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EVALUATION OF THE PHARMACOLOGICAL ACTIVITIES OF BETA-SITOSTEROL ISOLATED FROM THE BARK OF *SARCOCEPHALUS LATIFOLIUS* (SMITH BRUCE)

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ABSTRACT: Beta Sitosterol was previously isolated from the bark of *Sarcocephalus latifolius (smith Bruce).* This was subjected to antimicrobial screening with the following microorganisms to determine its pharmacological activities; Methycilin Resistant *Staphylococcus aureus (MRSA), Staphylococcus aureus, Streptococcus pyrogenes, Bacillus subtilis, Corynebacterium ulcerans, Escherichia coli, proteus mirabilis, proteus vulgaris, Pseudomonas aureginosa, Salmonella typhi, Shigellia dysenteric, Candida albicans, Candida virusei,* and *Candida tropicalis*. The zone of inhibition was observed to be between 20 to 27 mm with *Bacillus subtilis* and *Salmonella typhi* to be the most sensitive organism with the highest zones of inhibition of 27mm. The Beta Sitosterol was found to inhibit *Staphylococcus aureus, Streptococcus pyrogenes, Bacillus subtilis, Corynebacterium ulcerans, vulgaris, Pseudomonas aureginosa, Salmonella typhi, Shigellia dysenteric, Candida albicans and Candida virusei at 12.*5µg/ml. While the MBC/MFC was minimum for *Bacillus subtilis ,Salmonella typhi* and *Shigellia dysenteric* at 25µg/ml, and *Staphylococcus aureus, Streptococcus pyrogene, Corynebacterium ulcerans, Proteus vulgaris, Proteus aureginosa, Candida albicans* and *Candida virusei* were at 50µg/ml. The result further shows that it could be compared with a standard drug.

KEYWORDS: Pharmacological, Beta-Sitosterol, Microorganism, Sarcocephalus, Anti-Cancer

INTRODUCTION

Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. The continual search for, and the interest in natural plant products, for use as medicines has acted as the catalyst for exploring methodologies involved in obtaining the required plant materials and hence probing into their constituents. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs (Dewick, **1996**.), antimicrobial drugs, and antihepatotoxic compounds (Phillipson and Wright, 1996).

World Health Organization (WHO) has revealed that medicinal plants would be the best source to obtain variety of drugs and that about 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants has to be investigated for better understanding of their properties, safety, and efficiency (Arunkumar, 2009). A large number of phytochemicals belonging to several chemical classes

have been shown to have inhibitory effects on all types of microorganisms when tested in vitro (Cowan, 1999; Kavitha and Kamalakannan, 2011; Chandrabhan and Sumint, 2011; Malpani and Rajput, 2012 and Tarik *et al*, 2012). Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds (Criagg and David, 2001). Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances (Mojab *et al*, 2003; Parekh and Chanda, 2007; Parekh and Chanda, 2008) .Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga, *et al 2005*; Mann, 1978).These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas (Vasu, 2009).

This paper reports on the studies carried out on the pharmacological activities of beta Sitosterol isolated from the bark of *Sarcocephalus latifolius* (Smith Bruce) on some selected microorganism's based on the traditional uses of the plant.

LITERATURE

Sarcocephalus latifolius is of the Rubiaceae family, of genus *Sarcocephalus* and species *latifolius*. It is a plant often used by traditional healers in Sierra Leone and neighboring countries as tonic and fever medicine, chewing stick for dental health, toothaches, dental cures, septic mouth, malaria, diarrhea and in the treatment of dysentery (Etkin *et al*, 1990; Lamidi *et al*, 1995). The leaves are used in the treatment of fever, while the roots and bark used for the treatment of venereal disease, wounds and as odontalgic remedy (Pedro and Antonio, 1998). It is a shrub or small spreading tree that is widely distributed in the Savanna and commonly found in tropical Africa.

The present study attempts to investigate the pharmacological activities of beta-Sitosterol (Fig.1) previously isolated (Isah *et al*, 2015) from the bark of *Sarcocephalus latifolius* (Smith Bruce) for possible pharmacological properties against some selected microorganism based on the plant traditional uses.

Beta-Sitosterol is widely distributed in the plant kingdom and found in vegetable oil, nuts, avocados and prepared foods, such as salad dressings. β-Sitosterol is being studied for its potential to reduce benign prostatic hyperplasia (BPH) (Wilt et al, 2000 and Kim *et al*, 2012) and high blood cholesterol levels (Rudkowska *et al, 2008*). High levels of β-Sitosterol concentrations in blood have been correlated with increased severity of heart disease in men having previously suffered from heart attacks (Assmann *et al, 2006*).

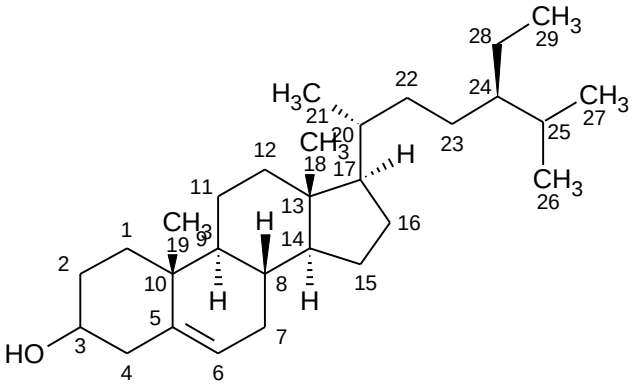

Fig (1) Beta Sitosterol

MATERIAL AND METHOD

Determination of microbial activities of the isolated beta Sitosterol using the well diffusion method.

The cork and bore diffusion method was used in the microbial screening (Barry and Thornsberry, 1985; Bauer et al, 1966). Inoculation of the prepared plates with the organism was done using a wire loop to transfer a strand of the organism into the plate followed by cross-streaking with the same wire loop to achieve uniform spread on the plate. A control was set up alongside using pure DMS0 for each strain of organism. The plates were incubated at 37^0C for 24 h after which they were examined for zones of inhibition of growth with a transparent ruler and a pair of divider and the result recorded in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was carried out on the test-organisms and this was done by the nutrient broth dilution method. Mueller Hinton broth was prepared according to the manufacturer's standard of 28 g/1000 ml. In this case double strength was prepared by dissolving 28 g in 500 ml of distilled water which was swirled and mixed thoroughly by heating to allow uniform dissolution after which 5 ml of it was dispensed into 30 sets of universal bottles and sterilized in an autoclave at 121^0C for 15 min. The agar was allowed to cool to 45^0C and each graded solution was then mixed gently with molten double strength nutrient agar in a Petri-dish and allow to solidify for one hour.

Two-fold serial dilution of the pure compound in the sterilized broth were made to obtained the concentrations of 40-, 20-, 10-, 5-, 2.5mg/ml and 50-, 25-, 12.5-, 6.25- 3.125µg/ml respectively. The initial concentration of the compound was obtained by dissolving 0.005g of the extract in 10mls of the sterile broth. Having obtained different concentrations of the pure compound in the broth, 0.1ml of the standard inoculums of the micro-organism in the normal saline was then inoculated into the different concentrations of the extracts in the test-tubes and the test-tubes were then incubated at 37^0C for 24hours and 30^0C for 1-7days for the fungi. The lowest concentration of the compound in the test-tubes which shows no turbidity is recorded as the minimum inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The minimum bactericidal and fungicidal concentrations of the extracts was carried out in order to determine whether the test micro-organisms were killed or only their growths were inhibited. Mueller Hinton agar was prepared according to manufacturer's instructions and sterilized at 121^0C for 15 minutes and poured into sterile Petri dishes to cool and solidify. The content of each MIC recorded was sub-cultured onto the prepared medium. Incubation was made at 37^0C for 24hours after which the plates were observed for colony growth. The MBC/MFC was taken as the plate with the lowest concentration of the pure compound without colony growth.

RESULTS AND DISCUSSION

The biochemical activities of beta Sitosterol was carried out in order to know if it has biological activities. From the result, It was observed that the compound was active on *Staphylococcus aureus, Streptococcus pyrogenes, Bacillus subtilis, Corynebacterium ulcerans, vulgaris, Pseudomonas aureginosa, Salmonella typhi, Shigellia dysenteric, Candida albicans, and Candida virusei* and not active on Methycilin Resistant *staphylococcus aureus, Escherichia coli, Proteus mirabilis* and *Candida tropicalis* (Table 1). The zone of inhibition was between 20 to 27mm, with *Bacillus subtilis* and *Salmonella typhi* to be the most sensitive organism with the highest zones of inhibition of 27mm (Table 2).

The Minimum Inhibition Concentration (MIC) was determined to ascertain the lowest concentration level at which this compound could inhibit the growth of these organisms. The Beta Sitosterol was found to inhibit *Staphylococcus aureus, Streptococcus pyrogenes, Bacillus subtilis, Corynebacterium ulcerans, vulgaris, Pseudomonas aureginosa, Salmonella typhi, Shigellia dysenteric, Candida albicans and Candida virusei at 12.*5µg/ml (Table 3). This result confirms the activities of the compound earlier determined.

The result from the MBC/MFC (Table 4) shows that *Bacillus subtilis ,Salmonella typhi* and *Shigellia dysenteric* were completely exterminated at a concentration of 25µg/ml, while *Staphylococcus aureus, Streptococcus pyrogene, Corynebacterium ulcerans, Proteus vulgaris, Proteus aureginosa, Candida albicans* and *Candida virusei* were exterminated at 50µg/ml.

This result was compared to a standard drug (Sparfloxacin) and was discovered that the isolated beta Sitosterol can be compared with the standard drug. This is because they both exterminated *Bacillus subtilis, Salmonella typhi* and *Shigellia dysenteric* at 12.5µg/ml showing that they can be used to treat such related ailment that can be caused by these microorganisms (Table 5).

The anticarcinogenic property of β-Sitosterol in 1, 2-dimethylhydrazine (DMH) induced colon carcinogenesis has being studied to be due to its antioxidant property and its ability to suppress the altered β-catenin and proliferating cell nuclear antigen (PCNA) expression in colonic mucosa of DMH-treated rats in short-term colon carcinogenesis. The studies suggests that β-Sitosterol exerts a chemo preventive effect in DMH-induced experimental carcinogenesis, indicating its potential as an anticancer drug (Albert et al 2010).

Table 1: Antimicrobial activities of pure isolated compounds

Test Organism	Sitosterol

MRS*A*	R
Staphylococcus aureus	S
Streptococcus pyrogenes	S
Bacillus subtilis	S
Corynebacterium ulcerans	S
Escherichia coli	R
Proteus mirabilis	R
Proteus vulgaris	S
Pseudomonas aureginosa	S
Salmonella typhi	S
Shigellia dysenteric	S
Candida albicans	S
Candida virusei	S
Candida tropicalis	R

Key: SB-C1= Sarcocephalus latifolius stem bark combination

Table 2: Zone of inhibitions (mm) of the pure isolated compounds on the test microorganism

Test Organism	**Beta-Sitosterol**
MRSA	0
Staphylococcus aureus	20
Streptococcus pyrogenes	24
Bacillus subtilis	27
Corynebacterium ulcerans	22
Escherichia coli	0
Proteus mirabilis	0
Proteus vulgaris	23
Pseudomonas aureginosa	20
Salmonella typhi	27
Shigellia dysenteric	25
Candida albicans	20
Candida virusei	21
Candida tropicalis	0

Key: SB-C1= Sarcocephalus latifolius stem bark combination

TABLE 3: Minimum Inhibition Concentrations (µg/ml) of the Beta Sitosterol on the test microbes

Test Organism	50	25.5	12.5	6.25	3.125l
MRSA					
Staphylococcus aureus	_	_	**O***	+	++
Streptococcus pyrogenes	_	_	**O***	+	++
Bacillus subtilis	_	_	**O***	+	++
Corynebacterium ulcerans	_	_	**O***	+	++
Escherichia coli					
Proteus mirabilis					
Proteus vulgaris	_	_	**O***	+	++
Pseudomonas aureginosa	_	_	**O***	+	
Salmonella typhi	_	_	**O***	+	++
Shigellia dysenteric	_	_	**O***	+	++
Candida albicans	_	_	**O***	+	++
Candida virusei	_	_	**O***	+	++
Candida tropicalis					

*Key: - =No colony growth, **O*** =MIC, + =light growth, ++ = Moderate colonies growth*

TABLE 4: Minimum bactericidal/fungicidal Concentrations (µg/ml) of the Beta Sitosterol on the test microbes

Test Organism	50	25.5	12.5	6.25	3.125
MRSA					
Staphylococcus aureus	**O***	+	++	+++	+++
Streptococcus pyrogenes	**O***	+	++	+++	+++
Bacillus subtilis	_	**O***	++	+++	+++
Corynebacterium ulcerans	**O***	+	++	+++	+++
Escherichia coli					
Proteus mirabilis					
Proteus vulgaris	**O***	+	++	+++	+++
Pseudomonas aeruginosa	**O***	+	++	+++	+++
Salmonella typhi	_	**O***	++	+++	+++
Shigellia dysenteric	_	**O***	++	+++	+++
Candida albicans	**O***	+	++	+++	+++
Candida virusei	**O***	+	++	+++	+++
Candida tropicalis					

*Key: - =No colony growth, **O*** =MBC/MFC, + = scanty colonies growth, ++ = Moderate colonies growth, +++ = Heavy colonies growth*

Comparison of isolated beta- Sitosterol with standard drug (fluconazole)

Table 5: Minimum Inhibition Concentrations (µg/ml) of Fluconazole on the test microbes

Test Organism	50	25.5	12.5	6.25	3.125
MRSA	**ɵ**	**ɵ**	**ɵ**	**ɵ**	**ɵ**
Staphylococcus aureus	**ɵ**	**ɵ**	**ɵ**	**ɵ**	**ɵ**

Streptococcus pyrogenes	**θ**	**θ**	**θ**	**θ**	**θ**
Bacillus subtilis	**θ**	**θ**	**θ**	**θ**	**θ**
Corynebacterium ulcerans	**θ**	**θ**	**θ**	**θ**	**θ**
Escherichia coli	**θ**	**θ**	**θ**	**θ**	**θ**
Proteus mirabilis	**θ**	**θ**	**θ**	**θ**	**θ**
Proteus vulgaris	**θ**	**θ**	**θ**	**θ**	**θ**
Pseudomonas aureginosa	**θ**	**θ**	**θ**	**θ**	**θ**
Salmonella typhi	**θ**	**θ**	**θ**	**θ**	**θ**
Shigellia dysenteric	**θ**	**θ**	**θ**	**θ**	**θ**
Candida albicans	_	_	**O***	+	++
Candida virusei	_	_	**O***	+	++
Candida tropicalis	_	_	**O***	+	++

Key: - =No colony growth, ***O**** *=MIC, + =light growth, ++ = Moderate colonies growth,* **θ***=Resistant.*

CONCLUSION

Medicinal plants used in the folk medicine may be an interesting and largely unexplored source for the development of potential new compounds. But it is necessary to isolate the active principles and characterize their constituents for the benefit of human being. In our attempt to identify new or bioactive compounds in this plant revealed the presence of beta Sitosterol. This plant have shown to have a high active component in it and hence the high microbial activities recorded for the isolated compound.

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