Journal of Advances in Medical and Pharmaceutical Sciences
7(1): 1-7, 2016, Article no.JAMPS.24422
ISSN: 2394-1111

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In vitro Antioxidant and Anti-bacterial Properties of a Contraceptive Herbal Mixture of *Zanthoxylum zanthoxyloides* Lam (Rutaceae) *Euphorbia hirta* Linn (Euphorbiaceae) and *Abrus precatorius* L. (Leguminosae)

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Authors' contributions

This work was carried out in collaboration among the authors. Author OEF designed the study and wrote the protocol. Author GFH wrote the first draft of the manuscript and collected all the data. Author TAA wrote part of the manuscript and performed the statistical analysis. Author EAA wrote part of the manuscript and did the literature search. All the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/24422
Editor(s):
(1) Dongdong Wang, Department of Pharmacogonosy, West China College of Pharmacy, Sichuan University, China.
Reviewers:
(1) Baokang Huang, Second Military Medical University, China.
(2) Maria Margarita Canales Martinez, Iztacala UNAM, Mexico.
Complete Peer review History: http://sciencedomain.org/review-history/13681

Original Research Article

Received 20th January 2016
Accepted 20th February 2016
Published 14th March 2016

ABSTRACT

Objective: The objective of this research was to evaluate, comparatively, the anti-oxidant and anti-bacteria properties of the mixture and the individual plants to ascertain if there is need for the continuous use of the herbal mixture as a contraceptive therapy.
Methods: The standard methods used to investigate the antioxidant activity were the standard methods of 1, 1-diphenyl-2-picrylhydrazyl and Ferric Reducing Antioxidant Property (FRAP). Anti-

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bacterial activity was evaluated by the Agar well Diffusion method.
Results: EH and ZX performed better as anti-oxidant agents than the herbal mixture. They compared favourably with ascorbic acid, the positive control. However, the herbal mixture performed better than AP. The herbal mixture did not show better inhibitory activity on the bacteria investigated than EH and AP.
Conclusion: In conclusion, the use of this herbal mixture as a contraceptive should be discontinued based on the results of the anti-oxidant and anti-bacterial activities. Both *E. hirta* and *Z. zanthoxyloides* performed better as antioxidant agents while *A. precatorius* and *E. hirta* performed better as anti-bacterial agents.

Keywords: Abrus precatorius; Euphorbia hirta; Zanthoxylum zanthoxyloides; contraceptive; herbal mixture; antioxidant; anti-bacterial.

1. INTRODUCTION

The present study deals with the investigation of the chemical and biological properties of a contraceptive herbal mixture and its constituent herbal plants used in South-Western Nigeria. The Yoruba tribe and their derived cultures are the main traditional inhabitants of South-Western Nigeria. For a long time, the contraceptive methods used by men are abstinence, withdrawal, condoms, vasectomy etc. to control fertility for ages [1]. These methods have been in use until the introduction of oral contraceptives and modern intrauterine contraceptive devices (IUDs) in the second half of the 1900s. With the development of certain successful methods on females, most of the burdens of contraceptive are now on women to control population. The use of herbs is almost universal among non-industrialized societies [2]. The larger part of the population in Nigeria still depend on the traditional herbal practitioners for their healthcare due to lack of access to orthodox medicine [3,4].

Zanthoxylum zanthoxyloides is a prickly shrub or low-branching shrubby, very prickly tree usually growing up to 12 metres tall, occasionally to 16 metres. The bole is armed with large, woody thorns that fall as the tree grows older. It is known as “Orin ata” or “Fagara” in the Yoruba speaking area of South-Western Nigeria. The tree provides medicines, food flavourings, timber and other commodities for the local people. It is sometimes planted. The bark of the roots and stems is toxic. A dioeciously species, both male and female forms need to be grown if seed is required. The seeds are used as a pepper-like condiment. The seeds are used as beads in necklaces. Young shoots and twigs are used as chew-sticks to clean the teeth and maintain oral hygiene [4]. The bark is aromatic and sudorific. The seeds are soaked in water and used in the treatment of rheumatism [5]. The plant (part not specified) is appetizer and galactagogue [6]. The yellow wood is close-grained and extremely hard. It is durable and termite-proof [7].

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 [8]. It is usually erect, slender-stemmed, spreading up to 45 cm tall, though sometimes can be seen lying down [9]. 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, anti-syphilitic and as a contraceptive [4,8-10].

Abrus precatorius, commonly known as ‘Oju Ologbo’ in South-Western part of Nigeria has many medicinal uses. The plant has a sweet taste as a result of the presence of glycyrrhizin, of which about 9–10% is found in the leaf. Among its numerous medicinal uses are in the treatment of coughs and vomiting in different animal species of which 0.01 mg/Kg body mass is the safe therapeutic dose. The seeds are used in the treatment of diabetes mellitus, chronic nephritis, scratches, sores and wounds caused by cats, mice and dogs. The seeds are brightly coloured, this makes them attractive to the children who play with them and use them in school in their handiwork and to count. The seeds are also used to make jewelries worn by both the young and the old [11]. In the South-Western part of Nigeria, the herbal medicine practitioners use the herbal plants being investigated here singly or as a mixture in birth control among other uses. Therefore, this

research work was carried out to compare the anti-oxidant and anti-bacteria properties of the mixture and the individual plants to justify the continuous use of either the mixture or the individual plant as a contraceptive herbal therapy [4].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh plant parts, root bark of *Zanthoxylum zanthoxyloides* (ZX), whole plant of *Euphorbia hirta* (EH) and leaf of *Abrus precatorius* (AP) 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, with voucher numbers; ACH 3215, ACH 3315 and ACH 3415 respectively. These were compared 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.

2.2 Extraction of Plant Material

2.2.1 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 soaked in ethanol for 48 h. A brownish colour extract was obtained from ZX while the extracts from AP and EH are green. ZX extract has a yield value of 7.94%, AP extract has a yield value of 2.8% while EH has a yield value of 7.8%. The solvent extracts were concentrated under reduced pressure and preserved at 5℃ 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 200 ml of ethanol in a conical flask, and the above procedure was repeated for its extraction.

2.3 Antioxidant Property

2.3.1 The ferric reducing antioxidant property

This was determined by assessing the ability of extracts to reduce $FeCl_3$ solution as described [12]. Briefly, extracts (0-250 µL of stock) were mixed with 250 µL 200 mM sodium phosphate buffer (pH 6.6) and 250 µL of 1% potassium ferrocyanide, the mixture was incubated at 50℃ for 20 min, thereafter 250 µL of 10% trichloroacetic acid was added, and subsequently centrifuged at 650 rpm for 10 min, 1000 µL of the supernatant was mixed with equal volume of water and 100 µL of 0.1 g/100 mL ferric chloride, the absorbance was later measured at 700 nm. A higher absorbance indicates a higher reducing power.

2.3.2 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. [13]. 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.

$$\text{Scavenging ability} = A - B / A \times 100$$

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

2.4 Antibacterial Activity

2.4.1 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℃ on nutrient agar slants.

2.4.2 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 [14]. 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℃ for 24 h in an upright position. The experiment was carried out in triplicates, and the zone of inhibition was recorded.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity

Experimental studies have shown that highly reactive oxygen species can be generated from the low reactive oxygen species. Hydroxyl radical, OH, Fe^{2+}(Ferryl ion) or $Cu(OH)^{2+}$ a copper III complex are formed when hydrogen peroxide (H_2O_2) reacts with low valence forms of the transition metal ions iron (Fe^{2+}) and copper (Cu^{2+}) ion. The abundance of hydroxyl radical. OH, under physiological conditions enhances its reactivity with any type of biological molecules in living cells, such as sugars, amino acids, phospholipids and nucleobases (the components of nucleic acids). The reducing power of substances is a fallout of their antioxidant activities.

As shown in Fig. 1, as the concentration of the extracts increases, the ferric reducing antioxidant property of all the samples increased. The antioxidant property of the herbal mixture is lower than those of EH and ZX, but higher than that of AP.

The radical scavenging activity of the extracts was observed to increase with increasing concentration. AP had a scavenging activity of 12.77% at 25 mg/ml and 79.50% at 100 mg/ml, for ZX, 32% was obtained at 50 mg/ml, and a maximum of 75% at 100 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 26.30% at 50 mg/ml, and a maximum of 71.30% at 100 mg/ml. Instead of showing antioxidant activity, both ZX and MIX are pro-oxidant at 25 mg/ml. This shows that both ZX and MIX are toxic at low concentration of 25 mg/ml.

As the concentration of the extracts increase, the antioxidant activity of the extracts which was determined by DPPH also increases. The decrease in absorbance of DPPH is directly proportional to concentration of free radical scavenger added to DPPH reagent solution but is inversely proportional to the DPPH scavenging activity [4].

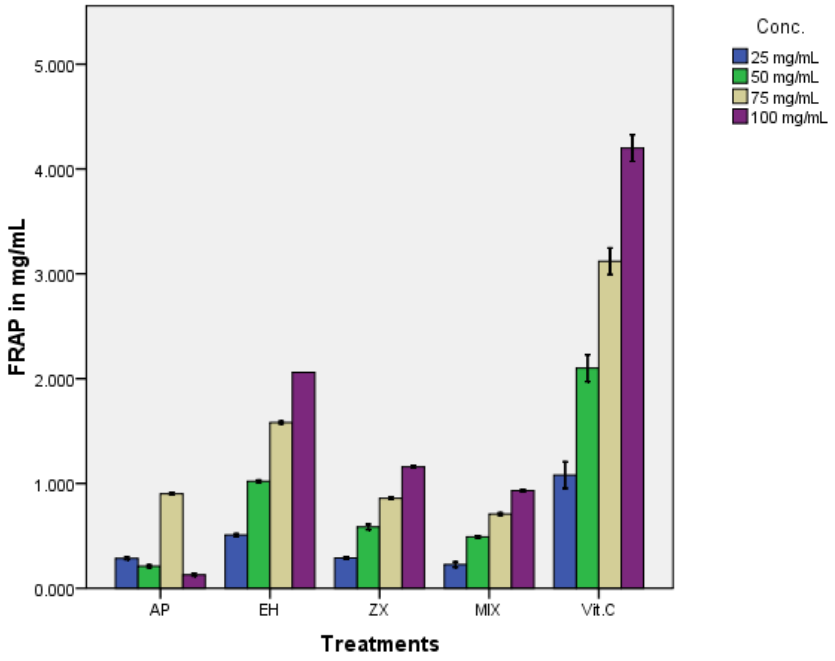

Fig. 1. Ferric reducing antioxidant property in mg/ml
MIX = Herbal mixture; AP = A. precatorius; ZX = Z. zanthoxyloides; EH = E. hirta

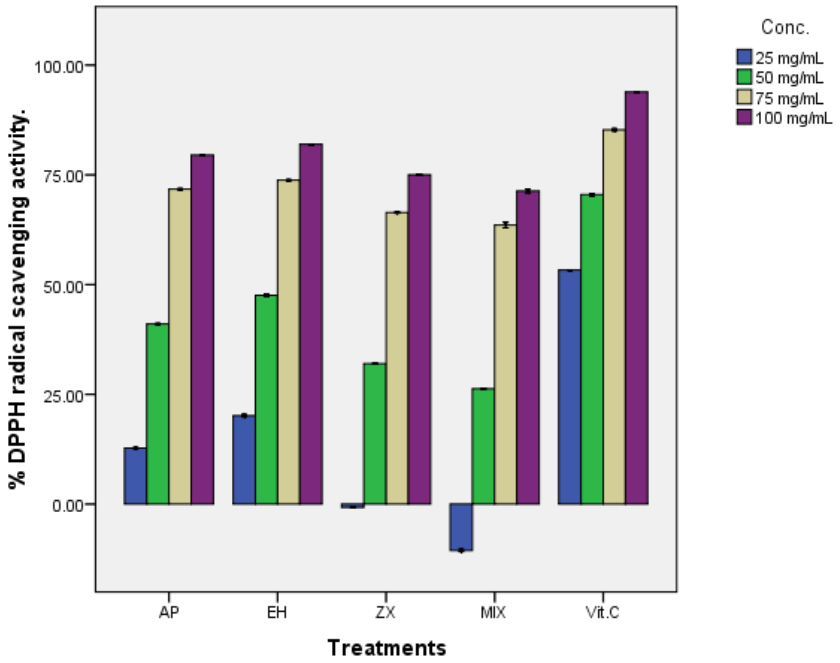

Fig. 2. DPPH radical scavenging in %

MIX = Herbal mixture; AP = A. precatorius; ZX = Z. zanthoxyloides; EH = E. hirta

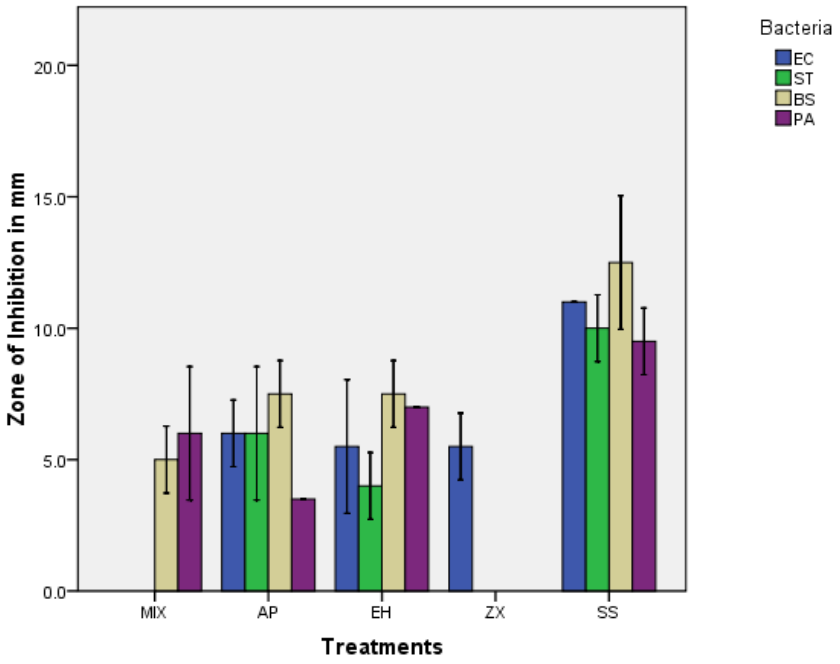

Fig. 3. Anti-bacterial activity of the extracts after 24 hr of incubation

MIX = Herbal mixture; AP = A. precatorius; ZX = Z. zanthoxyloides; EH = E. hirta ; SS= Streptomycin; EC= E. coli; ST= S. typhi; BS= B. subtilis; PA= P. aeruginosa

The results indicate that the ethanolic extracts possess capabilities to neutralize the free radicals and act as an antioxidant. The bioactivities attributed to these plants may be as a result of their anti-oxidant and anti-bacterial activities. The extracts compared favourably with the standard.

3.2 Anti-bacterial Activity

All the samples had appreciable inhibitory activity on *E. coli* except the herbal mixture. This bacterium, *S. typhi*, was not inhibited by both the mixture and ZX, but was inhibited by AP and EH. Both EH and AP inhibited *B. subtilis* equally, this was followed by the mixture, while ZX had no activity against this bacterium. EH inhibited *P. aeruginosa* comparatively with the standard, streptomycin, this is followed by the mixture, while ZX had the least activity as shown in Fig. 3. Higher plants appear a good source of antimicrobials to act against microbes. Plant-based antimicrobials are better than synthetic ones as they can serve the purpose with lesser side effects [15,16]. Further research is necessary to isolate and characterize the antibacterial compounds from these plants and also to determine their full spectrum of efficacy. The present study of *In vitro* antimicrobial evaluation of these plants presents a platform for further studies [17].

4. CONCLUSION

In conclusion, the use of this herbal mixture as a contraceptive should be discontinued based on the results of the anti-oxidant and anti-bacterial activities. Both *E. hirta* and *Z. xanthoxyloides* performed better as antioxidant agents while *A. precatorius* and *E. hirta* performed better as anti-bacterial agents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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