. 1 ml of the extract was concentrated to yield 10mg/kg.

2.7. Experimental animals

Eighty-five healthy adult Swiss albino mice and twenty Guinea pigs, of both sexes were obtained from the College of Medicine, Ambrose Alli University, Ekpoma, Edo State,

Nigeria. The mice weighed 20-30 gr, while the Guinea pigs weighed 250-600 gr. The mice and Guinea pigs were kept in the animal facility of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin and were given two weeks to adjust to the standard laboratory settings with a 12-hour light/dark cycle. They were feeding with regular animal food pellets regularly. The animals were treated according to standard procedures for laboratory animals

2.8. Quantification of flavonoids, alkaloids, and total phenolic compounds

The flavonoids, alkaloids, and total phenolic compounds of the polyherbal formulated tea was analyzed through the described method by Shazia et al.[15].

2.8.1. Alkaloids

An amount of 1g of plant extract was measured into a beaker with a capacity of 100 ml and dissolved in 50 ml of 2N hydrochloric acid. The liquid was agitated with a stirring device and left undisturbed for 10 minutes. The solution was strained using a special filter (0.5 ml) into a new container with a capacity of 100 ml. Sterilized test tubes were labeled as blank, standard, and test. The liquid was filtered and poured into the labeled test tube. Then, 2.5 ml of phosphate buffer with a pH of 4.7 and bromocresol green (2.5 ml) were added. Finally, chloroform (5 ml) was added. The solution was thoroughly agitated. The test tube containing bromocresol green (2.5 ml), phosphate buffer (2.5 ml) at PH 4.7, and chloroform (5 ml) was filled with water (0.5 ml) to create the blank. The liquid above the sediment of the empty, experimental, and reference samples was measured for its absorbance at a wavelength of 470 nm. A common, widely used graph of Quinine (a graph showing absorbance against concentration, R^2= 0.9755) was previously created, and the concentrations of alkaloids were estimated from it.

2.8.2. Flavonoids

The sterilized test tubes were labeled as blank, standard, and test. An amount of 0.5 ml of extract was placed into the test tube designated. Then, sodium nitrate (0.15 ml) and 10% aluminum chloride (0.15 ml) were added. The mixture was left undisturbed for 6 minutes. Next, a solution of sodium hydroxide 4% was added, and the total volume was adjusted to 5 ml using purified water. The test tube containing 0.15 ml of sodium nitrate (5%) and 0.15 ml aluminum chloride (10%), and water except the sample to create the blank. It was permitted to stand for 6 minutes, then 2 ml of 4% sodium hydroxide was addeds and brought up to 5 ml with distilled water. The blank test tube, reference samples, and experimental sample were measured for absorbance at a wavelength of 510 nm using a UV spectrophotometer. A common, widely used curve of quercetin (a graph showing absorbance versus concentration with a R^2 value of 0.9166) was previously created, and the amounts of flavonoids were estimated based on the curve.

2.8.3. Total phenolic compound

The sterilized test tubes were labeled as blank, standard, and test. At first an amount of (0.5 ml) of the sample was poured in the labeled test tubes, then folin-ciocalteu's solution, 2 ml (1:10), and 4 ml of a saturated solution of sodium carbonate (7.5%) was added. Then the test tubes were filled with water (0.5 ml) to create the blank. The blank test tubes, reference samples, and test were left for 30 minutes. The measurement of absorbance was conducted at a wavelength of 765 nm using a UV spectrophotometer (Model 501, UK). A common, widely used graph of garlic acid (absorbance vs. concentration, R^2= 0.9358) which was previously created, and the concentrations of total phenolic components were estimated from it.

2.9. Determination of non-Enzymatic antioxidant

Vitamin C, Total Carotenoids, and Lycopene were analyzed using the method of Moron et al.[16-19].

2.9.1. Determination of vitamin C

A 1 g of sample was added to the 1 ml of 4% TCA. A centrifugation process was performed at 2000 revolutions per minute for a duration of 10 minutes. The supernatant was treated with a small amount of activated charcoal. The solution was forcefully shaken and left for 5 minutes. The charcoal particles were separated through centrifugation. Two milliliters of TCA 4% were mixed with 0.5 ml of the supernatant, 0.2 ml of DNPH 2% in 9 N H_2SO_4, and two drops of a thiourea solution 10%. The substances were combined and kept at a temperature of 37°C for 3 hours, which led to the formation of formosazone crystals. The crystals were dissolved in 2.5 ml of sulfuric acid 85% at a low temperature. After adding sulfuric acid, DNPH reagent and thiourea were added to the blank. The tubes were chilled in ice, and the absorbance was measured at 540 nm in a spectrophotometer. A typical graph was created and presented in units of mg/g of sample.

2.9.2. Determination of total carotenoids and lycopene

Carotenoids and Lycopene were analyzed[16,17] according to the method of Palghat et al.[18].

The current study was carried out in poor lighting to avoid the degradation of carotenoids caused by light exposure. A mixture of 0.1 gr of the material and 0.5 ml of alcoholic potassium hydroxide 12% was kept in a water bath at a temperature of 60°C for 30 minutes. The transformed portion was transferred to a distinct funnel with 2–3 ml of petroleum ether and thoroughly combined. Then, the lower water layer was moved to a different separating funnel, and the upper layer of petroleum ether with the carotenoids was gathered. The extraction was

done again until the water layer became colorless. A small quantity of sodium sulfate, in its dry form, was added to the petroleum ether extract in order to eliminate any remaining moisture. The brightness of the yellow color was measured using a spectrophotometer at wavelengths of 450 nm and 503 nm, with petroleum ether as a reference. The overall carotenoid content was originally calculated using the formulas:

$$\text{Total carotenoids} = \frac{\text{A450 x Volume of the sample} \times 100 \times 4}{\text{Weight of the sample}}$$

$$\text{Total Lycopene} = \frac{3.12 \text{ x A503 Volume of the sample} \times 100}{\text{Weight of the sample}}$$

The Total Carotenoids and Lycopene were categorical as mg/g of the sample.

2.10. Antitussive

The antitussive property of polyherbal tea was evaluated as described by Uwaya et al.[20,21] using ammonium (NH4OH)-induced cough and citric acid-induced.

2.11. Ammonium induced-cough

Four groups were formed by randomly dividing 20 healthy adult mice, with 5 of both sexes' animals in each group.

Group 1 was given distilled water (10 ml/kg), group 2 was given 5 mg/kg of the polyherbal-formulated tea, group 3 was given 10 mg/kg of the polyherbal-formulated tea, and group 4 was given 25 mg/kg of dihydrocodeine.

One hour after giving mice distilled water, extract, or standard at varied doses, mice were placed in a glass chamber the size of 10 cm x 10 cm x 10 cm and treated with 0.3 ml of NH4OH 25% for 45 seconds. The number of coughing bouts was recorded for each mouse during a 5-minute period of exposure.

2.12. Citric acid-induced cough

The day before the test, animals were placed alone in a chamber (24 x 12 x 24 cm) and subjected to citric acid mist (7.5% w/v) to test for sensitivity using a nebulizer for 5 minutes. The animals which coughed 10-30 times were chosen for antitussive testing and randomly assigned to groups.

A total of 20 adult healthy guinea pigs of both sexex were divided randomly into 4 groups. Each group included 5 mice and 5 guinea pigs.

Group 1 received distilled water, group 2 was given 5 mg/kg of the polyherbal-formulated tea, group 3 was given 10 mg/kg of the polyherbal-formulated tea, and group 4 received 5 mg/kg of dihydrocodeine.

An hour later, the animals were exposed again to citric acid aerosol, and the number of coughsing was recorded for 5 minutes.

2.13. Expectorant

The mucus expectorant activity of a polyherbal tea formulation was evaluated by modifying the method described by Uwaya et al.[21].

Five groups of healthy mice were randomly formed, including 5 mice in each. Group 1 was given distilled water, group 2 was given 5 mg/kg of the polyherbal formulated tea, group 3 received 10 mg/kg of the polyherbal formulated tea, group 4 received 15 mg/kg of bromohexane, and group 5 was given 50 mg/kg of sodium cromoglycate.

The animals received treatment for a duration of five days. On the fifth day, after a period of fasting overnight, the animals were administered doses of the experimental compound 30 minutes prior to being exposed to a substance that induces the release of a specific compound, such as ammonium chloride (5 mg/kg). After 30 minutes, phenol red (500 mg/kg) was given to each mouse. Within 30 minutes of the phenol red injection, all animals were cervical dislocated. A 2 cm section of the trachea was excised (from the thyroid cartilage to the major stem bronchi). Each section of the trachea is stored for 30 minutes in a simple container with 2 ml of normal saline. An amount of 0.1 ml solution of 1 M/4% NaOH was added to the saline, and the absorbance of the mixture at 460 nm was measured with a spectrophotometer. The amount of phenol red was determined from a standard plot of absorbance against concentration. A commonly used curve (graph of absorbance against concentration, R^2=0.9681) was previously produced, to estimate the amounts of phenol red.

2.14. Analgesic

According to Uwaya et al.[20,21], the analgesic property of polyherbal tea was evaluated through hot plate and acetic acid induced pain.

2.15. Hot plate-induced pain

A total of 20 mice in good health were separated randomly into 4 groups. Each mouse was placed on a hot plate (not turned on) for 10 minutes to acclimatize to the environment. Group 1 was given distilled water, group 2 received 5 mg/kg of the polyherbal-formulated tea, group 3 was given 10 mg/kg of the polyherbal-formulated tea, and group 4 received 3 mg/kg of pentazocine intraperitoneally. The hot plate was adjusted to a temperature of 55 °C. After 30 minutes the animals were placed one by one on the hot plate. The time took to hop off the surface or lick their paws was recorded, using a stopwatch. An hour after taking the medicine orally, and then at 30, 60, 90, and 120 minutes interval, the animals were put on the hot plate and their response times were measured.

2.16. Acetic acid-induced pain

A total of 20 healthy mice were randomly divided into 4 groups, each group included 5 animals. Group 1 was given distilled water, group 2 received 5 mg/kg of the polyherbal-formulated tea, group 3 was given 10 mg/kg of the polyherbal-formulated tea, and group 4 received 100 mg/kg of aspirin. An hour after oral administration, the animals received 10 ml/kg of a acetic acid solution 0.6% through the peritoneum. The number of writhing by each mouse was counted for 30 minutes.

2.17. Statistical analysis

Data were presented as the average ± standard deviation. The data were examined using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical analysis was conducted using GraphPad Prism V.6.01, with a significance level of $p < 0.05$.

3. Results

Some phytochemicals and non-enzymatic antioxidants in polyherbal-formulated tea (*Moringa oleifera, Citrus limon, Allium sativum. Curcuma longa, Zingiber officinale*) were quantified.

Table 1 indicates the quantification of alkaloids, flavonoids, and total phenolic compounds in polyherbal-formulated tea. Polyherbal formulated tea contained 53.57 ± 1.96 mg/g phenolic compound, 40.93 ± 5.96 mg/g alkaloid, and 99.44 ± 1.96 mg/g flavonoid. Table 2 presents the quantification of 862 ± 18.76 mg/g Vitamin C, 52.00 ± 6.93mg/g Carotenoid, and 9.50±1.35 mg/g Lycopene in polyherbal formulated tea.

Table 1. Quantification of alkaloids, flavonoids and total phenolic compounds in poly-herbal formulated tea (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale*)

Constituents	Polyherbal formulated tea
Phenolic compound (mg/g)	53.57±1.96
Alkaloid (mg/g)	40.93±5.96
Flavonoid (mg/g)	99.44±1.96

n = 3

3.1. The effect of polyherbal-formulated tea (Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale) on cough

3.1.1. Citric acid-induced cough

As can be seen in Table 3, the effect of polyherbal-formulated tea (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale*) on citric acid induced cough in Guinea pig is indicated. An amount of 5 mg/kg, 10 mg/kg of the poly herbal aqueous extract, and 25 mg/kg of Dihydrocodeine significantly reduced the cough bouts and increased the percentage of cough suppression compared to the control group (**P < 0.01; *P < 0.05).

Table 2. The content of Vitamin C, Carotenoid, and Lycopene in polyherbal formulated tea (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale)*

Constituents	Polyherbal Formulated tea
Vitamin C (mg/g)	862±18.76
Carotenoid (mg/g)	52.00±6.93
Lycopene (mg/g)	19.50±1.35

n = 3

Table 3. The effect of polyherbal-formulated tea (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale*) on citric acid-induced cough in Guinea pig

Treatment	Cough bout before treatment	Cough bout after treatment.	Cough suppression (%)	cough inhibition (%)
Control	17.50±4.48	12.50±0.87	33.01±9.72	0.00
Extract (5mg/kg)	15.25±1.8	4.75±0.75**	71.93±4.34*	0.62
Extract (10mg/kg)	17.00±2.92	5.75±0.25**	65.01±6.22*	0.54
DHC (25mg/kg)	11.25±1.32	4.25±1.97**	70.51±7.66*	0.66

DHC: Dihydrocodeine, n = 5

3.1.2. Ammonium-induced cough

Figure 1 indicates the effect of polyherbal-formulated tea (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale)* on Ammonium induced cough in mice. The poly herbal tea formulation at 5 mg/kg and 10 mg/kg and dihydrocodeine (25 mg/kg) significantly reduced cough bouts in mice in comparison with control (**p<0.01 and p<0.05).

3.1.3. Phenol red dye secretion (Mucus expectorant)

Figure 2 indicates the effect of polyherbal-formulated tea (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale*) on phenol red dye secretion in mice. The extract at 5mg/kg and 10 mg/kg and standard

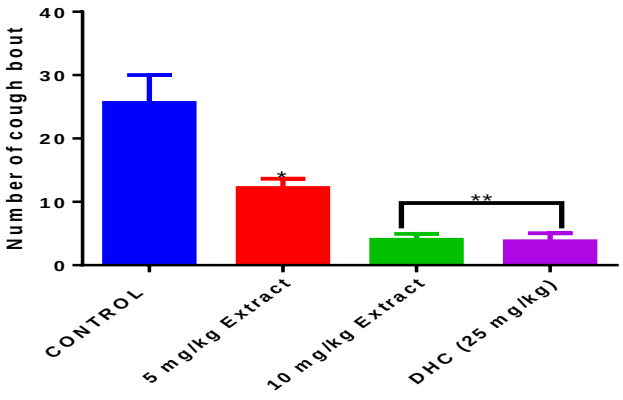

Figure 1. The effect of polyherbal-formulated tea (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale)* on ammonium induced cough in mice. DHC: Dihydrocodeine, n = 5.

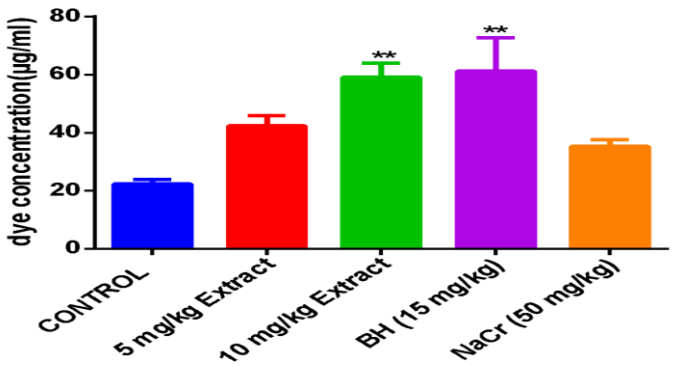

Figure 2. The effect of polyherbal tea formulation (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa,* and *Zingiber officinale*) on phenol red dye secretion in mice. BH: Bromohexiene.

(bromhexine, 15 mg/kg) increase phenol dye secretion compared with control group ($p < 0.01$).

3.2. The effect of polyherbal-tea formulation (Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale) on pain.

3.2.1. Hot plate-induced pain

The effect of polyherbal-formulated tea (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale*) on hot plate-induced pain in mice is indicated in Figure 3. The extract (5 mg/kg and 10 mg/kg) and Pentazocine (3 mg/kg) significantly elevate the latency of pain threshold in 2 hours after administration in mice (**P < 0.01;*P < 0.5)

3.2.2. Acetic acid-induced pain

Table 4 indicates the effect of polyherbal-formulated tea (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale*) on Acetic acid-induced pain in mice. The poly herbal extract and aspirin significantly reduced the number of writhing and increased the percentage of pain inhibition compared to control group (**P < 0.01).

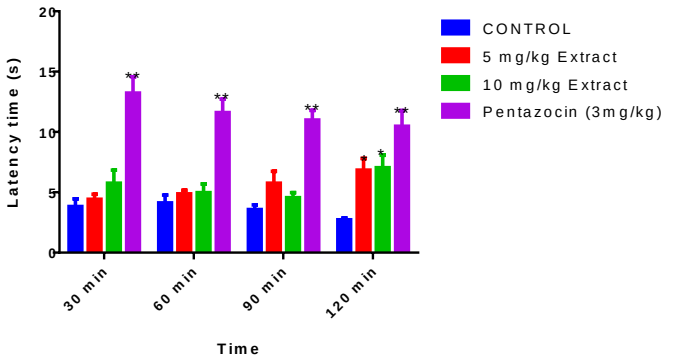

Figure 3. The effect of polyherbal-formulated teas (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, and Zingiber officinale*) on hot plate-induced pain in mice.

Table 4. The effect of polyherbal-formulated tea (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, Zingiber officinale*) on Acetic acid-induced pain in mice

Treatment	No. of writhing	Pain inhibition (%)
Control	118.00±7.60	0.00
5mg/kg of Extract	105.80±1.32	10.00
10mg/kg of Extract	70.80±7.59**	40.00
Aspirin (2mg/kg)	43.20±11.65**	63.00

n = 5

4. Discussion

The considerable chemical and biological impacts of bioactive plants has played a vital role in worldwide to prevent and treat diverse human illnesses[22]. Medicinal plants contain phytochemicals, which are vital compounds for plant growth and well-being. These phytochemicals provide various benefits for human health, such as managing blood sugar levels, decreasing inflammation, addressing diabetes, managing respiratory conditions, fighting against microorganisms, and delivering other advantageous effects[23,24]. The amount of phenolic compounds, alkaloids, and flavonoids in the polyherbal tea was measured and found as 53.57±1.96 mg/g for phenolic compounds, 40.93±5.96 mg/g for alkaloids, and 99.44±1.96 mg/g for flavonoids (Table 1). Due to the diverse pharmacological effects of Alkaloids, some alkaloids, such as morphine and codeine, are used as pain killers and analgesics[25]. Phenolic compounds and flavonoids have been reported to manage colds, coughs, dental and oral care, deodorants, perfumes, digestion, insect repellants, lice infection, menopause, menorrhagia, and sore throat[27]. The quantification of Vitamin C, carotenoid, and lycopene content in the polyherbal-formulated tea indicated in Table 2. Since some phytochemicals and some non-enzymatic antioxidants present in the polyherbal-formulated tea, it may be effective at preventing some conditions like inflammation, respiratory tract disorders, high cholesterol levels, heart conditions, colds, coughs, and stress.

In the present study, the polyherbal-formulated tea at doses of 5 mg/kg and 10 mg/kg, as well as the standard drug dihydrocodeine, exerted a cough-suppressing influence by reducing the cough bouts in mice with ammonium-induced cough (Figure 1) and citric acid-induced cough in guinea pigs (Table 3). According to Adejayan *et al.*[27] as Guinea pigs' airways have the necessary afferent nerves and they are capable of producing coughs similar to humans, they were selected for the citric acid-induced cough experiment. As stated by Lin *et al.*[28] ammonium hydroxide is known to cause irritation to the bronchial mucosa, and as a result, it stimulates the overproduction of fluid in the airways, which in turn assists in the easier removal of mucus. When inhaled, citric acid acts as a cough-inducing agent by stimulating the C-fibers' transient receptor potential. This leads to the release of tachykinins, which then trigger bronchoconstriction and mucus secretion. These processes stimulate the rapidly adapting receptors, which are known as well-researched cough receptors[29,30]. Researchers have

found that Vitamin C lowers the levels of histamine and 5-hydroxytryptamine or increases the production of prostaglandins to help people with coughing and catarrh[31]. It is found that the poly-herbal tea helped with coughing by reducing the number of coughing in mice (Figure 1) and Guinea pigs, as well as increasing the percentage of cough suppression in both groups (Table 3). These effects were similar to those of the standard drug, dihydrocodeine. Dihydrocodeine is a type of opioid medication derived from morphine, which attaches to the receptors in the central nervous system, thereby inhibiting the cough reflex. This is achieved by directly impacting the cough center in the medulla[32]. The poly-herbal-formulated tea may exert its mechanism of action through receptor activation, similar to dihydrocodeine, and due to the inclusion of Vitamin C and Carotenoids, and the phytochemicals present.

In order to evaluate the effectiveness of the poly-herbal-formulated tea as an expectorant, the tracheal phenol red secretion assay was used. The current study involves injecting phenol red after seven days of giving an expectorant. The increased secretion of phenol red from the trachea indicates improved expectorant activity. The poly-herbal-formulated teas (*Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, and Zingiber officinale*), as well as the standard drug bromohexane, were significantly impressive to increase phenol red secretion in mice (Figure 2), which was further enhanced by the presence of ammonium chloride. It is suggested that these substances aid in the secretion of mucus from the airway[21]. Bromohexane, a known expectorant, works by stimulating the vagus nerve in the gastric mucosa, resulting in thinner and easier-to-cough-up mucus. In addition, it alters the components of mucus by breaking chemical bonds, thereby reducing viscosity[33]. According to Wang *et al.*[34], the antitussive and expectorant activities of alkaloids might treat the cough. Flavonoids and triterpenoids present in plant extracts have been shown to decrease inflammation, thereby aiding in alleviating cough and promoting lung health. The mechanism of action of the poly-herbal-formulated tea is thought to be attributed to the presence of alkaloids, flavonoids, and phenols in plants[35].

According to the obtained findings, the poly-herbal-formulated tea at a dosage of 5 mg/kg and 10 mg/kg and pentazocine increased the latency time of pain in the hot plate-induced pain in mice (Figure 3). It is recommended that the poly-herbal-formulated tea may affect the central pain pathway, potentially involving various complex processes in the central nervous system, such as the opiate, dopaminergic, descending noradrenergic, and serotonin systems[36]. Pentazocine, being an opioid drug, produces analgesic effects by acting on μ opioid receptors in the central nervous system[37]. It is indicated that the poly-herbal tea had pain-relieving effects that were similar to pentazocine (Figure 3). Accordingly, the poly-herbal tea might have an effect on the opioid receptors in the brain and spinal cord, which could help alleviate pain. Plant secondary metabolites, including flavonoids and other phenolic chemicals, are commonly known[14]. These chemicals found in food and natural remedies. Both flavonoids and other phenolic compounds have been researched for their antioxidant, anticancer, antibacterial, cardioprotective, anti-inflammatory, and immune system-enhancing activities[38,39]. Flavonoids contain qualities that can relieve pain, reduce inflammation, and act as antioxidants, these effects are linked to their capacity to inhibit the synthesis of pro-inflammatory cytokines, which relies on the NF-KB pathway. Half of a dozen the existence of flavonoids and phenols in this tea created with many herbs (Table 1) is thought to be accountable for the way the tea works.

The polyherbal tea at dosage of 5 mg/kg and 10 mg/kg, along with aspirin, were able to lower the number of times that acetic acid made mice writhe (Table 4). The effects of the plant extracts were similar to those of aspirin. The release of serotonin, histamine, prostaglandins, bradykinins, and substance P, all known as pain mediators, is responsible for the pain caused by acetic acid[36]. Aspirin as a type of medication in the category of non-steroidal anti-inflammatory drugs, works by suppressing the activity of the cyclooxygenase enzymes. These enzymes play a crucial role in the arachidonic pathway, which is responsible for generating pain and inflammatory mediators, such as prostaglandins and bradykinins[40]. The ability of the polyherbal-formulated tea to increase the pain threshold and reduces writhing indicates could be as a pain reliever, both centrally and peripherally. Lycopene and carotenoid have been reported to exert anti-inflammatory and anti-cancer effects and are known to prevent chronic vascular diseases and cancer[41] and the mechanism of action of this polyherbal-formulated tea is believed to be the presence of these antioxidants (Table 2).

The present study indicates that the tea made from Moringa oleifera, citrus limon, Allium sativum, Curcuma longa, and Zingiber officinale could stop coughing, like the opioid receptor agonist dihydrocodeine, and ease pain, like the opioid agonist pentazocine. It is suggested that the polyherbal-formulated tea may work by interacting with opioid receptors or inhibiting the cyclooxygenase enzymes. Furthermore, the polyherbal-formulated tea possesses expectorant properties, which makes it suitable for treating coughs. Overall, the current study supports the use of these plants in traditional medicine to treat the cough and pain.

5. Conclusion

The results of the present study demonstrated that the polyherbal-formulated tea (Moringa *oleifera, citrus limon, Allium sativum, Curcuma longa, and Zingiber officinale*) contains phenolic compounds (53.57 ± 1.96 mg/g), alkaloids (40.93 ± 5.96 mg/g), flavonoids (99.44 ± 1.96 mg/g), Vitamin C (862 ± 18.76 mg/g), carotenoid (5200 ± 6.93 mg/g), and lycopene (19.50 ± 1.35 mg/g) and possesses antitussive, expectorant, and analgesic properties. Nonetheless, additional investigations should be conducted for better understanding of the mechanism, action, and the ascertained safety profile of the tea.

Declarations

Competing interests

According to the authors, there is no conflict of interest.

Authors' contributions

The authors affirm their involvement in the paper as follows: Examine development and planning: Dr. Dickson O. Uwaya. Data collection, analysis, and interpretation of results: Dr. Dickson O. Uwaya and Offiong Nnom Effiong. Draft manuscript preparation: Offiong Nnom Effiong and Dr. Dickson O. Uwaya. The final draft of the manuscript was approved by the authors after they had evaluated the findings.

Funding

This study did not receive any particular support from public, commercial, or not-for-profit organizations.

Availability of data and materials

The manuscript contains all datasets generated and/or analyzed in the current study.

Ethical considerations

The authors have examined the work for plagiarism, data falsification, multiple publications, and redundancy. The ethics committee of the Faculty of Life Sciences, University of Benin, with registration number LS23012, also authorized the study.

Acknowledgments

The authors would like to express their gratitude to Mr Lamidi-Afegbua Barnabas, a member of the laboratory staff in the department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Nigeria for his valuable assistance in conducting the work.

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