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ESTIMATIONS OF PHYTOCHEMICAL COMPOSITION AND NON-ENZYMATIC ANTIOXIDANT, AND ANTITUSSIVE, EXPECTORANT AND ANALGESIC PROPERTIES OF POLYHERBAL-FORMULATED TEA.

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ABSTRACT

For centuries, people have utilized combined herbal teas to address a diverse range of human health conditions like cough, cold and catarrh, pneumonia, pain arthritis, and cardiovascular problems. This study aims to enumerate phytochemical composition and non-enzymatic antioxidants while also assessing the antitussive, expectorant, and analgesic properties of a polyherbal-formulated tea (*Zingiber officiale*, *Moringa olifera*, *Allium sativum*, *Curcuma longa, Citrus limon*, and *Syzygium aromaticum*). Colorimetric methods were used to evaluate the phytochemical composition and non-enzymatic antioxidants. The antitussive efficacy of the tea was tested using citric acid, and ammonium-produced cough. Researchers investigated the expectorant activity by secreting phenol dye in mice. The analgesic effect of the tea was investigated utilizing a hot plate and an acetic acid-induced writhing animal model. The findings demonstrate that the polyherbal tea includes flavonoids (143.10±7.71mg/g), alkaloids (58.33±8.34mg/g), phenolic compounds (92.83±2.33mg/g), vitamin C (474.70±27.42mg/g), carotenoids (36.00±1.16mg/g), and lycopene (47.58±8.56mg/g). The tea reduced the number of cough bouts in mice ($p<0.05$) compared to the control in ammonium-induced cough in mice and citric acid-induced cough in guinea pigs. It also enhanced phenol red dye secretion ($p<0.05$) compared with the control in the expectorant trial. In analgesic experiments, the polyherbal tea increased the pain latency time compared to the control group ($p<0.05$) in hot plate-induced pain in mice. It also decreased number of writhing mice compared to controls ($p<0.01$) in acetic-induced pain in mice. In conclusion, our data demonstrate that polyherbal tea has antitussive, expectorant, and analgesic properties. The antitussive, expectorant, and analgesic activity might be because of the phytochemicals and antioxidants present.

Keywords: *Analgesic, Antitussive, Expectorant, Tea, Antioxidant*

INTRODUCTION

The World Health Organization (WHO) asserts that medicinal plants are the finest sources for obtaining a variety of medications. India, often known as the world's botanical garden, is the country that produces the most medicinal plants worldwide (Vijayalakshm and Selvaraj, 2018). Since ancient times, people have utilized herbal combinations with varied active principles and qualities to cure a wide spectrum of human ailments. Polyherbal formulations, as defined by Barik*et al.* (2016), are groups of medicines that combine and prepare composition to heal various illnesses. Herbal elements with varied pharmacological activity mainly interact together in aenergetic way to provide maximum healing advantages with the fewest negative effects. Researchers are studying mixtures of herbs to treat various lung diseases. These include asthma (Barik*et al.*, 2014), cough (Rui-zhi*et al.*, 2013), upper respiratory tract infections (Rudraswamy*et al.*, 2013), bronchitis (Tariq *et al.*, 2014), severe acute respiratory syndrome (Joseph *et al.*, 2005), tonsillitis (Yong *et al.*, 2013), pneumonia (Barik*et al.*, 2014), and other illnesses (Barik*et al.*, 2015).

Ginger (*Zingiberofficinale*), a genus of rhizomatous herbs, belongs to the family Zingiberaceae. Various foods commonly use ginger as a condiment and preservative (Devi *et al.*, 2017). Ginger is used as an antiemetic, antipyretic, antiasthma, antiarthritis, antistroke, antidiabetes, analgesic, and anti-inflammatory agent, as well as to treat gastrointestinal problems, toothache, and paralysis (Sharma *et al.*, 2020; Bhandari and Sethiya, 2018). *Moringaolifera,* a well-known Nigerian plant, has found widespread use and applications in traditional medicine for the treatment and management of a wide range of disease conditions (Iwara*et al.*, 2014*).* It belongs to the familyMoringaceae. Allium sativum, or garlic, is a member of the Alliaceae family (Singh and Singh, 2019). Globally, people widely cultivate and use garlic as a spice, additive, and medicinal plant to treat various ailments and physiological disorders (Tesfaye and Mengesha, 2015; Alare *et al*., 2020). Garlic is used to treat and prevent malaria, common colds, wound infections, coughs, lung TB, sexually transmitted infections, hypertension, mental problems, renal and liver diseases, asthma, and diabetes are among the conditions it addresses. It reduces common cold and flu symptoms by stimulating the immune system and demonstrating anticancer and chemopreventive activities.

In addition, aged garlic extract possesses hepatoprotective, neuroprotective, and antioxidative properties, whereas other preparations may stimulate oxidation (Amagase, 2006). A garlic extract may enhance immune cell function, which may be responsible for reducing the severity of colds and flu (Nantz*et al.*, 2012). Allicin, a chemical constituent of garlic, is an effective remedy for the common cold (Nahas and Balla, 2011). *Curcuma longa* is a flowering plant from the *Zingiberaceae* (Banik*et al.,* 2017). The active ingredient is curcumin (diferuloylmethane)(Shahrajabian and Sun, 2024). Traditional Indian medicine (Banik*et al.*, 2017; Briskey*et al.*, 2019) says that turmeric powder can help with inflammatory diseases, anorexia, coryza, cough, diabetic sores, hepatic diseases, stomach pain, gingivitis, periodontitis, wound and bacterial infections. *Citrus limon,* belongs to the *Rutaceae* family, and contains high-quality flavonoids, ascorbic acid, minerals, citric acid, and other crucial natural compositions (Mohanapriya*et al.,* 2013). It possesses antioxidant, anti-inflammatory, hypoglycemic, anticancer, and antibacterial properties (Kawaii *et al.,* 2000). According to studies, the major component of *C. limonene* essential oil, D-limonene, significantly reduces anxiety by triggering serotonin and dopamine reactions. It also inhibits pain receptors in a manner akin to indomethacin and hyoscine (Okwu, 2008). Clove (Syzygiumaromaticum) belongs to the family Myrtaceaceae (Batiha *et al.*, 2019).

Traditionally, people used cloves as a nerve stimulant and to treat flatulence, nausea, vomiting, liver, bowel, and stomach issues. Researchers in tropical Asia have documented the use of cloves to alleviate various microorganisms, including scabies, cholera, malaria, and tuberculosis. Traditionally, Americans have used clove to inhibit food-borne pathogens such as viruses, worms, candida, and various bacterial and protozoan infections (Bhowmik*et al.*, 2012). This study aims to enumerate certain phytochemical composition and non-enzymatic antioxidants while also assessing the antitussive, expectorant, and analgesic properties of a polyherbal-formulated tea *(Zingiberofficiale, Moringaolifera, Allium sativum, Curcuma longa, Citrus limon,* and *Syzygiumaromaticum)*. The polyherbal tea is composed of *Zingiberofficiale, Moringaolifera, Allium sativum, Curcuma longa, Citrus limon,* and *Syzygiumaromaticum.* The composition of each of the plants is 200 mg of*Zingiberofficiale,* 200 mg of *Moringaolifera,* 200 mg of *Allium sativum,* 200 mg of *Curcuma longa,* 200 mg of *Citrus limon,* and 50 mg of *Syzygiumaromaticum.*

MATERIALS AND METHOD

Plant collection

We purchased a lemon from the New Benin Market located in Oredo Local Government Area. We purchased garlic, cloves, turmeric, and ginger from Oregbeni Market in IkpobaOkha Local Government Area. Moringa leaf was obtained from a farmland of the Faculty of Agriculture, University of Benin, in Ovia North East Local Government Area, all in Edo State. The plants were identified by Dr. H.A. Akinnibosun of the Department of Plant and Biotechnology, University of Benin.

Preparation of plant material

Lemon, garlic, turmeric, and ginger were washed and chopped into smaller bits. The *moringa olifera* leaf was removed from the stalk and washed. The clove was washed. The chopped lemon, garlic, turmeric, ginger, clove, and washed moringa leaves were dehydrated using a dehydrator (Model No.: Sf-4006). After dehydration, the dehydrated lemon, garlic, turmeric, clove, ginger, and moringa leaf were ground to powder separately using an impact mill. The powdered lemon, garlic, turmeric, ginger, and Maringa leaf 200 mg of each and clove 100 mg of were weighed and mixed in a proportion of (1:1:1:1:1:0.5) to formulate the tea(Uwaya and Effiong, 2024).

Polyherbal tea extraction

The formulated polyherbal tea (3 g) was weighed into 150 ml of warm distilled water in a 250 ml beaker. The combination was stirred using a stirrer and allowed to stand for 10 minutes. The solution was filtered using a cosmonice filter or macron filter into a 100-ml sample bottle with a lid. 1 ml of the extract was concentrated to yield 10 mg/kg (Uwaya*et al.*, 2024).

Experimental Animals

Mice weighing 20–35 g and guinea pigs weighing 250-400 g were purchased in the animal house of Ambrose Ali University, located in Ekpoma, Edo State, Nigeria. The Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, housed the mice within its animal facility for two weeks, allowing them to acclimatise to normal laboratory conditions. Animals were given a regular grinder pellet and H_2O

spontaneously. With registration number LS23012, the ethical committee of the University of Benin's Faculty of Life Sciences gave its approval to this project.

Quantitative phytochemical analysis of the polyherbal-formulated tea

The flavonoids, alkaloids, and total phenolic compounds of the polyherbal-formulated tea were analysed according to the method below:

Determination of Flavonoids

The tea (0.5 ml), distilled water (1 ml), sodium nitrate (0.15 ml), and 10% ACl_3 (0.15 ml) were measured into a tube and kept to stand for 6 minutes. Next, 2 ml of 4% NaOH was added, and the volume was adjusted to 5 ml with distilled H_2O. The absorbance is read at 510 nm using the spectrophotometer (Model 501, UK). A curve of quercetin (a graph showing absorbance versus concentration with an R-value of 0.9166) was plotted, and the amounts of flavonoids were estimated based on the curve (Uwaya and Effiong, 2024; Shazia*et al.,* 2016).

Determination of alkaloid

One gram of the tea was mixed with 2N HCL and filtered. Filtrate (0.5 ml), bromocresol green (2.5 ml), and phosphate buffer (PH of 4.7), 2.5 ml were measured into a test tube. Chloroform (5 ml) was added and vigorously shaken to extract the alkaloid. The absorbance is read at 470 nm using the spectrophotometer (Model 501, UK). A graph of Quinine (a graph showing absorbance versus concentration with an R = 0.9755) was plotted, and the concentrations of alkaloids were estimated from it (Uwaya and Effiong, 2024; Shazia*et al.,* 2016).

Determination of total phenolic compound

The tea (0.5 ml), folin C (2 ml), and 7.5% sodium carbonate (4 ml) were mixed in a tube. The tube was incubated for 30 minutes at room temperature. Absorbance was read at 765 nm using the spectrophotometer (Model 501, UK). A graph of garlic acid (absorbance vs. concentration, R = 0.9358) was plotted, and the concentrations of total phenolic components were estimated from it (Uwaya and Effiong, 2024; Shazia*et al.,* 2016).

The determination of nonenzymatic antioxidants

Ascorbic acid, total carotenoids, and lycopene of the polyherbal-formulated tea were analysed according to the method bellow:

Determination of Ascorbic Acid

The tea (1 g) was added to the 1 ml of 4% TCA. The combination was centrifuged at 2000 rpm for 10 minutes. A nip of activated charcoal was added to the filtrate and vigorous shaking for 5 minutes. Centrifugation was done to remove charcoal particles. The supernatant (0.5 ml), added to 4% TCA (2 ml), and 2% DNPH (0.5 ml) in 9N H_2SO_4, followed by two drops of 10% thiourea solution, and incubated at 37 $^{\circ}C$ for 3 hours, resulting in formosazone crystals. The crystals were dissolved in 2.5 ml of 85% sulfuric acid in cold. The tubes cooled in ice, and the absorbance was read at 540 nm with a spectrophotometer. A standard graph for ascorbic acid was constructed for which vitamin C was extrapolated (Uwaya and Effiong., 2024; Palghat and Matheswaran, 2016; Russo *et al.,* 2000)

Determination of Total Carotenoids and Lycopene

The tea (0.1 g) was added to 12% alcoholic KOH (0.5 ml) in a water bath at 60 °C for 30 minutes. The saponified extract was transferred to a separating funnel containing 2-3 milliliters of petroleum ether and thoroughly mixed. Next, the top petroleum ether layer, which contained the carotenoids, was collected. Extraction was repeated until the aqueous layer became colorless. A tiny quantity of anhydrous sodium sulfate was added to the petroleum ether extract to moisten. The absorbance of the yellow shade was read in a spectrophotometer at 450 nm and 503 nm with the utilization of petroleum ether as the blank. The amount of full carotenoids and lycopene was determined using the equations: (100/ Sample Volume / A450 / 4) / Sample Weight and (A503 x 3.12 x Sample Volume x 100) / Sample Weight, respectively (Uwaya and Effiong., 2024; Palghat and Matheswaran, 2016; Russo *et al.,* 2000).

The antitussive properties analysis.

The antitussive effect of the polyherbal-formulated tea was studied using the methods stated below:

Ammonium-induced cough in mice

Mice weighing 25–40 g were put into five groups (n = 5). Group one obtained distilled water (10 ml/kg), Group two obtained 25 mg/kg of dihydrocodeine, and Groups Three and Four obtained 5 mg/kg and 10 mg/kg of the polyherbal tea. Mouse utilized was placed in a 1,000-ml unique chamber and subjected to 25% ammonia (0.3 ml)for 45s. Mice were taken out and put in a chamber with an aperture at the top, and cough frequency was counted for 5 minutes (Uwaya and Effiog. 2024; Uwaya*et al.*, 2023; Uwaya*et al.*, 2022; Ozolua*et al.*, 2012; Xu *et al.*, 2005).

Citric acid-induced cough

An ultrasonic nebulizer delivered 7.5% citric acid aerosol to the guinea pigs in a 24 x 12 x 24 cm Perspex. The frequency of coughs was counted, also known as the basal values for 5 minutes. We considered the guinea pigs with more than 10 bouts to have passed the test. The guinea pigs that passed the test were further divided into four groups (n = 5), treating group I with 10 ml/kg of distilled water after a single day of fasting (with water ad libitum). Group 2 was treated with 25 mg/kg of dihydrocodeine (DHC), and Groups 3–4 were treated with 5 mg/kg and 10 mg/kg of polyherbal-formulated tea 1 hour before exposure to citric acid. The percentage of cough suppression was calculated for every animal as follows: Percentage Cough Suppression (1- (CIC2)/C1) = 100 (where C1 is the number of coughs before medication administration and C2 is the number of coughs after drug administration) (Uwaya and Effiog. 2024; Uwaya*et al.*, 2023; Uwaya*et al.*, 2022; Ozolua*et al.*, 2012; Xu *et al.*, 2005).

Phenol red dye secretion in mice (expectorant)

Mice (25) were randomly assigned to the following groups: (1) 10 ml/kg distilled water; (2) 5 mg/kg polyherbal-formulated tea; (3) 10 mg/kg polyherbal-formulated tea; (4) 15 mg/kg bromohexine hydrochloride; and (5) 50 mg/kg of sodium cromoglycate (i p.). Ammonium chloride (5 mg/kg) was administered 30 minutes after administration of the test substance. Each mouse received an intraperitoneal injection of phenol red (500 mg/kg) 30 minutes later. Twenty minutes later, we sacrificed all the mice. The trachea was removed from the thyroid cartilage to reach the essential stem bronchi. Each trachea was once stored for 30 minutes in 2 ml of normal saline. 0.1 ml of IM sodium hydroxide was delivered to the fluid to stabilise the pH. A spectrophotometer was used to examine the absorbance of

phenol pink released from the trachea at 460 mm (Uwaya and Effiog. 2024; Uwaya*et al.*, 2023; Uwaya*et al.*, 2022; Ozolua*et al.*, 2012; Xu *et al.*, 2005)..

Analgesic activity of the polyherbal-formulated tea

The analgesic activity of the tea was evaluated using the method stated bellow:

Hot plate test

The temperature of the warm plate remained constant at 55°C. Adult Swiss mice (25 to 35 g) of each sex, were screened for appropriate response time 24 h before drug administration. The animals were divided into four groups of five mice each, as follows: The mice received four different treatments: (1) distilled water (10 ml/kg); (2) 5 mg/kg polyherbal-formulated tea; (3) 10 mg/kg polyherbal-formulated tea; and (4) 3 mg/kg pentazocine. Thirty minutes later, the animals were placed on the hot plate and recorded the index of response latency, which is the time between placement and licking, biting the hind paws, or jumping. Response latencies were recorded at 30, 60, and 90 minutes (Uwaya and Effiong, 2024; Uwaya*et al.,* 2023; Uwaya *et al.,* 2022).

Acetic acid-induced writing in mice

Twenty mice were divided into four groups, each containing five animals: Group 1 received distilled water (10 ml/kg) orally, while groups 2 and 3 received oral doses of polyherbal-formulated tea at 5 mg/kg and 10 mg/kg, respectively. Group 5 obtained aspirin (100 mg/kg). Every mouse received an intraperitoneal injection of acetic acid (10 ml/kg) thirty minutes later. The number of writhings was counted, characterised by constriction of the abdominal muscle and stretching of the hind limbs, for 30 minutes after the acetic acid injection. The study defined pain inhibition as a percentage of protection. % inhibition of pain = [mean writhing (control) - mean writhing (tested)] [Mean writhing (control)] 100 Where The term "mean writhing (control)" refers to the average writhing of animals treated with distilled water, while "mean writhing (treated)" refers to the average writhing of animals given the polyherbal-formulated tea (*Zingiberofficinale, Moringaolifera, Allium sativum, Curcuma longa, Citrus limon,* and *Syzygiumaromaticum*) (Uwaya and Effiong, 2024; Uwaya*et al.,* 2023; Uwaya *et al.,* 2022).

Statistical Analysis.

Data were expressed as mean ± standard error of the mean (SEM), and 'n' reflects the number of guinea pigs or mice per experimental group. One-way analysis of variance (ANOVA) was done using Newman Keuls' post hoc test. All data were examined using GraphPad Prism (UK) software version 6. $P<0.05$ denotes a substantial difference between the compared data.

RESULTS

Quantification of some phytochemical and Non-antioxidants polyherbal formulated tea

Table 1 shows that the polyherbal tea composed of *Zingiberofficinal*e, *Moringaoleifera, Curcuma longa, Citrus limon, Allium sativum, and Syzygiumaromaticum* contains flavonoids (143.10±7.71 mg/g), alkaloids (58.33±8.34 mg/g),

phenolic compounds (92.83±2.33 mg/g). Table 2 indicates that the polyherbal tea contains vitamin C (474.70±27.42 mg/g), carotenoids (36.00±1.16 mg/g), and lycopene (47.58±8.56 mg/g).

Table 1: Quantifiable phytochemical content of total phenol compound, flavonoid, alkaloid of the polyherbal-formulated tea

Constituents	Polyherbal Formulated tea
Phenolic compound (mg/g)	92.83±2.33
Alkaloid (mg/g)	58.33±8.34
Flavonoid (mg/g)	143.10±7.71

n = 3

Table 2: The content of Vitamin C, Carotenoid, and Lycopene in the polyherbal-formulated

Constituents	Polyherbal Formulated tea
Vitamin C (mg/g)	474.70±27.42
Carotenoid (mg/g)	36.00±1.16
Lycopene (mg/g)	47.58±8.56

n = 3

The effect of polyherbal-formulated tea on cough

Citric acid-induced cough in guinea pigs

Table 3 shows the effect of administering the polyherbal-formulated teas on citric acid-induced cough in guinea pigs. The polyherbal-formulated tea at 5 mg/kg, 10 mg/kg, and DHC at 30 mg/kg (standard) increased the percentage suppression of cough bouts (*p<0.05, **p<0.01) compared to the control.

Table 3: The effect of administering polyherbal-formulated teas on citric acid-induced cough in guinea pigs

Treatment	**Number of coughs before**	**Number of coughs after**	**% suppression**	**% inhibition**
Control	14.50 ± 2.02	11.50 ± 0.95	15.85 ± 12.67	0
PTea(5mg/kg)	11.75 ± 0.75	6.00 ± 1.80*	53.10 ± 6.15*	47.82
PTea(10mg/kg)	14.75 ± 3.84	3.75 ± 0.63**	70.88 ± 7.24**	67.39
DHC (25mg/kg)	16.50 ± 3.88	4.75 ± 1.75**	72.78 ± 7.17**	58.69

The polyherbal-formulated tea at 5 mg/kg, 10 mg/kg, and DHC at 25 mg/kg (standard) increased the percentage suppression of cough bouts (*p<0.05, **p<0.01) compared to the control, n = 5.

Note: DHC is Dihydrocodeine; PTea is Polyherbal tea extract

Ammonia-induced cough in mice

Figure 1 shows the effect of administering a polyherbal-formulated tea on mice's ammonia-induced cough. The polyherbal-formulated tea at 5 mg/kg, 10 mg/kg, and DHC at 25 mg/kg (standard) reduced the number of cough bouts in mice (**p<0.01) compared to the control.

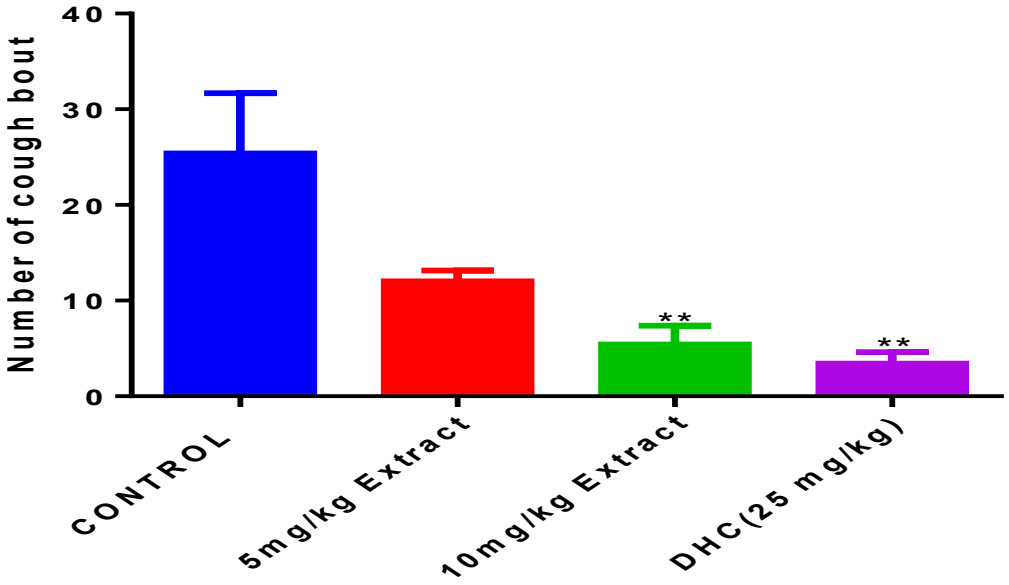

Figure 1: The effect of administration of polyherbal-formulated tea on ammonia-induced cough in mice. The polyherbal-formulated tea at 5 mg/kg, 10 mg/kg, and DHC at 25 mg/kg (standard) reduced the number of cough bouts in mice (**p<0.01) compared to the control, n = 5 per group. DHC is Dihydrocodeine

Phenol red dye secretion in mice (Expectorant)

Figure 2 shows the effect of the polyherbal-formulated tea bromohexine and sodium cromoglycate on phenol red dye secretion in mice. The polyherbal-formulated teas at 5 mg/kg, 10 mg/kg, and standard (bromohexine, 15 mg/kg) increased phenol red dye secretion (*p<0.05, **p<0.01) compared with control.

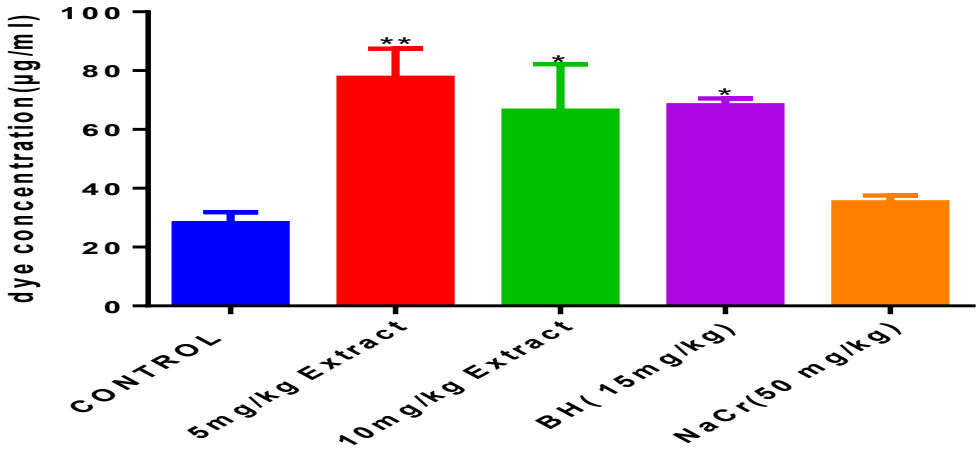

Figure 2: The effect of the polyherbal-formulated tea BH, bromohexine, and NaCr, sodium cromoglycate, on phenol red dye secretion in mice. The polyherbal-formulated teas at 5 mg/kg, 10 mg/kg, and standard (bromohexine, 15 mg/kg) increased phenol red dye secretion (*p<0.05, **p<0.01) compared with control, n = 5 per group.

Analgesic effect of the polyherbal-formulated tea

Hot Plate Test

Figure 3 shows the effect of the polyherbal-formulated teas on hot plate-induced pain in mice. The polyherbal-formulated tea and standard (pentazocine, 3 mg/kg) significantly increased the latency time of pain compared to the control (*p<0.05, **p<0.01;***$p<0.*0001). At all doses, the polyherbal-formulated tea increases the threshold for pain.

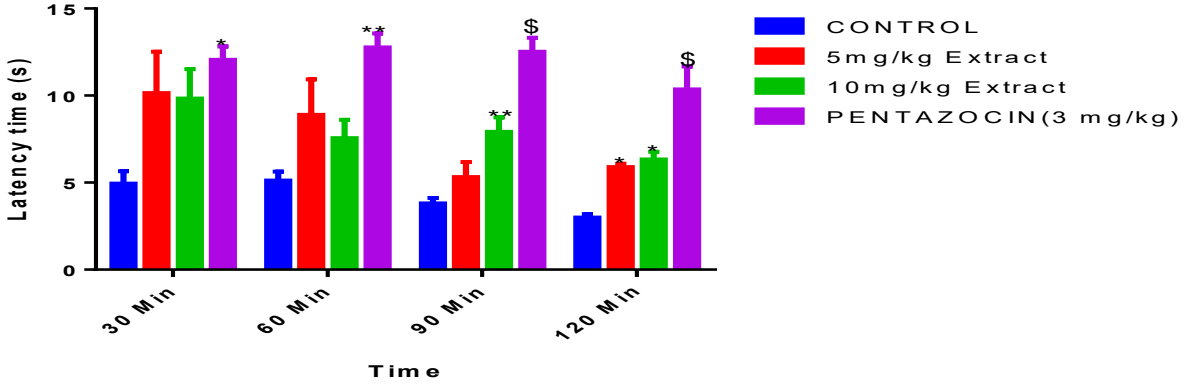

Figure 3 The effect of polyherbal tea on hot plate induced pain in mice. The polyherbal formulated tea and standard (pentazocine, 3 mg/kg) significantly increased the latency time of pain compared to the control (*p<0.05, **p<0.01, $p<0.0001). The polyherbal-formulated tea at all dose increases the threshold of pain, n=5 per group.

Acetic acid-induced writhing in mice

Table 4 indicates the effect of polyherbal tea on acetic acid pain. The polyherbal-formulated tea and standard (aspirin, 100 mg/kg) significantly reduced the number of writhing mice compared to controls (**p<0.01, ***p<0.001).

Table 4: The effect of polyherbal-formulated teas on acetic-induced pain in mice.

Treatment	Number of writing	%inhibition
Control	119.20 ±7.38	0
PTea(5mg/kg)	56.60 ± 7.18***	52.46
PTea(10mg/kg)	74.80 ± 8.20**	37.25
Aspirin (100mg/kg)	49.40 ± 10.82***	58.56

The polyherbal formulated tea and standard (aspirin, 100 mg/kg) significantly reduced the number of writhing mice compared to controls (**p<0.01, ***p<0.001), n = 5 per group. PTea is a Polyherbal tea extract.

DISCUSSION

Research has proven that phytochemical constituents of plants handle health benefits (Ozcan*et al.*, 2014). This study shows that the polyherbal-formulated tea comprises *Zingiberofficinale, Moringaolifera, Allium sativum, Curcuma longa, Citrus limon,* and*Syzygiumaromaticum* contains flavonoids (143.10±7.71 mg/g), alkaloids (58.33±8.34 mg/g), and phenolic compounds (92.83±2.33 mg/g) (Table 1). The amount of ascorbic acid present is 474.70±27.42 mg/g, the carotenoid present is 36.00±1.16mg/g and the amount of lycopene is 47.58±8.56 mg/g (Table 2). Studies have demonstrated the biological properties of these secondary metabolites, including anti-diabetic, antimicrobial, antioxidant, anti-carcinogenic, hypoglycemic, antipyretic, analgesic, and anti-malaria activities (Negi*et al.*, 2011). Several studies, have shown that flavonoid and phenolic compounds can help with pain, inflammation, allergies, asthma, arthritis, and other respiratory problems(Uwaya and Effiong, 2024; Ahmed *et al*., 2016). They can also lower fever, reduce inflammation, protect the heart, kidneys, liver, and brain, and kill microbes (Uwaya and Effiong., 2024; Hossain *et al.*, 2011; Spencer *et al.*, 2008). Alkaloids are effective at fighting malaria, asthma, tumors, high cholesterol, narrowing blood vessels, pain, and high blood sugar (Uwaya and Effiong., 2024; Kittakoop*et al.*, 2014; Cushnie*et al.*, 2014; Qiu*et al.*, 2014; Russo *et al.*, 2020). Vitamin C is a strong antioxidant and helps protect molecules in the body, such as proteins, lipids, carbohydrates, and nucleic acids, from damage by free radicals and reactive oxygen species that can be generated during normal metabolism and through exposure to toxins and pollutants (Halliwell, 2014; Li and Schellhorn, 2007).

It also fights molecules that trigger rheumatoid inflammation and is an effective adjunctive therapy for specific pain relief (Carol, 2007; Anitra and Cate, 2017; Pong, 2014). Carotenoids and lycopene are powerful antioxidants. They protect against many degenerative conditions, including heart disease, diabetes, cancer, macular degeneration,

cataracts, arthritis, and skin damage, and promote male fertility (Perusek and Maeda, 2013; Wang *et al.*, 2013; Mora-Esteves and Shin, 2013). The formulated tea, containing some phytochemical and non-enzymatic antioxidants, can effectively manage conditions such as arthritis, respiratory disorders, colds, coughs, catarrh, hypertension, stress, lower cholesterol, boost immunity, relieve pain, and promote male fertility.

In this study, we found that 5 and 10 mg/kg of polyherbal tea and dihydrocodeine, as well as 25 mg/kg, reduced cough frequency and increased the percentage of cough suppression in guinea pigs with citric acid-induced cough (Table 3). Researchers have used a citric acid-induced cough model in conscious guinea pigs as a sensitive model to evaluate some of the centrally acting opioid antitussive drugs like codeine and morphine (Uwaya and Effiong., 2024; Adejayan*et al.*, 2019). Citric acid can affect cough receptors directly or through the mucosa's release of an intermediate agent (Adejayan*et al.*, 2019). When inhaled, citric acid acts as a cough-inducing agent by stimulating the C-fibres' 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 (Uwaya and Effiong, 2024; Uwaya*et al.*, 2023; Uwaya*et al.*, 2022; Myers *et al.*, 2002; Canning *et al.*, 2001).

In mice, polyherbal-formulated tea (10 mg/kg) and dihydrocodeine (25 mg/kg) reduced cough bouts in ammonia-induced coughs (Figure 1). The ammonia-induced cough model is a common chemical stimulus model that can be used easily to test the cough-relieving effects of bioactive components in new drug development from traditional medicine (Wu*et al.*, 2018). Several investigators have previously used this model as a valid method (Wang *et al.*, 2012; Liu *et al.*, 2015). The polyherbal tea's ability to reduce citric acid and ammonia-induced cough bouts, along with an increased percentage of cough suppression, indicates its antitussive activity. Table 3 and Figure 1 indicate that the antitussive effect of the formulated tea is dose-dependent. There were similar results for the tea and the standard drug dihydrocodeine in terms of how well it stopped coughing (53.10 ± 6.15% and 47.82% for 5 mg/kg, 70.88 ± 7.24% and 67.39 % for 10 mg/kg, and 72.78 ± 7.17% and 58.69% for dihydrocodeine, 25 mg/kg, respectively). This suggests that the formulated tea may work similarly. Dihydrocodeine is a drug that is derived from morphine, which acts on the opioid receptor. Vitamin C reduces the levels of histamine and 5-hydroxytryptamine or increases the production of prostaglandins (Konya and Ferdinandy, 2006). The anticough activity of the polyherbal-formulated tea might be due to its interface with opioid receptors or the presence of phytochemicals (alkaloid, flavonoid, and phenolic compounds) and antioxidants (vitamin C, carotenoids, and lycopene) in the tea.

To determine the expectorant activity of the polyherbal-formulated tea, a trachea phenol red secretion assay was used. The development of this model is based on the principle that injecting phenol red after giving an expectorant for four consecutive days will enhance phenol red secretion from the trachea (Uwaya and Effiong, 2024; Uwaya*et al.*, 2023; Uwaya*et al.*, 2022). The polyherbal-formulated tea *includes Zingiberofficinale, Moringaolifera, Allium sativum, Curcuma longa, Citrus limon, and Syzygiumaromaticum* (5 and 10 mg/kg) along with bromhexane (15 mg/kg) to enhance the emission of phenol red dye from the trachea (Figure 2). The tea's ability to secrete phenol red dye from the trachea indicates that it may serve as a good expectorant.

An anti-nociceptor prevents the perception of pain by acting on peripheral or central pain receptors, either by enhancing their threshold or by preventing the propagation of their reflexes. Pain is associated with various clinical conditions like arthritis, cancer, and vascular diseases (Sharma *et al.*, 2019). The current study investigated the analgesic activity of the polyherbal extract using acetic acid-induced writhing and the hot plate model. Aspirin, 100 mg/kg, and polyherbal tea(5 and 10 mg/kg)diminished the number of abdominal constrictions or writhes (Table 4). The extract also increased the percentage inhibition of abdominal constriction from 0% to 52.46%. This effect was closely similar to that of the reference drug aspirin, which has a percentage inhibition of abdominal constrictions of 58.56% (Table 4). Acetic acid-induced writhing reflex is a model of visceral pain that is highly useful for screening analgesic drugs (Raquibul*et al.,* 2010). Researchers have reported that an intraperitoneal injection of acetic acid significantly increases the level of prostanoids, specifically PGE2 and PGF2, and lipoxygenase products in the peritoneal fluid, thereby inducing pain in the rodent (Dhara*et al.*, 2000; Brignola*et al.*, 1994). The polyherbal extract's ability to attenuate acetic acid-induced writhing in mice suggests that it possesses analgesic activity. The percentage of pain inhibition also shows that the tea and aspirin standard drugs are comparable. This result aligns with the investigations conducted by Antomisamy*et al.* (2017) and Odoma(2019), which suggest that the extract's ability to inhibit acetic acid-induced writhing is a sign of its analgesic potential.

Lee and Choi (2008) found that the hot plate method, originally described by Woolfe and MacDonald (1994), is suitable for the evaluation of centrally acting analgesics. Thermally induced nociception suggests narcotic involvement (Uwaya and Effiong, 2024). Centrally acting analgesics usually raise the pain threshold in mice. Sensory nerves appear to sensitize the nociceptors, while endogenous peptides like prostaglandins may have minimal involvement in this model (Mohan *et al.*, 2009). In this study, the polyherbal-formulated tea and standard (pentazocine, 3 mg/kg) increased pain latency time. The tea at all doses increased the threshold of pain (Figure 3). Pentazocine has a better analgesic effect compared to the tea (Figure 3). The formulated tea's ability to increase the pain threshold demonstrates its analgesic activity. The polyherbal-formulated tea, containing *Zingiberofficinale, Moringaolifera, Allium sativum, Curcuma longa, Citrus limon, and Syzygiumaromaticum*, effectively reduces acetic acid-induced pain and increases the pain threshold in hot plate-induced pain, indicating its potential as a peripheral and central analgesic. Flavonoid, alkaloid, lycopene, carotinoid, and vitamin C may also be present in the tea's analgesic effect (Uwaya and Effiong, 2024; Carol, 2007; Anitra and Cate, 2017; Peng, 2014). The analgesic activity of the tea could be the presence of ginger, moringa, and clove. Research has it that ginger, moringa, turmeric, and clove possess analgesic properties (Sun *et al.*2018; Ajanaku*et al.,*2202; Li et al., 2008). The antitussive activity of the tea may be attributed to ginger, garlic, moringa, and lemon. It has been demonstrated that turmeric, moringa, and ginger possess anti-allergic, anti-inflammatory, antioxidant, and immunomodulatory effects (Bhattacharya et al., 2018; Sun *et al.*2018). Lemon has been shown to have an abundance of vitamin C, which may play a role in neutralizing free radicals and **possess** cold and flu activities (Nisar*et al*., 2018).

CONCLUSION

Polyherbal-formulated tea comprises *Zingiberofficinale, Moringaolifera, Allium sativum, Curcuma longa, Citrus limon, and Syzygiumaromaticum.* has demonstrated the occurrence of numerous phytochemicals, such as flavonoids (143.10±7.71), alkaloids (58.33±8.34), and total phenolic compounds (92.83±2.33), vitamin C (474.70±27.42), carotenoids (36.0±1.16), and lycopene (47.5±8.56). The formulated tea possesses antitussive, expectorant, and analgesic activities.

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CONFLICT OF INTEREST

The authors disclose no conflict of interest.

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