International Journal of
Advanced Research in Environment, Agriculture and Physical Sciences
Volume 3, Number 1 April, 2025

IJAREAPS
p-ISSN: 2636-6016
e-ISSN: 2636-6908

Comparative Study on Acetylsalicylic Acid, Flavonoid, and Cardiac Glycoside Contents of Vasoprin and Natural Antioxidant Mixed Tea

[1]Obiora, Joy O., [2]Ezeokafor E. N. & [3]Dike Vivian N.
[1&3]Department of Science Laboratory Technology, Federal Polytechnic Oko
[2]Department of Human Physiology, Faculty of Basic and Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus Science

Article DOI: 10.48028/iiprds/ijareaps.v3.i1.03

Abstract

Cardiovascular diseases (CVDs) and their complications are the most common causes of death in industrialized countries and investigations into human heart failure and animal models of heart failure have provided substantial evidence that oxidative stress increases during heart congestion and contributes to disease progression. This study aimed at investigating the synergistic effects of natural products in formulated aspirin rich tea using standard method. The formulated teas were in different ratios of formula ratio 1, 2 and 3 respectively. The biomaterials used were subjected to solvent extraction method to obtain their active constituents and maximize their presence in the formulated teas the preliminary qualitative phytochemical analysis results gave: the secondary metabolites (formulas 1 (++), formula 2(++) and formula 3(+++) respectively. The quantitative phytochemical analysis of the standard (vasoprim) and produced natural antioxidant rich tea formulations gave concentrations as acetyl salicylic acid (0.61mg/g), flavonoid (0.75mg/g) and cardiac glycoside (0.65mg/g) and acetyl salicylic acid (0.62mg/g), flavonoid (0.78mg/g) and cardiac glycoside(0.76mg/g) in that order showing high level of active component of the standard drug in the formulated tea. This could be as a result of synergistic effects of the bio extracts hence could be used in the strategy for the controlling pains due to inflammations and supply of lack of enough anti oxidant needed in the body. Therefore, in synergy with natural products an anti oxidant, analgesic and anti-cancer tea was explored. We recommend this product to pharmaceuticals and research institutes.

Keywords: *Acetylsalicylic Acid, Synergistic Effects, Phytochemical and Analgesic*

Corresponding Author: Obiora, Joy O.

Background to the Study

Antioxidants are compounds that inhibit auto oxidation being chemical reaction that can produce free radicals (Hunyad, 2019). According to Helberg and Pratt, 2021, antioxidants are man-made or natural substances that may prevent or delay some types of cell damage and are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Examples of antioxidants include: Beta-carotene, Lutein, Lycopene, Selenium, Vitamin A, Vitamin C and Vitamin E (Karpinska *et al*, 2001). Known dietary antioxidants are vitamins A, C, and E, but the term antioxidant has also been applied to numerous other dietary compounds that only have antioxidant properties in vitro, with little evidence for antioxidant properties in vivo (NCCIH, 2013).

Acetylsalicylic acid (ASA) is an anti-inflammatory and anti platelet drug administered orally to patients with acute coronary syndrome. Its protective role results from inhibiting cyclooxygenases (COXs) that metabolize arachidonic acid and produce prostaglandin (Kawabata *et al.,* 2016).

Vasoprin is a medication that contains acetylsalicylic acid (ASA), commonly known as aspirin. It is primarily used for its antiplatelet effects, meaning it helps prevent blood clots from forming by inhibiting the aggregation of platelets. This property makes it valuable in the prevention of heart attacks, strokes, and other cardiovascular events in individuals at risk or those who have already experienced such events.

Cardiovascular diseases (CVDs) and their complications are the most common causes of death in industrialized countries (WHO, 2022). Over the past several decades, investigations into human heart failure and animal models of heart failure have provided substantial evidence that oxidative stress increases during heart failure and contributes to disease progression (Sawyer, 2011). Reactive oxygen species directly injure the cell membrane and cause cell death (Hori and Nishida, 2019). The therapeutic effects of antioxidants on heart failure progression have already been reported (Sawyer, 2011), hence this study on comparative study on acetylsalicylic acid, flavonoid and cardiac glycoside content of vasoprin and natural antioxidant mixed tea. Acetylsalicylic acid (ASA) rich tea was produced and its flavonoid and cardiac glycoside content compared with that of Vasoprin tablet sold in the pharmaceutical stores.

Mechanism of Action of Aspirin

According to Vane and Bottling, (2013) Vane and Botting, 2013 acetylsalicylic acid has ability to suppress the production of prostaglandins and thromboxanes by irreversible inactivation of the cyclooxygenase (COX; officially known as prostaglandin-endoperoxide synthase, PTGS) enzyme required for prostaglandin and thromboxane synthesis. Aspirin acts as an acetylating agent where an acetyl group is covalently attached to a serine residue in the active site of the COX enzyme (Suicide inhibition). This makes aspirin different from other NSAIDs (such as diclofenac and ibuprofen), which are reversible inhibitors (Tohgi *et al.,* 2012). Flavonoids are secondary metabolites, which mainly consists of a benzopyrone ring bearing a phenolic or poly-phenolic groups at different positions (Cavalcante *et al.,* 2018). They are most commonly found in fruits, herbs, stems, cereals, nuts, vegetables, flowers and seeds (Shan *et al.,* 2017).

Cardiac glycosides affect the sodium-potassium ATPase pump in cardiac muscle cells to alter their function (Patel, 2016). Normally, these sodium-potassium pumps move potassium ions in and sodium ions out. Cardiac glycosides have long served as the main medical treatment to congestive heart failure and cardiac arrhythmia, due to their effects of increasing the force of muscle contraction while reducing heart rate. Heart failure is characterized by an inability to pump enough blood to support the body, possibly due to a decrease in the volume of the blood or its contractile force (Triana-Martínez *et al.,* 2019).

Medicinal Properties of the Natural Products Used

According to Thomas 2012, Ginger (*Zingiber officinale*) has been used to handle ailments including arthritic, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases. Clove, *Syzygium aromaticum* essential oils are used as analgesic due to eugenol is effective for toothache pain and other types of pain. Orange *Citrus sinensis*. According to Carr *et al*., 2017, oranges are an excellent source of vitamin C, which helps strengthen the immune system. Plant Carrot *Daucus carota* helps in improved Vision, promotion of good eyesight due to their high content of beta-carotene, (Haskell *et al.*, 2012). Carrots contain antioxidants and phytochemicals, such as beta-carotene and falcarinol, which have been associated with a reduced risk of certain cancers. Cinnamon *cinnamomum cassia*, cinnamon extract exhibited significant anti-inflammatory activity, antioxidants and enhance insulin sensitivity (Shan *et al.*, 2007). *Cassia occidentalis* Coffee senna contains bioactive compounds with antioxidant and anti-inflammatory properties, such as flavonoids and phenolic compounds. These properties may help protect against oxidative stress and reduce inflammation in the body (Kumar *et al.*, 2011).

Methodology Collection and Preparation of Sample Materials

Vasoprin tablets was obtained from Chukwunso Pharmaceutical store in Oko Orumba North, Anambra State, whereas the biomaterials were gotten from Eke Oko market in Oko. Vasoprin tablets were crushed into powered form and dissolved in a suitable solvent for analysis. Aspirin-rich tea was prepared by steeping aspirin-containing plant material in (100%) hot water according to standard protocols. Also, the asprin rich tea were made from different ratios of the dried and ground biomaterials.

Identification of the Compounds (Qualitative Analysis Harbone Method 2014)

Test for Flavonoids

Small amount of each aqueous extract (1ml) in a test tube was added equal volume of 10% ferric chloride. The formation greenish-brown or black precipitate or colour is taken as positive for a phenolic nucleus.

Test for Cardiac Glycosides

Small amount of the extract (1ml) was dissolved in 2ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface indicates the presence of glycone of the cardiac glycosides.

Acetylsalicylic Acid (ASA)

Five (5) grams of the powdered Vasoprin tablet was added into the test tube labeled "Vasoprin".

For aspirin-rich tea, five (1) gram volume of the prepared hot water extract was put into the test tube labeled "Aspirin-rich tea". To each test tube, a few drops of ferric chloride solution was added using a dropper or pipette. A reddish-purple colour was observed which indicated the presence of salicylates, suggesting the presence of ASA in the tested sample.

Quantification of Compound

Determination of Cardiac glycosides (Colorimetric Method)

Eight (8) ml of the extract was transferred to a 100ml volumetric flask and 60ml of H_2O and 8ml of 12.5% lead acetate was added, mixed and filtered.50ml of the filtrate was transferred into another 100ml flask and 8ml of 47% Na_2HPO_4 was added to precipitate excess Pb^2+ ion. This was mixed and completed to volume with water. The mixture was filtered twice through same filter paper to remove excess lead phosphate. 10ml of purified filtrate was transferred into clean Erlyn – Meyer flask and treated with 10ml Baljet reagent. A blank titration was carried out using 10ml distilled water and 10ml Baljet reagent. This was allowed to stand for one hour for complete colour development. The colour intensity was measured colorimetrically at 495nm with a photoelectric colorimeter Calculation

Concentration of cardiac glycoside (steroid) = Absorbance of unknown x concentration of standard Absorbance of standard

Determination of Flavonoids (Gravimetric Methods of Analysis)

Ten (10) grams of the sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature for 4hours and the whole solution was filtered through Whatman filter paper, the filtrate was later transferred in a crucible and evaporated into dryness over a water bath and was reweighed to a constant weight

$$\textbf{\% Flavonoids} = \frac{(W_2\text{-}W_1) \quad X \quad 100}{W_t}$$

W_1 = Weight of the Empty crucible + lid;
W_2 = Weight of crucible + sample + lid;
W_t = Original weight of the sample.

Determination of Acetylsalicylic acid (ASA) (Colorimetric Method)

The photoelectric colorimeter was set to the appropriate wavelength around 510 nm. The absorbance of the standard ASA solutions and the prepared sample solutions was measured. A calibration curve was plotted using the absorbance values of the standard solutions against their known concentrations. The concentration of ASA in the samples was determined by comparing their absorbance values to the calibration curve and extrapolation on the calibrated graph.

Table 1: Formulation of the Natural Analgesic Rich Tea

S/N	Natural Products	Formula Ratio 1(g)	Formula Ratio 2(g)	Formula Ratio 3(g)
1	Cassia Occidentals	2	4	6
2	Orange Pulp	4	6	8
3	Clove	6	8	10
4	Cinnamon Stem bark	8	10	4
5	Garlic/Ginger/Onions	3:3:04	3:3:04	3:3:04
6	Pineapple	10	10	10
Total		40g	48g	48g

Table 2: Qualitative Analysis of the Formulated (ASA) Teas

S/N	Test	Spot Test	Observation	1	2	3
1	Acetyl Salicyclic Acid	Added 5% Ferric Chloride Sol	Reddish- Purple Colour	++	++	+++
2	Flavonoid	10% Ferric Chloride	Black Precipitate	+	++	+++
3	Cardiac Glycoside Test	Chloroform + Conc H2SO4	Dark- Brown Colour	+	++	+++

Table 3: Quantitative Analysis of the Best Formulated (ASA)Tea Formula Ratio 3

S/N	Samples	Acetyl Salicylic Acid(mg/g)	Flavonoid (mg/g)	Cardiac Glycoside(mg/g)
1	**Vasoprin**	0.61	0.75	0.65
2	**natural(ASA) tea**	0.62	0.78	0.76

Discussion

From the results shown in Table 1, the formula ratio 3 of the tea gave the best quality in terms of the intensity of the precipitate and colour of the secondary metabolites (formulas 1(++) 2(++) and formula 3(+++) respectively (Table .2). The quantitative phytochemical analysis of the standard (vasoprim) and produced natural antioxidant rich tea formulations gave concentrations as acetyl salicylic acid (0.61mg/g), flavonoid(0.75mg/g) and cardiac glycoside(0.65mg/g) and acetyl salicylic acid(0.62mg/g), flavonoid(0.78mg/g) and cardiac glycoside(0.76mg/g) in that order showing high level of active component of the standard drug in the formulated tea. This could be as a result of synergistic effects of the bio extracts hence could be used in the strategy for the controlling pains due to inflammations and lack of enough anti oxidant therapy in the body. The phytochemicals present in the cassia occidentalis leaves, *Zingiber officinale* (ginger Oleoresin), *Syzygium aromaticum*, orange and lemon extracts showed anti inflammatory and analgesic properties as shown in research by Obiora *et al.*, 2024 in research on phytochemical and analgesic properties of *cassia occidentalis* leaves

Phytochemical compounds are known to be biologically active and thus aid antibacterial activities of plants. The phytochemical activities of mixed antibacterial tea sample have been allied with medicinal uses for centuries. Most common biological properties of alkaloids are their toxicity against cells of foreign organisms, anti-inflammatory, anti-asthmatic, and anti-anaphylactic properties. The addition of alkaloids on the mixed antibacterial tea entails that it interferes with the division of cells thus inhibiting their growth.

The addition of the high level of cardiac glycoside showed that the tea could replace the vasoprim sold in the market because of its natural flavonoids and phenolics whose duty is to improve human health by regulating cellular processes and serving as antioxidants. Researchers are growing interested in phenolics found in various sources of dietary supplements due to their numerous health benefits such as free-radical scavenging, coronary heart disease prevention, anticancer activity, and antiviral properties. The high bioflavonoid proved the efficacy of the tea in immune system boosting as they supply with the necessary antioxidant that can canter the free radical accumulation in the body. Finally, the acetyl salicylic acid test conducted and the result gave the confirmatory result that the formulated aspirin rich tea can serve the same purpose like the synthetic tea without any adverse effect.

Conclusion

Comparative studies on acetylsalicylic acid, flavonoid, and cardiac glycoside contents of vasoprin and natural antioxidant mixed tea showed that the the phytochemical screening (qualitative analysis), quantitative analysis conducted on the formulated and standard teas showed that the environmentally friendly sustainable formula can replace the synthetic chemicals. It is easily assessable and cheap compared to vasoprim sold in the market because of that progress we recommend that the tea should be formulated using medicinal plant mixture that contains analgesic, anti inflammable and antibacterial properties, also to pharmaceuticals in production of new analgesic novel drug and encourage further studies in understanding synergistic effects in inhibition of microorganisms against pathogens and infections.

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