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RESEARCH ARTICLE

ANTIBACTERIAL SCREENING OF ALKALOID EXTRACTS FROM *WALTHERIAINDICA*LEAVES.

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Abstract

Leaves of Waltheriaindica is used for the treatment of various illness via traditional medicine practices. Studies have described a range of bioactive compounds extracted from different plant parts which include alkaloids. The effect of these alkaloids alone against gram positive, gram negative bacteria and pathogenic fungi as well as the nutritional value of the leaves was evaluated. Extracted alkaloid base displayed resistance to Candida krusei (25±0.10mm), Candida tropicalis (23±0.25mm), Klebsiella pneumonia (24±0.45mm), Staphylococcus aureus (24±0.75mm) and Streptococcus pneumonia (28±0.90mm) using Ciprofloxacin (0.1 mg/ml) and Fluconazole (0.3mg/ml) as controls. Minimum Inhibitory Concentrationvalues in the range of 0.25 – 0.75 mg/ml were observed. Minimum Bacterial Concentrations of 1.25mg/ml, 1.0mg/ml and 0.75mg/ml were observed against K. pneumonia, S. aureus and S. pneumonia respectively.
TheMinimum Fungal Concentrations of 0.75mg/ml and 1.25mg/ml were observed against C. krusei and C. tropicalis respectively. Proximate analysis revealed that Waltheriaindica leaves contain crudeprotein(18.74%), crude fiber (15.06%), moisture (8.23%), total ash (7.37%), total carbohydrate(55.95%). The results obtained suggested that the alkaloid base of Waltheriaindica may proffer alternative lead compounds towards the development of new plant-based antibiotic and anti-fungal agents.

Introduction:-

For several decades, the world has witnessed a continuous onslaught of infectious diseases derived from of bacterial or fungal origins. The worst affected areas is Africa for which the distribution of poverty and dilapidated infrastructure serve as external vectors that collectively weaken treatment and effective primary healthcare delivery (Mabhiza*et al.,* 2016; Ngwu*et al.,* 2016; Okwute*et al.,* 2016).Research show that the reoccurrence of several pathogenic microorganisms which defy most commonly known therapeutic agents particularly amongst immune-compromised patients leads to high incidences of morbidity and mortality (Ara *et al.,* 2009; Shakeri*et al*., 2012).The failure of such therapeutic agents to mount an effective defense against such invading pathogens has led to the development and spread of antibiotic resistant species of several microbial pathogens (Beltrame*et al.,* 2007). Furthermore, such events areworsen by recently discovered unforeseen side effects as a result of consuming certain drugs of synthetic origin (Doughart and Okafor, 2007). In addition to human health, nosicomal infections also affect livestock thus posing a serious negative impact on commerce, economy and tourism in the affected locale (Ngwu*et al.,* 2016).

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Data of the prevalence of infectious disease reoccurrence in Africa includes upper respiratory tract, gastro-intestinal and urinary tract infections, all of which possess a rapid means of transmission between humans and animals via coughing in a crowded place, sexual contact, congenital or vertical transmission (mothers to their children or fetus), etc (Carol, 2005; Nichol et al., 2005). Studies have elucidated that certain microbial pathogens like Aspergillus sp., Candida sp., Chlamydia trachomatis, Corynebacterium sp., Escherichia coli, Klebsciellasp, Mycoplasma pneumonia, Neisseria gonorrheae, Proteus sp., Pseudomonas sp, Staphylococcus sp, Treponema pallidum, etc are common culprits behind a number of ailments associated with the different tracts of the human body already mentioned (mcginn and Ahlawa, 2003; Ahameethunisa and Hoper, 2010; Shim et al., 2010).Protractedillness triggered by these pathogens creates an ideal launch pad for the raid of the host body by more lethal pathogens considered to be opportunistic and multi-drug resistant (Zignolet al., 2006).

The combined nuisance of ineffective antibiotic use as well as the associated side effects of their synthetic variants has caused researchers to screen for nature' suitable alternatives that treat or diminish the spread of pathogenic microorganisms. One solution has been to screen several plant families for the presence and activities of bioactive compounds which serve the therapeutic purpose of treatment, prevention and synthesis of a much more powerful drug candidate via plant-based precursor molecules (Ramar*et al*., 2008). Whole plants or their parts have been applied for centuries towards the treatment of infectious diseases, a feat achieved owing to their unique arsenal of effector eliciting compounds that serve as both sources for food and health to mankind (Balunas and Kinghorn, 2005; Tanaka *et al.,* 2006; Gautam*et al.,* 2007). Plant based medicines serve as a suitable therapeutic alternative particularly for Africa as they are more readily available, cheaper and from decades of use are said to proffer minimal to no side effects on patients compared to its western counterparts (Ibrahim *et al*., 2007). Validating the use of plant-based medicine against targeted microbial pathogens has been studied thereby providing scientific validation for their use in tradition African medicine. Phytochemical compounds produced by medicinal plants differ in concentration and complexity across taxa as well as the metabolic pathways that lead to their synthesis in response to a range of environmental stimuli (Reymond*et al.,* 2000; Kennedy and Wightman, 2011). Such plant secondary metabolites, grouped according to their unique chemical nature of their recognized active components include but is not limited to alkaloids, flavonoids, glycosides, phenolic acids, saponins, steroids, terpenoids, etc all of which have been studied and associated with the immune-stimulatory events within the body upon consumption of different indigenous medicinal plants (Duraipandiyan*et al.,* 2006).

Plants like *Glossonemaboveanum*, *Tributus sp.*, *Premnabarbata*, *Clerodendrumviscosum*, *Canthiumparviflorum* and *Pergulariadaemia*were studied for their respective compounds and how they collectively elicit effective inhibitory response to different disease causing agents like *Staphylococcus sp.*, *Klebsiella sp.*, etc (Usman et al., 2007; Tamta et al., 2012; Ramanathan*et al.,* 2013; Uttu*et al.,* 2015).Medicinal indigenous to Nigeria and other African countries such as *Waltheriaindica*of the Sterculiaceae family is used for the treatment of diarrhea, stomach ache, wounds, cough, haemorrhage, fever and malaria (Olowokudejo et al., 2008; Ayantunde*et al.,* 2009).Other reports revealed that this plant can be used to treat skin diseases, impotence and can be used as an aphrodisiac (Mohammed *et al.,* 2007; Gbadamosi*et al*., 2012). Studies into the general phyto-chemistry and antimicrobial activity of this plant has been reported form different geographical locations (Olajuyigbe*et al.,* 2011; Zailani*et al.,* 2011). One unique feature from all reported data is that the plant leaf extracts contain a rich concentration of alkaloids. This study would attempt to illuminate the antimicrobial activity of the alkaloid and crude leaf extract for *Waltheriaindica* against a selection of pathogenic microorganisms associated with common ailments across Nigeria. Furthermore, this study would evaluate its nutritional composition as a food or forage material.

Materials and Methods:-

Plants were collected from the south western region of Nigeria from local markets and transported to the National Institute for Pharmaceutical Research and Development, Abuja for taxanomic identification. Extracts of the plant leaves were prepared using existing methods (Chitemerere and Mukanganyama, 2011).

Plant Material and Preparation of Extracts:-

Leaves of *Waltheriaindica* were dried in an oven at 50^{o}c then ground to powder using a mortar and a pestle. 5g of the powdered sample was first mixed 15mls of ammonia (10% v/v) solution. Alkaloid extraction was performed by adding 30ml of ethanol to the ammonia treated sample. The reaction was monitored in a water bath (40^{o}c) for 10 minutes. The mixture was then filtered using awhatman filter paper no. 1 and the filtrate was collected in a 50ml falcon tube then kept in an incubator (50^{o}c) for 48 hours (Mabhiza *et al.,* 2016).

Antimicrobial screening:-
All the Media were purchased from Sigma-Aldrich and were prepared in accordance with manufacturer instructions. The clinical bacterial and fungal isolates were obtained from department of medical microbiology Ahmadu Bello University Teaching hospital, Zaria, Kaduna state, Nigeria. The identities of all isolates used were confirmed using standard biochemical tests (Cheesbrough, 2002). The antimicrobial activity was determined using agar well diffusion technique with little modification (Ngwu*et al.,* 2016). Sterilized Mueller Hinton agar plates were inoculated with 0.1ml of the test bacterial culture while Sabourand dextrose agar inoculated with the test fungi respectively. 0.1ml of the solution of the alkaloid extract (2mg/ml) was then introduced into their respective wells, bored with a sterile 6mm cork borer, on the inoculated medium. The samples were then incubated for 24 hours at 37^{o}c for the bacteria and at 30^{0}C for 24-168 hours for the fungi, after which each plate of the media were observed for the zone of inhibition of growth recorded in millimeters.Ciprofloxacin (0.10 mg/ml) and Fluconazole (0.30 mg/ml) were used as controls.

Minimum Inhibitory Concentration (MIC):-
The minimum inhibitory concentration (MIC) was determined on the test organisms that were sensitive to the extracts and was done by broth dilution method (Suffredini*et al.,* 2004). Mueller Hinton broth was prepared, dispersed into test tubes and the broth was sterilized at 121^{o}c for 15mins, the broth was allowed to cool. Normal saline was prepared, 10mls was dispersed into sterile test tube and the test microbes was inoculated and incubated at 39^{o}c for 6hrs. Dilution of the test microbes was done in the normal saline until the turbidity marched that of the mcfarland's standard scale by visual comparison at this point, this test microbes has a concentration of about 1.5 x 10^{8}CFU/ml. Two fold serial dilution of the extract in sterilized broth was made to obtain the concentration of 5mg/ml, 2.5mg/ml, 1.25mg/ml and 0.625mg/ml. Media containing extract only and extract-free broth media were also used as negative controls. All broths were observed for turbidity (growth), the lowest concentration of the extract in the broth which shows no turbidity was recorded to the MIC.

Determination of minimum bacteriacidal/fungal concentrations:-
Minimum bactericidal concentration (MBC) and Minimum fungal concentration (MFC) were evaluated by plating the bacterial suspensions from individual well at the beginning and at the end of the experiments on Mueller Hinton agar medium for estimation of MBC (Suffredini*et al.,* 2004). The culture from MIC well was taken and streaked on the surface of fresh Mueller Hinton agar in a 90-mm plate with division and incubated at 37^{o}c for 24 hours (bacteria) and 30^{o}c for 1-7 days (fungi) after which the plates of the medium was observed for colony growth, the MBC/MFC were the plates with lowest concentration of the extract without colony growth.

Proximate Analysis:-
The Moisture, protein, fat, ash, crude fibre and carbohydrate content of the dried leaves was determined. For moisture content, 5 g of dried leaf samples was weighed and dried in an oven at 105°C to a constant weight. The percentageweight loss was determined. Fat content was determined by extracting 5 g of dried leaf sample with hexane or petroleum ether in a Soxhlet apparatus for 8 hours. The ash content was estimated by incinerating 5 g of dried sample in a muffle furnace (Carbolite-RHF 1600) at 550°C for 4 hours, and then the percentage ash content was determined. The micro-Kjedahl method was employed for estimation of crude protein by determining total nitrogen and converting to crude protein by multiplying with 6.25 and carbohydrate was determined by difference.
All experiments were done in triplicate and results were expressed as the averages on dry weight basis.

Results And Discussion:-

Results obtained by disc diffusion method measured by zones of inhibition against 3 bacterial and 2 fungal species showed that the alkaloids obtained from *Waltheriaindica* leaves possessed sufficient antimicrobial activity (Table 1). In the bacteria screening, the alkaloid extract of the leaves were found more active against S. Pneumonia with 28±0.90 mm zone of inhibition, 24 mm against *S. Aureus* and *K. Pneumonia*. The highest zone of inhibition recorded for the fungal screen of the same alkaloid extract was 25±0.10 mm against *C. Krusei.* Against *C. Tropicalis,* the measure inhibitory zone was measured at 23±0.25 mm (Table 1).

Table 1:- Antimicrobial activity of Alkaloid leaf extract (mm).

Test microorganisms	Test sample	Control
Candida Krusei	**25 ±0.10**	**33 ±0.40 (F)**
Candida tropicalis	**23 ±0.25**	**32 ±0.65 (F)**

Klebsiella pneumonia	**24 ±0.45**	**34 ±0.20 (C)**
Staphylococus aureus	**24 ±0.75**	**31 ±0.55 (C)**
Streptococus pneumonia	**28 ±0.90**	**30 ±0.15 (C)**

A= Antibiotic (0.10mg/ml Ciprofloxacin), F= Anti-fungal (0.30 mg/ml Fluconazole).

Alkaloids represent one of the largest components of phytochemical compounds which are being extensively studied for their diverse therapeutic applications (Chen and Fadamiro, 2009; Rao *et al.*, 2009). From this large group of phytochemicals, different new drug compounds have been obtained such as Ibogaine, Voacangine, Graveolinine, etc isolated from the medicinal plants *Tabernaemontanacitrifolia* and *Lunasiaamara* respectively (Kishore *et al.*, 2009). In that report, these purified products as well as their unpurified alkaloid plant extracts were reportedly active against a number of infectious diseases causing pathogens plaguing developing countries. Such findings prompt the screening of alkaloid extracts from promising medicinal plants across the globe. In this study, the inhibitory range of 23-28mm, measured against Ciprofloxacin (10µg/ml) and Fluconazole (30 µg/ml) is in support of other reported studies that indicated the alkaloid fraction from dozens of different plant species and taxa inhibit the growth of pathogenic microorganisms serving as a target for drug development (Okunade*et al.*, 2004; Lim *et al.*, 2009; Okwute*et al.*, 2016).

Values obtained for measurement of the minimum inhibitory, minimal bacterial and minimal fungal concentrations (MIC, MBC and MFC) revealed that the alkaloid leaf extracts were most active against all *S. Pneumonia* as the MIC values of 0.25mg/ml was observed while an MIC value of 0.5 was attained when tested against *C. Krusei* and 0.75 against all other studied microorganisms (Table 2). The MBC results of 0.75mg/ml against *S. Pneumonia* and an MFC of 0.75mg/ml against *C. Krusei* was observed (Table 2).

Table 2:-Minimum Inhibitory/Bacterial/Fungal Concentrations (MIC, MBC, MFC) (mg/ml ± SD).

Microorganisms	MIC (mg/ml)	MBC (mg/ml)	MFC (mg/ml)
Candida krusei	0.50	-	0.75
Candida tropicalis	0.75	-	1.25
Klebsiella pneumonia	0.75	1.25	-
Staphylococcus aureus	0.75	1.0	-
Streptococcus pneumonia	0.25	0.75	-

Each value represents mean (n = 3).

The data in this study (table 2) showed that disease causing pathogens like those selected in this study, commonly associated with respiratory diseases, urinary tract and gastrointestinal infections, can be thwarted and prevented with minimal concentration of the alkaloid base extracts from the plant leaf in this study displayed that it required small amounts to elicit a response(Trautmann*et al.*, 2008; Akoachere*et al.*, 2012).

As a prophylactic measure, consumption of this plant part as part of our diet may serve to prevent unwanted attacks from such prokaryotic pathogenic bacteria. The nutritional composition analysis of *Waltheriaindica* leaves revealed that they are rich in protein (18.74% w/w) and fiber (15.06% w/w) as depicted in table 3. The leaves also possess a high carbohydrate content (55.95% w/w). The leaves also possess a low fat content of 2.88 (% w/w).

Table 3:- Proximate composition data of leave samples

Components	% Content (w/w)
Ash	**7.37**
Carbohydrate	**55.95**
Crude Fat	**2.88**
Crude Fiber	**15.06**
Crude Protein	**18.74**
Moisture	**8.23**

An ash content of 7.37 (% w/w) suggests the presence of a moderately high concentration of minerals that would aid in the disruption of microbial activity as well as bolster the interruption of both bacterial and fungal cell membrane integrity. Furthermore, the synergy of minerals within the leaf as well as the alkaloid base may trigger other structural conformations to the invading pathogen by changing their fluidity and disrupting their respiratory

chain (Mirsha*et al.*, 2009).The results also indicate that the relatively low moisture content of *Waltheriaindica* leaves also account for a moderate to high antimicrobial activity whereby the higher the moisture content, the more microbial growth it would encourage. Furthermore, the high percentage crude fiber (15.06%) is indicative the high digestibility of the leaf. The low fat content (2.88%) hints towards their use as a good source of animal feed coupled with the proteincontent (18.74%).Since these substances are essentialfor growth, metabolism and other body functions,the data in this study supports the consumption of *Waltheriaindica*as a good food source for mankind.

Conclusion:-

The leaves of *Waltheriaindica* contain a unique alkaloid base that confer resistance to disease causing microorganisms like *Streptococcus sp., Staphylococcus sp.,Klebsiella*sp., and *Candida sp.,* which can be studied towards drug development. Also, this study authenticates traditional knowledge and adds to the overall knowledge base of phytodrugs and plant use as prophylactics.From the data obtained in this preliminary study, further work should be done to purify, characterize and identify the activeprinciples to add to the known antibiotic spectrum.

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