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IN VITRO ANTIOXIDANT AND ANTI-BACTERIAL PROPERTIES OF AN ANTI-INFLAMMATORY HERBAL MIXTURE OF *C. JAGUS L.* (AMARYLLIDACEAE*) E. HIRTA LINN* (EUPHORBIACEAE) AND *T.TETRAPTERA TAUB* (MIMOSACEAE).

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ABSTRACT

The potential of most herbal preparations to prevent and cure diseases and disease conditions may be largely attributed to the anti-oxidant and anti-microbial activity of the herbal plants. The present study was undertaken to evaluate, comparatively, the antioxidant and anti-bacterial properties of the anti-asthmatic herbal mixture and its constituent herbal plants; *Crinum jagus* (CJ), *Euphobia hirta* (EH) and *Tetrapleura tetraptera* (TT). Antioxidant activity was evaluated using the standard methods of 1, 1-diphenyl-2-picrylhydrazyl and Ferric Reducing Antioxidant Property (FRAP). Anti-bacterial activity was evaluated by the Agar well Diffusion method. The antioxidant assay results showed the higher antioxidant capacity of EH and CJ than the mixture and compared favourably with the positive control, ascorbic acid, while TT showed a lower antioxidant capacity than that of the mixture. The anti-bacterial activity results showed that both EH and CJ have higher inhibitory activity than the herbal mixture. We may, therefore, conclude that there is no scientific justification for the continuous use of this herbal mixture as an herbal therapy for the treatment of inflammation related diseases, since both EH and CJ performed better than the herbal mixture as anti-bacterial and antioxidant agents.

KEYWORDS: *Crinum jagus, Euphorbia hirta, Tetrapleura tetraptera,* Herbal mixture, Antioxidant, Anti-bacterial.

INTRODUCTION

Inflammation is the body's response to disturbed homeostasis caused by infection, injury or trauma resulting in systemic and local effects. An inflammatory reaction prevents the spread of infections and promotes the healing of any destroyed tissue.[1] Inflammation hastens the healing of wounds and infections and unchecked destruction of the tissues will lead to extinction of the organism. However, inflammation which runs unhindered can lead to numerous diseases, such as hay fever, atherosclerosis and rheumatoid arthritis. An inflammatory reaction may be propelled by infection trauma, thermal injury, chemical injury and immunologically mediated injury.[2] Some of its symptoms are excessive heat, swelling, pain and redness. It is a common factor in arthritic diseases or osteoarthritis. The rapid response to an injurious agent that serves to deliver mediators of host defence leukocytes and plasma proteins to the site of injury is known as acute inflammation. It has three major components: vasodilation, vascular leakage, oedema and leukocyte emigration (mostly polymorphonuclear cells). When a host encounters an injurious agent, such as an infectious microbe or dead cells, phagocytes that reside in all tissues try to eliminate these agents. Asthma is one of the disease conditions that are inflammation-related.[3]

Crinum jagus, commonly known as "Ogede odo" in South Western part of Nigeria, belongs to the family *Amaryllidaceae*. About seven species of Crinum are found in West Africa, all being plants of relatively damp soils with showy flowers. *C. jagus* (Christopher lily) in particular is a common plant found in swampy locations with white flowers that appear in the dry season. It is a tender perennial bulb that is native to tropical Africa with tulip-like white flowers, which bloom in clusters during drier season atop leafless stalks typically growing up to about 1 m tall from a clump of strap-shaped green leaves. Other important genera in the family include *Allium L*. (the genus of onion, which is widely cultivated throughout the drier parts of West Africa) *Hymenocallis Salisb.*, *Hipeastrum Herbert* and *Zephyranthes Herbert*, many species of which are ornamental plants.[4]

Euphorbia hirta Linn. known locally in South Western Nigeria as "Emi-ile" or "Iroko Iju" and popularly called asthma weed, is one of such herbs belonging to the family *Euphorbiaceae* which is frequently seen occupying open waste spaces and grasslands, road sides and pathways. Though a native of Central America, the herb is widely cultivated throughout the tropics, especially in West, Central and East Africa.[5] It is

usually erect, slender-stemmed, spreading up to 45 cm tall, though sometimes can be seen lying down.[6] Some of the reported phytoconstituents of the herb included triterpenoids, sterols, alkaloids, glycosides, flavonoids, tannins, phenols, choline and shikimic acid, while some of the reported scientific uses include its use as an anti-spasmodic, anti-asthmatic, expectorant, anti-catarrhal and anti-syphilitic.[5;6;7]

Tetrapleura tetraptera popularly called Aridan, in the Yoruba speaking area of South West Nigeria. It is a perennial, single-stemmed plant with dark green leaves. It is found in the rain forest belt of West Africa. The plant has many ethno-medicinal and non-medicinal uses such as anti-ulcer, anti-microbial, anti-convulsant, emulsifying, contraceptive, and as a nutritive agent.[8,9]

In the South-Western part of Nigeria, the herbal medicine practitioners use the herbal plants being investigated here singly or as a mixture in the treatment of asthma and other inflammation related diseases.

Hence, our objective was to evaluate, comparatively, the anti-oxidant and anti-bacteria properties of the mixture and the individual plants to ascertain if the active constituents in the herbal plants are in synergy to give a better herbal therapy.

MATERIALS AND METHODS

Collection of plant material

Fresh plant parts, bulb of *Crinum jagus* (CJ), whole plant of *Euphorbia hirta* (EH) and fruit of *Tetrapleura tetraptera* (TT) were collected from plantations in Ondo, South-west, Nigeria. Authentication was carried out by Mr. R.A. Sanni of the Department of Biology, Adeyemi College of Education, Ondo, by comparing with voucher specimens deposited at the Herbaria of the Department of Crop Protection and Pest Management, Federal University of Technology, Akure, Nigeria and the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction of plant material

Solvent Extraction

The solvent and chemicals used for this work were of analytical grade. Thoroughly washed plant parts were dried in shade for five days and then powdered with the help of blender. The powdered plant parts were extracted successively with ethanol in Soxhlet extractor for 48 h. A brownish colour extracts were obtained. TT extract has a yield value of 15.94%, CJ extract has a yield value of 1.8% while EH has a yield value of 7.8%. The solvent extracts were concentrated under reduced pressure and preserved at 5ºC in airtight bottle until further use. For the herbal mixture, 5 g of each of the air-dried powder of the herbal plant was mixed and taken in 200ml of ethanol in a conical flask and the above procedure was repeated for its extraction.

ANTIOXIDANT PROPERTY

The Ferric Reducing Antioxidant property was determined by assessing the ability of extracts to reduce $FeCl_3$ solution as described.[10] Briefly, extracts (0-250µL of stock) were mixed with 250µL 200 mM sodium phosphate buffer (pH 6.6) and 250µL of 1% potassium ferrocyanide, the mixture was incubated at 50ºC for 20 min, thereafter 250µL of 10% trichloroacetic acid was added and subsequently centrifuged at 650 rpm for 10 min, 1000µL of the supernatant was mixed with equal volume of water and 100µL of 0.1g/100mL ferric chloride, the absorbance was later measured at 700 nm. A higher absorbance indicates a higher reducing power.

1, 1-diphenyl-2 picrylhydrazyl free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2- picrylhydrazyl) free radical was evaluated as described by Halliwell et al.[11] Briefly, appropriate dilution of the extracts (1 mL) was mixed with 1 mL of 0.4 mM methanol solution containing DPPH (20 mg/L) free radicals, the mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Scavenging ability = A – B/A x 100

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

ANTIBACTERIAL ACTIVITY

Bacterial strains

In vitro antimicrobial activity was examined for the ethanol extracts of the stem bark of the plants used by traditional healers. Microorganisms were obtained from the Department of Crop Protection and Pest Management of the Federal University of Technology, Akure, Nigeria. Among the four microorganisms investigated, one Gram-positive bacterium was *B. subtilis* while three Gram-negative bacteria were *P. aeruginosa, E. coli and S. typhi*. All the microorganisms were maintained at 4°C on nutrient agar slants.

Antibacterial activity of ethanol extracts

The antibacterial activity was tested against *E. coli, S. typhi, B. subtilis* and *P. aeruginosa* by the agar well diffusion method.[12] 24 h old Muller-Hinton broth cultures of test bacteria were aseptically swabbed on sterile Muller-Hinton agar plates. Wells of 9 mm diameter were made aseptically in the inoculated plates and the ethanol extract (20 mg/ml of 10% dimethyl sulfoxide [DMSO]), standard (streptomycin sulfate, 1 mg/ml) and control (10% DMSO) were added to the respectively labeled wells. The plates were incubated at 37°C for 24 h in an upright position. The experiment was carried out in triplicates and the zone of inhibition was recorded.

RESULTS AND DISCUSSION

Antioxidant activity

Studies have shown that the reactive oxygen species of low reactivity can be converted to a highly reactive species. Reaction of hydrogen peroxide (H_2O_2) with low valence forms of the transition metal ions iron (Fe^{2+}) and copper (Cu^{2+}) ion lead to the formation of .OH (Fenton reaction) or species of comparable reactivity such as Fe^{2+}(Ferryl ion) or $Cu(OH)^{2+}$ a copper III complex. The hydroxyl radical .OH, abundant under physiological conditions are quite reactive, reacts rapidly with any type of biological molecules in living cells, such as sugars, amino acids, phospholipids and nucleobases (the components of nucleic acids).[13] The antioxidant activities have been reported to be the concomitant development of reducing power.[14]

As shown in Figure 1, the ferric reducing antioxidant property of all the samples increased with an increase in concentration of the extracts. The values are almost at par with that of the mixture, but EH and CJ still had slightly higher antioxidant property than the mixture.

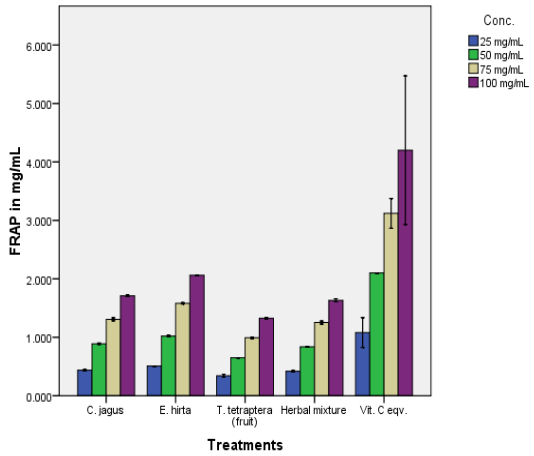

Figure 1: Ferric Reducing Antioxidant Property in mg/ml.

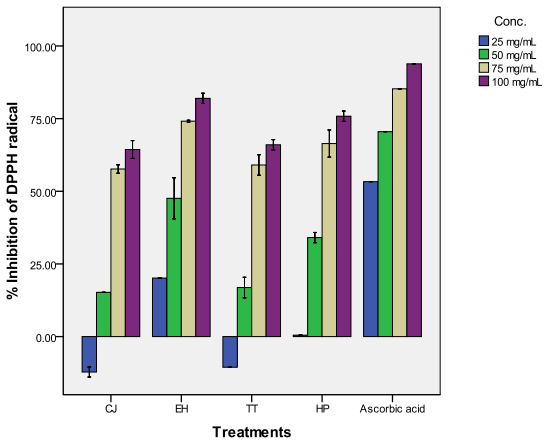

Figure 2: DPPH radical scavenging in %.

The radical scavenging activity of the extracts was observed to increase with increasing concentration. The minimum scavenging activity of 15.23% was obtained at a lower concentration of 50 mg/ml and a maximum scavenging activity of about 64% was obtained at a concentration of 100 mg/ml by CJ, for TT, 17% was obtained at 50 mg/ml, and a maximum of 66%. Conversely, both CJ and TT are pro-oxidant at 25 mg/ml. This implies that both CJ and TT are not safe at low concentration of 25 mg/ml. EH had a minimum of 20% at 25 mg/ml and a maximum of 82% at 100 mg/ml while the herbal mixture had a minimum of 0.5% at 25 mg/ml and a maximum of 75% at 100 mg/ml. Similarly, maximum activity of 94% was also obtained from standard ascorbic acid.

The antioxidant activity of the extracts which was determined by DPPH increases with a corresponding increase in the concentration of the extracts. The decrease in absorbance of DPPH is proportional to concentration of free radical scavenger added to DPPH reagent solution. Decrease in the DPPH solution absorbance indicates an increase in the DPPH scavenging activity.[15]

The results indicate that the ethanolic extracts possess hydrogen donating capabilities and act as an antioxidant. The efficacy of these plants in some of their bioactivities may be attributed to this encouraging antioxidant potential. Though the scavenging activity of the standard was higher than those of the extracts, they are potential antioxidant drugs.

Anti-bacterial Activity

All the samples inhibited *E. coli* appreciably with the mixture and CJ having the higher activity followed by EH then TT. For *S. typhi*, CJ had the highest activity followed by TTF and then EH. This bacterium was not inhibited by the mixture. For *B. subtilis*, EH had the largest zone of inhibition followed by the mixture, while CJ had the least. TTF had no inhibitory activity against B. subtilis. EH inhibited *P. aeruginosa* comparatively with the standard, streptomycin, this is followed by CJ and then the mixture, while TTF had the least activity (Figure 3). The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that incubation (zone of inhibition in mm) are often associated with synthetic antimicrobials.[16] Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plant parts and also to determine their full spectrum of efficacy. However, the present study of *In vitro* antimicrobial evaluation of these plants forms a primary platform for further phytochemical and pharmacological studies.[3] These extracts possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissory extracts open the

possibility of finding new clinically effective antibacterial compounds.

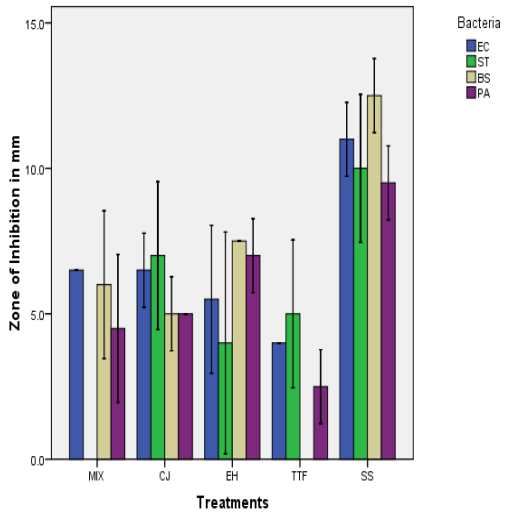

Figure 3: Anti-bacterial activity of the extracts after 24 hr of incubation.

MIX = Herbal mixture; **TTF** = *T. tetraptera*; **CJ** = *C. jagus*; **EH** = *E. hirta;* **SS**= Streptomycin; **EC**= *E. coli*; **ST**= *S. typhi*; **BS**= *B. subtilis*; **PA**= *P. aeruginosa*.

CONCLUSION

We may, therefore, conclude that there is no scientific justification for the continuous use of this herbal mixture as an herbal therapy for the treatment of inflammation related diseases, since both *E. hirta* and *C. jagus* performed better as anti-bacterial and antioxidant agents.

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