Antipyretic And Antinociceptive Activities of Ethanolic Extract Of Eugenia Aromatica Baill Seeds

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Abstract: Eugenia aromatica Baill popularly known as Kanafuru is said to be useful in food processing, cooking as spicy, perfumery cosmetic. Its oil extract (Eugenol) and ground powdered have been used in folk medicine for manifold conditions such as in dental care, headache, blood purifier, cough, fever, pain, malaria, stomach pain, etc. This study was designed to determine the antipyretic and antinociceptive property of ethanolic extract of Eugenia aromatica, in laboratory wistar rats. Ten groups of four animals each weighing between 100-120g were studied. Antinociceptive test was performed by using acetic acid solution to induce pain in form of Writhes in wistar rat intraperitoneally (i.p); 30 minutes after: group I was administered orally with (p.o) normal saline solution. Standard aspirin (100mg/kg) and diclofenac solution (100mg/kg) to group II and III respectively, ethanolic extract of Eugenia aromatica to group IV, V and VI at dose of 50,100 and 200mg/kg respectively. The Baker’s yeast induced anal temperature showed a dose dependent and temperature decrease as ethanolic extract have 37.4, 37.2 and 37.1°C at dose of 50, 100 and 200mg/kg which is more effective than standard and control group having 37.8°C and 38.7°C respectively. The acetic acid induced Writings count for the control standard and test sample with Normal saline having 133, Aspirin 100mg/kg 120 Diclofenac 100mg/kg, 128 ethanolic extract 50, 100 and 200mg/kg at 116, 110 and 105 respectively. However, the antinociceptive effect was markedly observed with ethanolic extract of Eugenia aromatica more effective as it increases. Based on the result obtained, Eugenia aromatica has the ability to control or prevent fever and pain and could be recommended for fever and pain treatment. Efforts should be made on the production of oil extracts of Eugenia aromatica [Eugenol] for intake as daily diet. However, the dosage should be controlled.

I. INTRODUCTION

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological function and to defend attack from predators such as insects, fungi and herbivorous mammals (Tapsell et al., 2006). According to a WHO report, about 70-80% of the world’s populations rely on non-conventional medicine mainly from herbal sources in their primary health care. It is especially the case in developing countries where the cost of consulting Western style doctor and the price of medication are beyond the means of most people (Chan, 2005). A medicinal plant is factually any plant which in one or more of its parts contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of direct therapeutic purposes or which are precursors for the synthesis of direct therapeutic agents. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total chemical compounds in plants mediate than effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs in terms of how they work. This enables herbal medicines to have beneficial pharmacology, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effect (Lai and Roy, 2004).

Eugenia aromatica (Clove) is an unopened flower bud growing on a tree belonging to the family of mytaceae which is same as that of guavas. Some varieties of clove are Eugenia aromatica, Syzygium aromatica and Eugenia caryophyllata. Their aromatic dried flower buds are commonly used in biryanis, pickles, salads and garam masala. The aroma of the clove is pleasant yet spicy and can be used to make table drawers and closets smell nice (Banerjee et al., 2006).

The tree that creates the miracle of nature originated from the Moluccas Islands, actually known as spicy island. It is the common product found in the spice rack around the world. Clove buds posses intense fragrance and burning taste. They have deep brown colour, powerful fragrant odour which is warm pungent, strongly sweet and slightly astringent (Banerjee et al., 2006). Indonesia uses half the world production of cloves to make Kretek cigarettes, generally contain 60% to 80% tobacco and 20% to 40% ground clove. Eugenol, one of the chemicals in clove acts like menthol to reduce the harshness of tobacco smoke. The clove tree is frequently cultivated in coastal areas at maximum altitude of 200 above the sea level. The production of flower buds, which is the commercialized part of this tree, starts after four years of plantation. Flower buds are collected in the maturation phase before flowering. The collection could be done manually or
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chemically-mediated using a natural phytohormone which liberates ethylene in the vegetal tissue, producing precocious maturation (Filho et al., 2013).

Figure 1: Clove tree

Figure 2: Clove flower

Figure 3: Clove wet pod

Figure 4: Dried seed

Nowadays, the larger producer countries of Eugenia aromatic (Clove) are Indonesia, India, Malaysia, Sri Lanka, Madagascar and Tanzania especially the Zanzibar Island (Kamatou et al., 2012).

Several researches had been conducted on the qualitative phytochemical screening in Eugenia Sp. and alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars have been detected (Bishnu et al., 2010; Fitrilia et al., 2015).

Eugenia aromatica (Clove) are used in Indian Ayurvedic medicine, Chinese medicines and Western herbalism and dentistry where the essential oil is used as anodyne (pain killer) for dental emergences. Eugenia aromatica are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis (Alqareer et al., 2012).

Pyrexia fever is a disease caused as a result of secondary impart of other disease states due to the resetting of the hypothalamic set-point from scientific discovery, antipyrhetic drugs such as aspirin, NSAIDS, opioids have been developed for use and of which mostly produces side effects including gastrointestinal bleeding, renal, hepatic effects e.t.c. therefore many herbal plants have been found to be having antipyretic effects.
Nociception is defined as “the neural processes of encoding and processing noxious stimuli.” It is the afferent activity produced in the peripheral and central nervous systems by stimuli that have the potential to damage tissue. The receptors involved in pain detection are aptly enough referred to as nociceptors-receptors for noxious stimuli. These nociceptors are free nerve endings that terminate just below the skin as to detect cutaneous pain. Nociceptors are also located in tendons and joints, for detection of somatic pain and in body organs to detect visceral pain. Common examples of nociceptive pain include brain fractures, burns, bumps, inflammation, bruises, obstructions, myofascial pain.

According to Hansel and Sticher (2001), Eugenia aromatica (Clove) is a natural antiviral, antimicrobial, antiseptic and anti-fungal and antinociception agent. The oil of clove (Eugenol) has been used in a variety of health conditions including indigestion, generalized stress, parasitic infestation, cough, toothaches, headaches and blood impurities (Alqareer et al., 2012). The U. S Food and Drug Administration (FDA) had reclassified eugenol, down grading its effectiveness as a mosquito repellent and its use to prevent premature ejaculation have been inconclusive (Medlineplus, 2014). With this previous observation, this study focused on the ethanolic extract of Eugenia aromatica which was furthered applied as a test sample on wistar rat antipyretic and antinoceptive activities.

II. MATERIALS AND METHOD

Sample Collection and Extraction
The whole seed of Eugenia aromatica flower bud (clove) was bought from Bodija Market, Ibadan, Oyo State, Nigeria. The dried seed of Eugenia aromatic was ground into fine powder by blending in high speed electric blender. They were separately kept in an airtight container to avoid the absorption of moisture. One thousand grams (1000g) of the powdered sample was soaked in 5litres of ethanol and left for 72 hours. The extract was then decanted from the container, distilled to concentrate; it was transferred into a beaker and placed in a water bath at 50°C until the solvent was fully evaporated.

Chemicals
Pharmaceutical standards were procured from reputable manufacturers these were: Aspirin (CDH Limited, New Delhi), Normal saline (Dana Pharmaceutical Limited), paracetamol (Emzor Pharmaceutical, Industries), Diclofenac sodium (Bliss GV Pharma Ltd, India), Baker’s yeast (CDH Limited, New Delhi).

Test animals
Male wister rats (100-120g) were used to assess the antipyretic and antinociceptee activity of ethanolic extract of Eugenia aromatica. Animal were procured from University of Ibadan Central Animal Household Physiology Department. The animals were acclimatized in The Polytechnic Ibadan animal house, where they were maintain on standard animal pellet and water ad libitum. The rats for antipyretic study were selected based on the measured basal anal temperature (using a thermistor probe) not exceeding 37°C and achieving anal temperature elevation of at least 0.1°C in response to intra peritoneal (i.p) administration of 15% /v saccharomyces cerevisiae at dose of 10ml/kg body weight. The animals for the antipyretic and antinoceptive studies were divided into five groups of the animals each and fasted overnight.

Preparation and Administration of Drug
All the standards and tested samples were prepared using sterile normal saline solution which also served as placebo or control in the different experiments. Oral (p.o) administration was carried out using a canula-syringe assemblage, and hypodermic syringe-needle assembly used for intra-peritoneal (i.p) administration.

Antipyretic study Baker’s Yeast-Induced pyrexia
Adopting standard procedure as described by a (Paul et al., 2012 ) previous study, Wister rats earlier selected and induced pyrexia were randomly divided into five groups of animals each (Okokon and Onah, 2004). The animals were allowed to starve overnight in their respective cages. Twenty hours after the administration of 15% saccharomyces cerevisiae (Baker’s yeast suspension) the anal temperature of each animal was measured to determine the initial temperature by insertion of digital telethermometer probe to a depth of 2cm into the rectum of the rats. Only animals which developed satisfactory pyrexia (1°C or more increase in rectal temperature) after twenty four hours were used. Fever induced animals were divided into five groups of five animals in each group. Group I served as pyrexia control receive normal saline. Group II to receive standard drugs paracetamol at an oral dose (P.O) while group III-IV received 50,100 and 200mg/kg oral dose of ethanolic extract of Eugenia aromatica respectively . Rectal temperatures of all rats were recorded using digital telethermometer four hours after the administration of the doses.

Antinociceptive Study
Adopting literature (Paul et al., 2012 ) documented technique, 0.6% acetic solution was administered intra peritoneally (i.p) at a dose of 10ml/kg was used to induce writhing (typical constriction response consisting of abdominal wall contraction, pelvic rotation and hind limb extension), after 30minutes, oral
administration (p.o) of Normal saline solution to Group I control, Group II served as positive control received acetic salicylic acid (100mg/kg p.o). Group III received Diclofenac sodium 100mg/kg. Group IV-VI received ethanolic extracts of *Eugenia aromatica* 50, 100 and 200mg/kg respectively and each animal was returned to cage. Five minutes after administration of acetic acid, writhing counts were taken. Antinociceptive effects were measured by comparing their mean abdominal writhes count to the mean writhe count of the placebo group (C-t or S)/C x 100. NB: C’s mean writhes count for placebo or control, t or S is mean writhes count for any of the tests or standards respectively.

**Statistical Analysis**

Using the t-test of independence to analyse the data and having a statistically a value of -8.065 with a critical value of 4.297 (at p<0.001), indicated that there is high level of significant in effect of antipyretic and antinociceptive properties of *E. aromatica* in fever and pain

### III. RESULT AND DISCUSSION

**Antipyretic Study**

The results of antipyretic activity of ethanolic extract of *Eugenia aromatica* was reported in Table 1. The basal body temperature of rats was elevated by 1.5°C after 20hrs of oral administration of 15% Baker’s yeast. There was a progressive reduction in the rectal temperature of rats after oral administration (p.o) of extract and standard drug paracetamol (500mg/kg). The reduction in the rectal temperature after treatments was significantly (P<0.001) high after 4hrs of Drugs administration. The reduction in temperature caused by the extract was dose dependent. The animal, receiving 50, 100 and 200mg/kg of ethanolic extract of *Eugenia aromatica* demonstrated high level of anal temperature reduction at 37.4°C, 37.2°C, and 37.1°C respectively compare to standard paracetamol 500mg/kg at 37.8°C and 5ml/kg normal saline 38.7°C. The statistical analysis of antipyretic study using student t-test analysis indicated P ≤ 0.001 as level of significance in the Baker’s yeast induced anal temperature. The t-test analysis showed a dose dependent and temperature decrease with ethanolic extract of *E. aromatica* more effective than the standard drug and control with significant value of (P ≤ 0.001).

**Antinociceptive study**

The antinociceptive activity of ethanolic extract of Eugenia aromatic was evaluated using acetic acid induced writhing test. The extract exhibited activity in dose dependent manner. Ethanolic extract of Eugenia aromatic has significantly (P < 0.001) reduced number of writhes i.e typical contraction response consisting of abdominal wall contraction, pelvic rotation and hind limb extension at an oral dose of 50mg/kg, 100mg/kg and 200mg/kg with percentage inhibition or antinociceptive effect of 12.78, 17.29 and 21.05 respectively, which is comparable with standard aspirin (100mg) Diclofenac sodium (100mg) and control having writhes count of 120, 128 and 133 respectively. Antinociceptive effect for Asprin and Diclofenac sodium were 9.77 and 3.75respectively. *Eugenia aromatica* extract effect increases as dosage increases. However, the administration of Diclofenac sodium (100mg/kg) has not altered the significant antinociceptive effects, as it was markedly observed having 3.75% which is very low to others.
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Writhing Test: The acetic acid induced writh count data for the standard, control and ethanolic extract of *E. aromatica* doses were (P ≤ 0.001). However, the effect of antinociceptive was markedly observed with diclofenac at 3.75% and acetic salicylic acid (ASA) at 9.77% while *E. aromatica* extract effect increases as dosage increases.

**Figure 6: Acetic Acid Induce Writhes Test**

Value (n=6) * significantly (P ≤ 0.001) different from group 1 placebo

### IV. DISCUSSION

*Eugenia aromatica* dried flower buds have been known to have health benefits over the centuries. It is beneficial as a home remedy in curing several ailments or diseases. In this study, the present result showed that, the effect of ethanolic extract of *Eugenia aromatica* on yeast induced pyrexia showed decrease in rectal temperature after 4hours of administration which is comparable to the standard drug and control, paracetamol and Normal saline respectively. The ethanolic extract of *Eugenia aromatica* at dose of 50, 100 and 200mg/kg body weight decreased rectal temperature significantly (P < 0.001) respectively. Treatment of the tested drug as a dose of 50, 100 and 200mg/kg produced a decrease in rectal temperature that was comparable to standard paracetamol.

In this study, ethanolic extract of *Eugenia aromatica* activity against pyrexia, the baker’s yeast (*Saccharomyces cerevisiae*) was used to act as pyrogen inform of an exogenous stimulus. This evaluated body temperature and intensified the process of lipid per oxidation, which indicated that increase of oxidative stress causes pyrexia. The supplementation of standard drugs, control and ethanolic extract of *Eugenia aromatica* decrease the lipid per oxidation processes (Sehgal et al., 2011). According to Parle and Khanna 2011 on a review article of clove as champion spice, *Eugenia aromatica* contains flavonoid that has antioxidant activity. Thus, its antioxidant activity may be one of the possible mechanisms to reduce the elevated body temperature (Nwafor et al., 2012). The ethanolic extract may reduce prostagladin PGE₂ by its action on cyclooxygenase (COX-2), after been inhibited by the arachidonic acid pathway. Such inhibitory usually decrease elevated body temperature .The antipyretic test indicated that the ethanolic extract of *Eugenia aromatica* was able to significantly (P ≤ 0.001) decrease the yeast induced and temperature elevation in the animals. This showed that ethanolic extract of *Eugenia aromatica* was more effective in reducing fever than standard paracetamol, or acetic salicylic acid and placebo which also showed its possession and inhibitory activity against arachidonic acid pathway.

The antinociceptive test was carried out using writhing test intra peritoneal administration (i.p) of 0.6% acetic acid was used to induce pain in form of writhes in laboratory wistar rat animals. The ethanolic extract of *Eugenia aromatica* at 50, 100 and 200mg/kg has showed significant reduction in the number of writhes count and the activity was dose dependent. The ethanolic extract of *Eugenia aromatica* at dose 50, 100, 200mg/kg has higher percentage of antinociceptive effect than standard aspirin 100mg/kg and Diclofenac sodium 100mg/kg 9.77 and 3.75 respectively. The acid is considered to produce nociception by stimulating the release of various endogenous pain mediators peripherally (Raj, 2006). Such mediators usually excite the nociceptors and produce increase in the peritoneal fluid level of prostagladins (PgEa and PgE2a) and lipoxygenase. Writhing test data showed that ethanolic extract of *Eugenia aromatica* was more effective than standard and placebo significantly (P ≤ 0.001) increases the number of writhes. This observation suggests that the possession of inhibitory property by the ethanolic extract and standard drugs against the activity of the endogenous pain mediators or their effect.
Prior study shows that writhing test is limited by its lack of bias for either central peripheral activity (Anoka et al., 2008).

V. CONCLUSION

The ethanolic extract of *Eugenia aromatica* has been studied to possess substantial antipyretic and antinociceptive activities where as data obtained may support the folkloric use of the herb, the extract is worth probing further for development into pharmacological constituent or phyto-drug with the hope that it will produce beneficial effect, similar to herb experience.

VI. RECOMMENDATION

The use of ethanolic extract of *Eugenia aromatica* as spices act as a primary and secondary antioxidants to satisfactorily meet nutritional needs combine with other food stuffs is recommended. However, the processing of this spice before consumption will help in reducing the anti-nutritional properties of some of the phytochemicals. Efforts should be made on the production of oil extracts of *Eugenia aromatica* (*Eugenol*) for intake as daily diet.

REFERENCE


[4]. Bishnu Joshi, Govind Prasad Sah, Buddha Bahadur Basnet, Megh Raj Bhatt, Dinita Sharma, Krishna Subedi, Janardhan Pandey and Rajani Mall (2011): Phytochemical extraction and antimicrobial properties of different medicinal plants: Ocimum sanctum (Tulsi), Eugenia caryophyllata (Clove), Achyranthes bidentata (Datiwan) and Azadirachta indica (Neem) *Journal of Microbiology and Antimicrobials* 3(1), 1-7.


