Evaluation of medicinal plants blend formulation for symptomatic relief, phytochemical and microbiological quality and safety

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Abstract

Background: Medicinal plants are employed for the primary health care by the community as a supportive care for symptomatic relief of generalized pain, headache, fever, cough and others symptoms. This study was therefore aimed to evaluate blends of traditionally used medicinal plants for symptomatic relief effects besides determining the microbial and, phytochemical qualities and sensorial acceptability.

Methods: Laboratory animals-based experiment was employed in this study. The medicinal herbs blend preparations used as tea infusion were Cymbopgon citratus, Menta spicata, Thymus schimperi, Ruta chalepensis and Stevia rebaudiana. The essential oil blends of Rosmarinus officinalis, Salvia officinalis and Cymbopgon citratus in aqueous solution were used as oral gargle. The tea infusion and essential oils were administered as a single dose by oral gavage in mice. The doses used for analgesics effect of tea infusion were 350, 700 and 1400mg/Kg, and for the antipyretic effect of the gargle solution was five drops essential oil blends in 100ml distilled water. Oral gargle containing 2100mg/Kg tea infusion and five drops of essential oil in 100ml distilled water were used to evaluate the anti-inflammatory activity in mice. The phytochemical and microbial quality and safety, and sensory acceptability were also investigated for medicinal plant blend preparations.

Results were summarized as mean ± standard error of mean. The analysis was done using one-way analysis of variance (ANOVA). The means values were compared using Dunnet’s t- test.

Results: The medicinal herb plant possessed analgesic, anti-pyretic and anti-inflammatory activities. The presence of bioactive compounds that was attributed for symptomatic relief was indicated by quantitative determination of total flavonoids and phenolic compounds in the tea infusion and oral gargle solution. The results also showed that the microbial quality was within prescribed limits of international pharmacopeia and WHO requirements. The tea infusion and oral gargle had good sensorial acceptability indicating accepted palatability.

Conclusion: The investigation verified the symptomatic relief effects of the medicinal plant blends besides meeting the phytochemical and microbial quality parameters requirements of the medicinal herb’s blends. The findings entails that the plants may be used to relieve pain, fever and inflammation.

Key words: Medicinal plants, herbal preparations, symptomatic relief, phytochemical, microbial quality

Introduction

In Ethiopia, traditional medicine appears to be the source of healthcare for large segment of the population. The people have relied for centuries on a system of traditional or indigenous health care in the prevention, diagnosis and treatment of ailments. Traditional medicine is an integral part of the local culture that is affordable and accessible even when there is demonstrably efficient and less costly alternative modern health care. Traditionally used medicinal plants are the mainstay of primary healthcare. They are also used as a source of income and improving livelihood for the community (Fassil 2003; Fulas 2012). Cognizant of these facts and cultural acceptance of using medicinal plants in the traditional health care system, there is a need to ascertain their safety, efficacy, dosage and quality through rigorous scientific evaluation using pre-clinical studies and clinical trials. This will facilitate to gain trust to promote the use of scientifically validated medicinal plants and mainstream into the health care system. Various medicinal plants are employed for the primary health care by the community commonly as a home remedy for supportive care through the relief of generalized pain, headache, fever, cough and others symptoms (Abdul and Mousa 2017; Mathens and Bellanger 2010; Abate 1989).

Different medicinal plants that are employed for various illness are sold by vendors during market days in the markets of various towns of Ethiopia (Kloos 1978). Among marketable medicinal plants, some are commonly used by the community such as Damakassie, Tenaadam, Tosige, Dingetiga, Zingebe, etc. are used for generalized or sudden pain, fever, cough, etc., The remedies are commonly administered as skin rubbing/painting or sniffing pressed leaves juice,
fumigation of the cooked leaves or other parts of the herbs, steam inhalation by soaking the herb in boiling water, drinking the tea infusion of the herbs, etc. (Abate 1989; Fulas 2001; Rusike and Mammen 2014).

With increased prevalence of viral respiratory tract infections such as influenza and influenza like illness including the recent pandemic Covid-19, that are commonly manifested by cough, pain, fever, inflammation, etc., the unavailability of optimal medications and vaccines shortage or accessibility challenge, the cultural acceptance and common use of traditional medicine by the community, stimulate and necessitate the need to investigate some of the claimed medicinal plants that relieve pain, fever, etc. this may support considerably the nursing and home care of the patient (Abdul and Mousa 2017). Simulating the traditional way of preparation for ease of administration, considering reports from previous studies in the extracts of the medicinal plants, as a means to enhance the acceptance or preference for the palatability, flavor and other physicochemical conditions, the selected medicinal plants were prepared as blends for herbal tea infusion and oral gargle. The current study was therefore intended to evaluate the analgesics, antipyretic and anti-inflammatory effects of the blends for herbal tea infusion and oral gargle besides the determination of the physicochemical and microbial quality of the formulations.

Materials and methods

Plant material and sample preparation

The medicinal plants used for used for the preparation of the herbal tea were Cymbopgon citratus (DC.) Stapf (voucher No, CC-2011), Menta spicata L. (voucher no. MS-2012), Thymus schimperi Roniger (voucher No, TS-2014), Ruta chalepensis L. (voucher No, RC-2013) as primary herbs for the desired biological effect, Stevia rebaudiana (Bertoni) Bertoni (voucher No. SR-2016) as secondary herb for pleasant test and fragrance. While the medicinal plants used for the preparations of the oral gargle blends were Rosmarinus officinalis L. (Voucher no. RO-2015), Salvia officinalis L. (voucher no. SO-2017) and Cymbopgon citratus (DC.) Stapf (voucher No, CC-2011), respectively. The indicated plants were cultivated and propagated as per good agricultural practice (GAP). The plants were collected from Wondo Genet agricultural research center experimental site between February 2019 and 2020, Wondo Genet, Sidama region.

The study area is geographically located at 7°192’ N latitude and 38° 382’ E longitudes with an altitudinal range of 1780-1920 meter above sea level. The collected fresh leaves of the plants were transported to EPHI using mobile refrigerators for processing and preparation. Voucher specimens of the collected samples were deposited in Traditional and Modern Medicine Research Directorate Herbarium, Ethiopian Public Health Institute, Addis Ababa.

Chemicals, reagents and standard drugs: Glacial acetic acid (Carlo Erba Spa Farmitalia, Lot. 0286N100) was diluted to with double distilled water. Carrageen (Sigma chemical company, batch no 16925A). Tramadol 50mg capsules (as Mabron6, Medochemie. Cyprus, Lot No. A3C022), Acetaminophen 500 mg tablets (as Panadol8, GSK, Kenya, Lot No. QWX208), Ibuprofen 400mg tablets (Ibuprofen denk6, Germany, Lot No. 3863), 0.9% sodium chloride 500 ml bag, sterile and non-pyrogenic for injection USP, lot no 533-JB1323, Nova-Tech, Inc. were used as standard drugs. Tablets or capsules of the standard drugs equivalent to the active ingredient was calculated and diluted with 0.9% normal saline to prepare the standard solution giving 30mg/kg in 0.2ml.

Test organisms: The following bacterial strains were used in the study. Indicator organisms, namely, Staphylococcus aureus (ATCC 25923), APC- Escherichia coli (ATCC 25922), Salmonella Spp Is. typhimurium (ATCC 13311), Shigella Spp/Shigella bodii (NCTC 9333), Pseudomonas aeruginosa (ATCC 27853), Klebsella pneumonia (ATCC 700603) and Candida albicans (clinical isolates), Aerobic bacteria count (NMKL no. 86), total mold (NMKL no. 98), Coliform (NMKL no. 44) and yeast count (NMKL no. 98) were determined to ensure the microbial quality of the formulated products.

Test sample preparation: The test samples used are oral gargle solution and tea infusion. The oral gargarling solution is prepared by adding five drops of the blended essential oils of Rosmarinus officinalis, Salvia officinalis and Cymbopgon citratus while stirring into 100 ml mildly hot water. The tea infusion of the fresh cleaned and comminuted leaves of the primary herbs, Cymbopgon citratus, Menta spicata and Thymus schimperi for the desired biological effect and Stevia rebaudiana as adjuvant (secondary herb) to improve the test and fragrance medicinal plants were blended as 80% of the primary and 20% of secondary herbs in 100 ml of water using juice mixer as per herbal tea formulation protocol (WHO 2018; Tea and herbal infusions Europe 2018).

Preparation of experimental animals: Swiss albino male and female healthy mice weighing 25-40g and rats weighing 140-160g were used to evaluate the symptomatic relief of the medicinal plant formulations experiment. All the animals were obtained from the animal unit in the Ethiopian Public Health Institute (EPHI). The animals were categorized randomly into five groups (six male mice and six female mice in each

https://ejphn.ephi.gov.et/
group, respectively) for evaluating the analgesics and antipyretic tests. The anti-inflammatory experiments were conducted in 3 male and 3 female rats, respectively. The animals were kept under standard conditions, temperature of 22± 2°C with 12h light and 12 hrs. dark cycle and provided with free access standard pellet laboratory diets and drink tap water ad-libitum. The animals were acclimatized to laboratory conditions for one week prior to the experiment to minimize any non-specific stress (OECD 452, 2009).

Assessment of anti-nociceptive effect: Screening of the oral gargle solution and the tea infusions for anti-nociceptive effect was done in mice by hot plate method following Connor et al. (2000). The experimental animals were fasted for two hours prior to the administration of the medicinal plant’s formulation. The oral gargle solution was prepared by dissolving five drops of essential oil in 100ml distilled water. The tea infusion, which was prepared by mixing 80% of the primary herbs and 20% secondary herbs blend in 100 ml hot water, were orally administered to the experimental mice in doses of 350, 700 and 1400 mg/Kg. The animals were then gently placed into a glass beaker whose bottom surface was maintained at about 55°C with a thermostat. The time between placing the animals on hot surface and licking of the limbs or jumping by the animals was recorded with a stop watch as response latency. Baseline latencies were determined twice at 15-minute intervals and the first reading was discarded. Latencies were then determined at 15, 30, 45, 60, 90, 120, 150 and 180 minutes after the tea infusions and oral gargle solution as test substance, 0.9% normal saline as vehicle, Tramadol 10 mg/Kg standard drug administration. A cut off time of three times the mean pre-drug latency was imposed to minimize tissue damage (Le Bars et al. 2001).

Assessment of anti-inflammatory effects: The test samples were oral gargle solution that was prepared by dissolving five drops of essential oil in 100ml distilled water, and the tea infusion prepared by mixing 80% of the primary herbs and 20% secondary herbs blend in 100 ml hot water (in doses of 350, 700 and 1400 mg/Kg). Normal saline (0.9%) as vehicle or negative control and Ibuprofen (10 mg/Kg) were employed as standard drug. The test samples, standard drugs and vehicle were orally administered to rats for anti-inflammatory effect by carrageen induced paw edema (Gokhale et al. 2002). The male and female rats were injected with 0.1 ml of carrageen (1%w/v in water) into the sub-plantar area of the right hind paw to induce edema. The medicinal plant formulations (tea infusion and oral gargle solution), standard drug and vehicle were given orally one hour before carrageen injection. The volume of rat paw was then measured using a plethysmometer (UgoBasile, Italy) at hourly interval for six hours. The difference between the paw edema after and before (basal volume) carrageen injection was taken as the volume of edema for each rat. The results were expressed as % reduction in the mean hind paw swelling for test samples or standard drugs as compared to the control or vehicle treated group with the initial hind paw thickness.

Assessment of antipyretic effects: Screening of the medicinal plants formulation for antipyretic effect was done in mice according to the method described by Williamson et al. (1982) and Gokhale et al. (2002). A thermistor probe was inserted about three cm into rectum of each mouse and their basal rectal temperatures were recorded on a digital thermometer. The mice were then injected with 30% (w/v) suspension of yeast in 0.9% sodium chloride in a dose of 10ml/Kg S.C. The temperature was recorded 16 hours after yeast injection. Mice that showed a rise in temperature of less than 0.5°C were excluded from the experiment. The remaining mice were dosed with 350, 700, 1400 and 2100 mg/Kg body weight of the tea infusion or 10mg/Kg body weight of acetyaminophen or equal volume of 0.9% Sodium Chloride/distilled water by the intra-gastric route. Sixteen hours after yeast injection, the rectal temperatures were measured at half hour and then every hour for six hours consecutively after dosing. The mean change of temperature was determined for the medicinal plant formulations, acetyaminophen (the standard drug) and 0.9% Sodium Chloride vehicle treated groups every hour for six consecutive hour’s period.

Phytochemical and microbial quality
Total flavonoids content: The total flavonoids contents of the tea infusion of the plants were determined according to the method outlined by Adom and Liu (2002) with slight modification. Catechin was used as a standard. Tea infusion (0.5ml) composed of the mixture of 80% of the primary herbs and 20% secondary herbs blend in 100 ml hot water, 0.5 ml of oral gargle solution prepared by dissolving five drops of essential oil in 100 ml water and 0.5 ml of the catechin standards were transferred in separate test tubes and mixed with 0.15ml of 5% (w/v) Sodium nitrite and 2.5ml of distilled water. The sample, standard and reagents mixtures were vortexed and left for six minutes at room temperature. 0.3ml of 10% (w/v) aluminum chloride was then added to each sample, mixed and kept for more than six minutes. This was followed by addition of 1ml of 1.0M Sodium hydroxide subsequently 0.5 ml of distilled water. The mixture was vortexed and kept for 15 minutes. Finally, the absorbance was measured using a UV-VIS spectrophotometer (Shimadzu, UV-1800, Japan) at 510 nm. The total flavonoids contents of the sample were expressed as mg catechin equivalent per ml of the blended formulation.
Determination of total phenol content: The total phenolic contents of the blended product were determined according to the procedure outlined by Singleton and Rossi (1965). Gallic acid standard was used to develop a calibration curve. Standard gallic acid (0.5ml), the tea infusion (0.5ml) composed of the mixture of 80% primary herbs and 20% secondary herbs blend in 100 ml water, oral gargle solution (0.5ml) composed of five drops essential oil in 100 ml water, the vehicle (water) that was used as a blank were transferred into three different test tubes. A volume of 2.5 ml of 10% aqueous Folin-Ciocalteu reagent (v/w) was then added to each test tube and mixed using a vortex mixer. The mixture solutions were kept for eight minutes at room temperature. Two ml of 7.5% (w/v) Sodium carbonate in water was added and mixed. The samples, standard and blank were then kept in the dark at room temperature for 2 hours. The absorbance of the samples, standard and the blank was measured at 765 nm using UV-VIS spectrophotometer (Shimadzu, UV-1800, Japan). The concentrations of total phenolic compounds in the blended tea infusion and oral gargle solution were determined from the standard curve and expressed as mg gallic acid equivalent per ml of the blended products.

Sensory evaluation: The tea infusion composed of the blend of 80% of the primary and 20% of the secondary herbs blend was prepared in 2250 ml of hot water. Similarly, the gargle solution was prepared by adding 68 drops essential oil in 2250 ml mildly warm water for the panelists to assess using sensory evaluation for odor, flavor, color, taste, mouth feel and over all acceptance using six hedonic scale points by volunteer panel assessors. The six-point hedonic scale, where 1= dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = neither like nor dislike, 5 = like moderately, 6 = like very much. The sensory acceptability of the tea infusion and oral gargle were determined by 45 volunteers’ panelists for the attributes of appearance, mouth feel, taste, flavor and overall acceptability. The average value for the filled sensory evaluation forms responses result of the 45 panelists is summarized using the six hedonic scale (Brich et al. 1981; Ayele 2014).

Assessment of microbial quality and safety: The test samples, the tea infusion composed of 80% of the primary herbs and 20% secondary herbs blend in 100 ml hot water, the oral gargle and steam inhalation solution of five drops essential oil in 100 ml water were subjected to the following examinations: total aerobic viable count (TAVC) and total yeast/mold count (TYMC) by spread plate technique and the presence or absence of pathogenic bacteria such as Salmonella spp. and Shigella spp., enumeration of Staphylococcus aureus, total and thermo tolerant Coliforms and E. coli by presumptive identification using differential and/or selective media. Methods prescribed in the (WHO 2011) were employed to test the microbial quality of the herbal tea infusion. Different selective and differential medias were used for the presumptive identification of the indicator organisms except for TAVC. Plate Count Agar media was used to enumerate the total bacterial population. Rose Bengal medium was used for the identification and enumeration of fungi. Violet red blue agar media was used for the enumeration of coliforms. In addition, Shigella-Salmonella. Agar and Xylose-Lysine Deoxycholate agar were respectively for the isolation of Salmonella spp. and Shigella spp. Manitol Salt agar was used for the enumeration of S. aureus.

Statistical analysis: The experimental data were entered and analyzed using SPSS-22 software. All the experiments were conducted in triplicate and the results were expressed as mean values (M) and standard errors of mean (SEM). The analysis was done using one-way analysis of variance (ANOVA). The mean values of test sample and control were compared using Dunnet’s t-test. Differences at p<0.05 were considered significant.

Ethical consideration: Ethical clearance letter was obtained from Institutional Review Board of Ethiopian Public Health Institute (Reference No 6.13/207). The experimental animals in this study were handled humanly, and kept from any unnecessary painful and terrifying situations as per the OECD guideline. The animals were also protected from pathogens and placed in an appropriate environment. All procedures were carried out by a well-trained person (OECD 452, 2009).

Results
Analgesic effect of the medicinal plants’ formulation: Evaluation of the herbal tea infusion for analgesics effects using hot plate method revealed good analgesic effect in comparison to the standard, tramadol. The analgesic effect of the tea infusion is dose dependent and the dose at 700mg/Kg and 1400 mg/Kg were found to have comparable effects. The analgesic effect increases in the reaction time at 15-, 30-, 60- and 120-min following administration of the tea infusion in mice as compared with the control animals which is statistically significant (P<0.05). The effect is maximized at 120 minutes in all doses while the effect was sustained up to 150 minutes for doses of 350 and 700 mg/Kg. The effect of treatment group at 700mg/Kg and 1400 mg/Kg were insignificant with the standard, tramadol 10mg/Kg at the reaction time of 90minutes as shown in Figure 1.
Figure 1: Analgesic effect of medicinal herbal tea infusion using hot plate method

Anti-pyretic effect of medicinal plants’ formulation:
The antipyretic effects of the herbal tea infusion and oral gargle were investigated after yeast inducing pyrexia in mice. The result for the tea infusion showed that it possessed an antipyretic effect that persists up to 6 hours following yeast induced pyrexia at dose of 2100mg/Kg. The antipyretic effect of the tea infusion was observed after two hours of inducing pyrexia. Similarly, the antipyretic effect of the oral gargle solution was observed after half hours of induced pyrexia and persists up to six hours. The tea infusion did not produce hypopyrexia (36.5°C to 36.8°C starting 3 hrs. to 6hrs). In similar way, the oral gargle solution also did not produce hypopyrexia (36.9°C to 37.2°C starting 2 h to 5h). Both the tea infusion and oral gargle solution reduced the pyrexia significantly (P<0.05) to a normal level unlike the standard acetaminophen 10mg/Kg that cause hypopyrexia (34.8°C to 34.7°C starting 3 h to 6h) (Figure 2 and 3).

Figure 2: Antipyretic effect of medicinal herbal tea infusion using yeast induced fever
Figure 3: Anti-pyretic effect of medicinal herbs essential oil steam inhalation using yeast induced fever

Anti-inflammatory effect of the medicinal plants’ formulation: The herbal tea infusion exhibited anti-inflammatory activity at all doses starting 3rd hour. The % inhibition for the anti-inflammatory effect observed for the herbal tea infusion at a dose of 350 mg/Kg and 700mg/Kg that suppressed the paw edema 8.2% to 53.6%, 2.05% to 33.6% and 29.1% to 52.5%, respectively at 3, 4, 5 and 6 hr. Maximum % inhibition for the anti-inflammatory effect was observed for the herbal tea infusion at dose of 350mg/Kg. There is a significant variation (p<0.05) between the standard Ibuprofen10mg/Kg and herbal tea infusion (700 mg/Kg) at the 4th and 5th hour (Figure 4).

Figure 4: Anti-inflammatory effect of medicinal herbal tea infusion using carrageen induced inflammation

Phytochemical, microbial quality and sensory evaluation: Herbal substances intended for the preparation of the tea infusion fresh comminuted leaves of the leaves of the medicinal plants. Organoleptic
features of the medicinal plants used for tea infusion showed color of pale yellow and pleasant odor. The medicinal plants tea infusion was observed to have pleasant taste. Sensory evaluation of the medicinal plant’s tea infusions and oral gargle by volunteered panelists using six points hedonic scale by volunteered panelists showed that hedonic scale value for taste and flavor acceptance were similar while the herbal tea has better odor and color acceptance. The overall acceptability for the herbal tea and oral gargle solution were 5.6 and 4.8, respectively (Figure 5).

**Figure 5: Sensory evaluation (using six-point Hedonic scale) of medicinal herbal tea infusion and oral gargle**

The total phenols and flavonoids contents for the medicinal plants blend tea infusion, oral gargle and steam inhalation solutions containing essential oil blends showed 153.9 and 106.27 µg/ml, 38.17 and 26.17 µg/ml, and 39.99 and 32.94 µg/ml, respectively. Herbal tea contained the highest total phenols and flavonoids contents compared to the oral gargle (P<0.05). Screening of the medicinal herbal blend tea infusion and the oral gargle solution showed free from microbial contaminants, i.e., molds, yeast, aerobic colony count, total *Coliforms*, fecal coliform, *E. coli*, *S. aureus* *Salmonella* Spp, and *Shigella* Spp that showed <1.0 × 10^3 cfu/g.

**Discussion**

Pain originates due to the sensitization of the nociceptors by various endogenous signaling molecules, like prostaglandins, leukotrienes, histamine, bradykinines, and monoamines. The mechanisms underlying pain involve both peripheral and central pain mechanisms (Manchikanti et al. 2002). The results of the present study showed that the medicinal herbal blends tea infusion possesses significant analgesic activities using the hotplate experimental model. Although there is a need to screen the analgesic effect using different anti-nociception models, the medicinal herbal blends tea infusion may resemble with that of the standard, tramadol which has central analgesic activity as screened using hot plate method. The anti-nociceptive activity may reside in the essential oil of di and sesquiterpenes and phenolic constituents mainly flavonoids as these are the major constituents of the medicinal herbs. The medicinal plants blend tea showed comparable analgesic property as that of the extract of the most commonly traditionally used analgesic plant known as “Dengetega”, *Taverniera abyssinica* constituents (Dagne et al. 1990) though there is variation in potency. The aqueous roots extract of *T. abyssinica* reported to contain flavonoids as major constituents (Duddeck 1987; Assefa and Admassie 2019).

It was found that the analgesic effect of tramadol is central acting with a multimode of action. It acts on serotonergic and noradrenergic nociception, while its metabolite O-desmethyl tramadol acts on the µ-opioid receptor. Its analgesic potency is claimed to be about one tenth that of morphine (Subedi et al. 2019). The hot plate produces two behavioral components paw licking and jumping, both of which are considered to be supraspinal integrated responses. Both the paw licking behavior and the jumping reaction are abolished by opioids explained by the central action (Le Bars et al. 2001). In the current study, the medicinal plants showed of paw licking and jumping reaction in the tested animals, it may tentatively suggest central effect. It may be also suggested that the analgesic effect of the tea infusion might resemble tramadol, however, further work on anti-nociception activity with different testing model, standard medicines and isolated compounds of the medicinal plants are required to get more idea to proclaim the mechanisms of action.

**Anti-inflammatory:** Inflammation is an immunological defense mechanism elicited in response to mechanical injuries, burns, microbial infections, allergens and another noxious stimulus (Yoon and Baek 2005).
Use of anti-inflammatory agents may, therefore, be helpful in the treatment of inflammatory disorders (Sosa et al. 2002). A number of traditionally used Ethiopian medicinal plants are reported to have anti-inflammatory effect with limited adverse effects (Yonathan et al. 2006). The medicinal plants blend tea at dose of 350 mg/Kg and 700mg/Kg found to suppress the paw edema at 2, 3, 4, 5 and 6 hr. There is not significant variation between the standard Ibuprofen 10mg/Kg and medicinal plants tea infusion at 700 mg/Kg (p<0.05). The tea infusion possesses significant anti-inflammatory activities indicating inhibition of inflammation produced by carrageen.

The anti-inflammatory effects of the medicinal plants blend tea infusion found to be similar with the reports on the aqueous tea infusion of Cussonia paniculate (Adedapo et al. 2008) and Tinospora cordifolia (Rao et al. 2005). The development of edema in the paw of the rat after injection of carrageen is a biphasic event. Inflammation induced by carrageen is mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-HT, prostaglandins and bradykinin are known to be involved for inflammation mediation (Wheeler-Aceto and Cowan 1991).

**Antipyretic effect:** Fever is a source of discomfort. Hence, antipyretic medication needs to be administered to manage the fever and minimize the consequences of increased metabolic demands (Aronoff and Nielson 2011). Febrifuge or fever reducing properties medicinal plants is attributed to their phytochemicals constituents that are commonly used by the community. The medicinal plant blends possess an antipyretic effect after half hours of inducing pyrexia and persists up to six hours. The tea infusion did not produce hypopyrexia (36.5 °C to 36.8 °C starting three h to six h). In a similar way, the steam inhalation also did not produce hypopyrexia (36.9 °C to 37.2 °C starting 2 hrs-5hrs). Both the tea infusion and steam inhalation reduced the pyrexia to a normal level unlike to the standard acetaminophen that caused hypopyrexia (34.8 °C to 34.7 °C starting 3 hrs. to 6hrs) (Figure 2 and 3). This may be attributed to the phytoconstituents of medicinal plants that reduces raised body temperature without inducing the lowering of body temperature below the normal level (36.7 °C). Literature reports indicated that acetaminophen causes hypothermia due to the development of congestion, hypovolemia and inflammation (Li et al. 2008). The findings are in line with the antipyretic effect produced by that of Ocimum lamifolium and O. suave (Makonnen et al. 2003).

**Phytochemical and microbial quality control and sensory evaluation of the medicinal herb’s formulations:** In recent years, plant derived products are increasingly being sought out as medicinal products, functional foods (nutraceuticals) and cosmetics commonly sold in healthy food shops and pharmacies over the counter as self-medications. Therefore, there is a need for standardization of herbal formulations, which is an important aspect for maintaining and assessing the quality, safety and acceptability of the herbal formulation to attain the desired biological effects through microbial and physicochemical analyses (Yadav and Dixit 2008; Sharma et al. 2009). Screening for microbial contaminants in the medicinal plant’s tea infusion and oral gargo revealed that the total count of aerobic microorganisms, the total count of yeasts and molds were found to be <1.0×10^8cfu/g each besides the absence of specific objectionable bacterial species.

The values indicated are within the prescribed limits of WHO (2011) and international pharmacopeia (2019). International standards for microbiological quality of non-sterile substances for oral use show that the total aerobic microbial count of 10^5cfu/g or cfu/ml and total combined yeasts/molds count 10^3cfu/g or cfu/ml (International Pharmacopoeia 2019; WHO 2011) are within acceptable range. Medicinal plants tea infusion composed of primary and secondary or adjuvant plants, the supporting or adjuvant plants of the blend, contributes for harmonization and preserve the primary plants through improving flavor and taste, preventing microbial growth and chemical stability. The supporting and activating herb may also have overlapping activities (Tea and herbal infusions Europe 2018).

Microbial screening is important to detect that the product is free from microbial contaminants and to ensure the safe use of the plant-based medicine. Inappropriate method of collection, transport, storage and processing can render raw material of herbal medicines prone to microbial contamination. Therefore, microbial screening is important to detect that the product is free from microbial contaminants to ensure the safe use of herbal medicines. Aerobic colony count is intended to indicate the level of microorganism in consumable products. In addition to this, microscopic yeasts and molds (fungi) could attack or infest consumable products due to their relatively versatile environmental requirements. Yeasts and molds cause various degrees of deterioration and decomposition of consumable. They can invade and grow virtually at any time and cause health hazards.

They also produce toxic metabolites known as mycotoxins. Most mycotoxins are stable compounds that are not destroyed during food processing or cooking. Certain food borne molds and yeasts may also elicit allergic reactions or may cause infections especially in immunocompromised populations, such as the aged and
debilitated, HIV-infected individuals, and persons receiving chemotherapy or antibiotic treatment (Milavec 1977; Beneke and Rogers 1971).

Phytochemicals standardization of medicinal plants is commonly undertaken on the basis of organoleptic properties and characteristics from chemical constituents’ perspectives. Markers which are chemically defined constituents have paramount importance for the quality control purposes, independent of whether they have any therapeutic activity or not. They are for identifying or quantifying the constituents of medicinal herbs (Satheesh et al. 2011; WHO 1992).

Determination of the total flavonoids and phenolics constituents of the medicinal plants blends that serve as markers were undertaken to ensure the quality of the medicinal plants blend products.

Masking the tastes of herbal medicines to have good attributes will facilitate compliance (act of conforming or agreeing with the substance) (Brich et al. 1981). Sensory evaluation of the medicinal plant tea infusion and oral gargle showed that the tea infusion and oral gargle have an overall acceptability hedonic scale value of 5.6 and 4.8, respectively. The secondary plant, is useful masking the bitter taste and producing the pleasant taste and flavor that may produce compliance during the administration of the medicinal herb blends is attributed for *Stevia rebaudiana* (Gasmalla et al. 2014; Abou-Arab et al. 2010) which is useful in masking the bitter taste of the primary medicinal plants (Figure 5). Herbal tea granule and infusion form is extremely versatile due to the ease of swallowing and greater patient acceptance (Allen 2008).

The importance of medicinal plants as mentioned from the literature sources indicated that a lot of herbs were known to be used for various ailments although there is a need to verify through proper scientific validation, if multifunctional properties are possessed by the medicinal substances. There is a necessity for a medicinal substance with a multi-pharmacological or dual effect on symptomatic relief (analgesic, antipyretic, anti-inflammatory) and antimicrobial agent that will create great benefit for human well-being. If a medicinal substance possesses dual properties of analgesic, antipyretic, anti-inflammatory and antimicrobial activities, it will then reduce the burden of polypharmacy. This in turn will reduce the incidence of adverse effects and treatment cost (Nadkarni and Nadkarni 1992; WHO 2020). In indigenous health care delivery of Ethiopia, numerous plant species are used to treat ailments and symptomatic problems associated with fever, pain, headache, etc. The mode of administration usually involves squeezing the fresh leaves and sniffing the juice, drinking or inhaling after boiling in water (Abate 1989; Kloos et al. 1978). The analgesics, antipyretic and anti-inflammatory effects may support the dual property of the medicinal herbs that could benefit the patient from point of view of safety, efficacy and cost effective.

**Limitation and implications:** Herbal infusion forms brewing rates and stability studies were not undertaken in this study to reinforce the current findings. The infusions of herbs could provide a useful supplementary approach to improving certain aspects of well-being.

**Conclusions**

This study demonstrates that the medicinal plants blended possessed analgesic, antipyretic and anti-inflammatory properties. Determination of total phenolic and flavonoid content in the tea infusion, gargle and steam inhalation solution indicated the presence of bioactive compounds that may be attributed for the antioxidant activity. The results also showed that the microbial quality was within prescribed limits of WHO requirements. The tea infusion and the oral gargle also showed good preference ranking for different sensory attributes indicating accepted administration. The phytoconstituents of the medicinal plant blends may have a role in symptomatic relief (pain, fever and inflammation). Further research is, however, required to establish the infusion forms brewing rates and stability

**Conflict of interests:** The authors declare that they have no conflicts of interest related to this article

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