ANTI-MICROBIAL AND SPASMOLYTIC EFFECTS OF *HOLERRHENA FLORIBUNDA.*

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Abstracts:
AIMS: To evaluate *Holerrhena floribunda*`s anti-microbial property and its effect on smooth muscles.
METHOD: Anti-microbial effect of *H. floribunda* was studied using the cup bore agar method and the effect of *H. floribunda* extract on guinea pig ileum was evaluated in-vitro.
RESULTS: *H. floribunda* did not inhibit the growth of *Pseudomonas aeroginosa, Staphylococcus aures, Bacillus subtilis* and *Proteus mirabis*. Gentamycin, the standard drug used inhibited the growth of these organisms to varying degrees. It was also observed that the methanolic extract of *H. floribunda* leaves did not have effect on the ileum when used alone but decreased acetylcholine, histamine and KCL contractile effects on the guinea pig ileum.
CONCLUSION: The results showed that *H. floribunda* is not effective in the treatment of infectious diseases as claimed by Traditional medical practitioners but may be useful in the management of colic (stomach) pain. The result on guinea pig ileum supports the claims of the actions of *H. floribunda* in folk medicine in the management of stomach pain.

Key words: Anti- microbial activity, *Holerrhena floribunda*, smooth muscles.

Introduction

The search for drugs and dietary supplements derived from plants has accelerated in recent years[1]. Ethnopharmacologists, botanists, microbiologists, and natural-product chemists are combing the Earth for phytochemicals and "leads" which could be developed for the treatment of infectious diseases. According to Cowan[5], while 25 to 50% of current pharmaceuticals are derived from plants, none are used as antimicrobial agents. Plants produce highly bioactive molecules that allow them to interact with organisms in their environment. Many of these substances are important in the defense against herbivores and contribute to the plant's resistance to diseases. Plants can therefore, be promising sources of antimicrobial agents.

Herbs are widely employed in folk medicine, mainly in communities with inadequate health care facilities and by low income earners in urban centers. Several medicinal plants have been extensively studied in order to find more effective and less toxic compounds with antimicrobial and other pharmacological effects.

Holerrhena floribunda (G. Don) Dur. and Schiz belong to the family Apocynaceae. It is commonly called baa`kin maayuu in Hausa and Nago irena ife-oju-ona in Yoruba. In English it is known as the false rubber tree. *H. floribunda* grows up-to 17m high by 1m girth. It is found in West Africa and other parts of Africa. *H. floribunda* is a soft wood which is use for carving and is ornamented with shining foliage and white fragrant flowers.
The bark yields resinous copious sticky latex which is used in Ivory Coast as arrow poisons. The bark decoction or macerate in palm wine is taken for the prevention and cure of dysentery, malaria and fever. The macerate of the root is used for the management of stomach pains, habitual abortion and for

treating renal infections[6]. The plant extract has been reported to contain 8-dihydrotrichothecinol and trichothecinol (with cytotoxic effect), 6 beta-hydroxyrosenonolactone (with anti-leishmaniasis effect), 3-O-(3'-hydroxyeicosanoyl)lupeol, 3-O-[(2'-(tetracosyloxy)acetyl]lupeol and 3-O-[(1"-hydroxyoctadecyloxy)-2'-hydroxypropanoyl]lupeol {effective against chloroquine resistant malaria parasite}[2,4,6]. It also contains flavonoside identified as isoquercitroside, (Paris RR, et al 1959). and steroids; including cycloartenol, nor-31 lanosterol, dehydro-24 lophenol, desmosterol conessine, isoconessimine, conamine, conarrhimine, methylholaphylline, holaphyllinol, holaphyllidine, dihydroholaphyllamine, holaline, holarrheline, holadienine, holaromine, holaline and progesterone[4,7,11,12].

In this research work we investigated the effect of *H. floribunda* on isolated guinea pig ileum and selected bacteria species.

MATERIALS AND METHODS

Collection of plant material

H. floribunda leaves was collected from Babale, Jos, Plateau State, Nigeria in July 2005 and identified by a taxonomist, Mr. Kareem of the federal college of forestry and Prof. Huseni of the Department of Botany, University of Jos, Jos. Plateau State.

Extraction

Powdered *H. floribunda* (leaves) was soxhlet extracted with methanol for 72 hours. The resultant solution was vaporized at 40°C to get the solid extract. The extract was refrigerated at -4°C until use. The percentage yield was 21%.

Chemicals

Acetylcholine, Atropine, Histamine, Promethazine, KCL, Nifedipine, *H. floribunda* extract and distilled water were used for this experiment.

Animals and Micro-organisms

Guinea pigs and selected micro-organisms were used. Guinea pigs were bred in the Animal House of the Department of Pharmacology, University of Jos, Jos, Plateau State, Nigeria, under standard environmental conditions, and fed with standard diet (Vital Products, Nigeria) and water *ad libitum*. Pure cultures of micro-organisms were obtained from the Department of Pharmaceutical Technology and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria.

Antimicrobial activity

The anti-microbial activity of the methanolic extract of *H. floribunda* leaves was assayed in-vitro using the cup bore method on *Pseudomonas aeroginosa, Staphylococcus aures, Bacillus subtilis* and *Proteus mirabis.* Aliquot of nutrient broth (5ml) was inoculated with test organism and incubated at 37° C for 24 hours. After 24 hours using a sterile pipette 0.2 ml of the broth culture of the test organism was added to 20ml of nutrient agar which had been cooled to about 23° C. This was mixed well and poured into a sterile Petri dish. The agar was allowed to set and harden after which five holes were bored using a sterile cup borer on each of the agar plate. Serial dilutions of the extract and standard drug (gentamycin) were prepared and using a dropper, 0.1 ml of each dilution was introduced into five holes on each plate. The five holes in the last plate contained Gentamycin, the standard drug while the first plate contained distilled water. The plates were left for 1 hour to allow for diffusion of the extract, distilled water or gentamycin before incubation at 35° C. After 24 hours, the zones of inhibition were observed, measured and recorded.

Gastro intestinal tract activity

Guinea pigs were sacrificed by a blow on the head, dislocating the neck, and exsanguination. Segments of the ileum, about 2.0 cm long, were removed and dissected free of adhering mesentery. The intestinal contents were removed by flushing with Tyrode's solution of the following composition in millimoles (mM): NaCl, 136.8; KCl, 2.7; CaCl, 1.3; NaHCO3, 12.0; MgCl, 0.5; NaPO4, 0.14; glucose, 5.5. The tissue was mounted in a 50 ml organ bath containing Tyrode's solution

maintained at 35^0 C and aerated with air. A load of 0.5 g was applied. Equilibration period of 60 minute was allowed during which the physiological solution was changed every 15 min. At the end of the equilibration period, the effects of graded doses of Acetylcholine ($1x10^{-5}$ g/ml), Potassium Chloride ($1x10^{-2}$) and Histamine ($1x10^{-5}$ g/ml) were determined. The effects of graded doses of the extracts of *H. floribunda* leaves were also determined. Also the effects of the various doses of the extract (which was incubated for 3 minutes prior to the introduction of the standard drug) on Acetylcholine, Histamine and Potassium Chloride induced contraction of guinea pig ileum was determined. The contact time for each concentration was 1 min, which was followed by washing three times. The tissue was allowed a resting period of 15 min before the next addition. Responses were recorded iso-metrically using Ugo Basile Unirecorder 7050 (Otimenyin et al., 2006).

Results

The results obtained from this study showed that methanolic extract of *H. floribunda* leaves is devoid of anti-microbial activity against *Pseudomonas aeroginosa, Staphylococcus aures, Bacillus subtilis and Proteus mirabis.* (Table 1). Gentamycin was shows to be effective against these organisms. Methanolic extract of *H. floribunda* leaves was shown from our results to have no significant contractile or relaxing effects on the guinea pig ileum (Fig. 1). *H. floribunda* extract inhibited Acetylcholine, (Fig.2) Histamine (Fig.3) and Potassium (Fig.4) induced contraction of the guinea pig ileum Similarly, nifedipine was more effective in blocking contraction induced by KCL than that induced by the methanoilic extract of *H. floribunda* (Fig 4).

Table 1; Anti-microbial properties of ethanolic extract of *Holerrhena floribunda.*

Treatments	Zones of Inhibition (mm)					
Extract. conc.	30mg	7.5mg	1.8mg	0.5mg	Gent. (2mg)	Dist. H_2O
B. subtilis	0.4±0.2*#	0.2±0.1*#	0.1±0.3*#	0.1±0.2*#	16±3.4	0±0*
P. aeruginosa	0.2±0.1*#	0±0*#	0±0*#	0±0*#	13±1.3	0±0*
P. mirabilis	0±0*#	0±0*#	0±0*#	0±0*#	23±2.4	0±0*
S. aureus	0±0*#	0±0*#	0±0*#	0±0*#	23±2.1	0±0*

*P<0.05. When compared with gentamycin., #P>0.05, When compared with distilled water.
mm = millimeter
Dist. H_2O = Distil water
Gent. = Gentamycin.
conc. = Extract concentration.

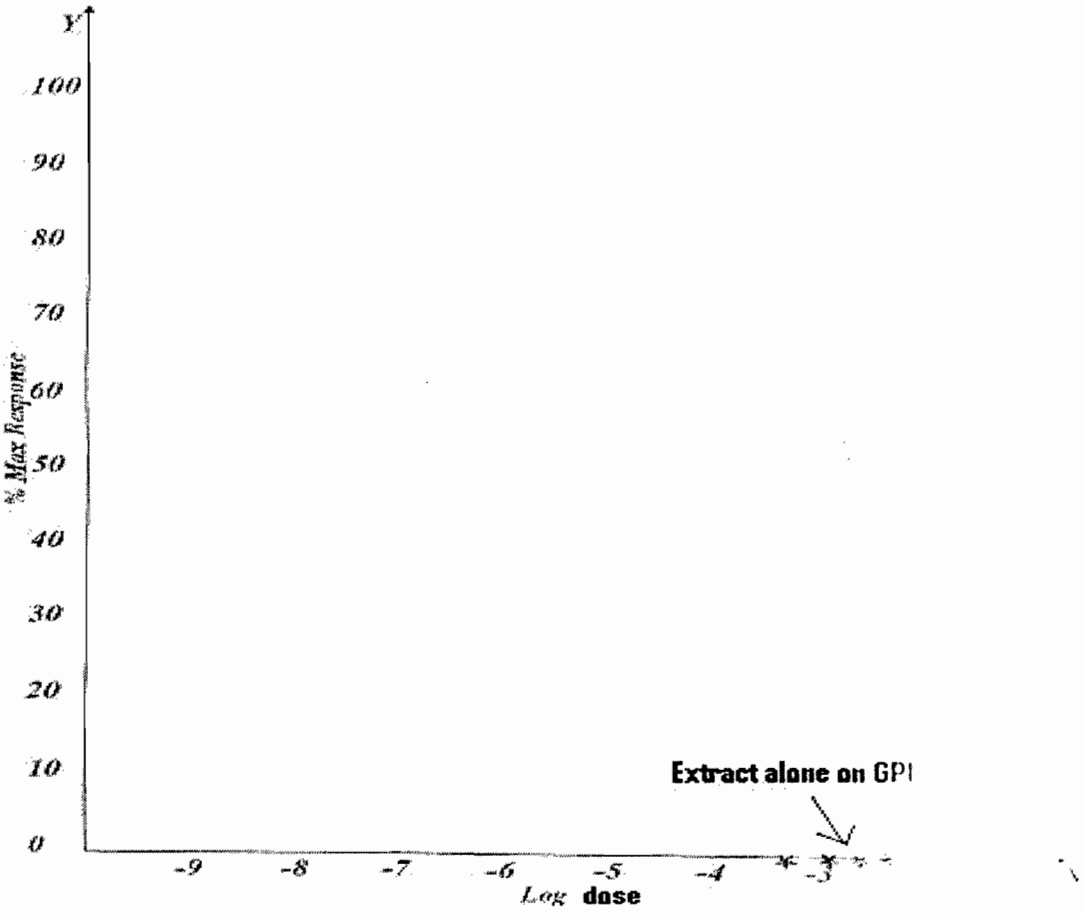

Fig 1. Effect of *H. floribunda* extract on guinea pig ileum (GPI).

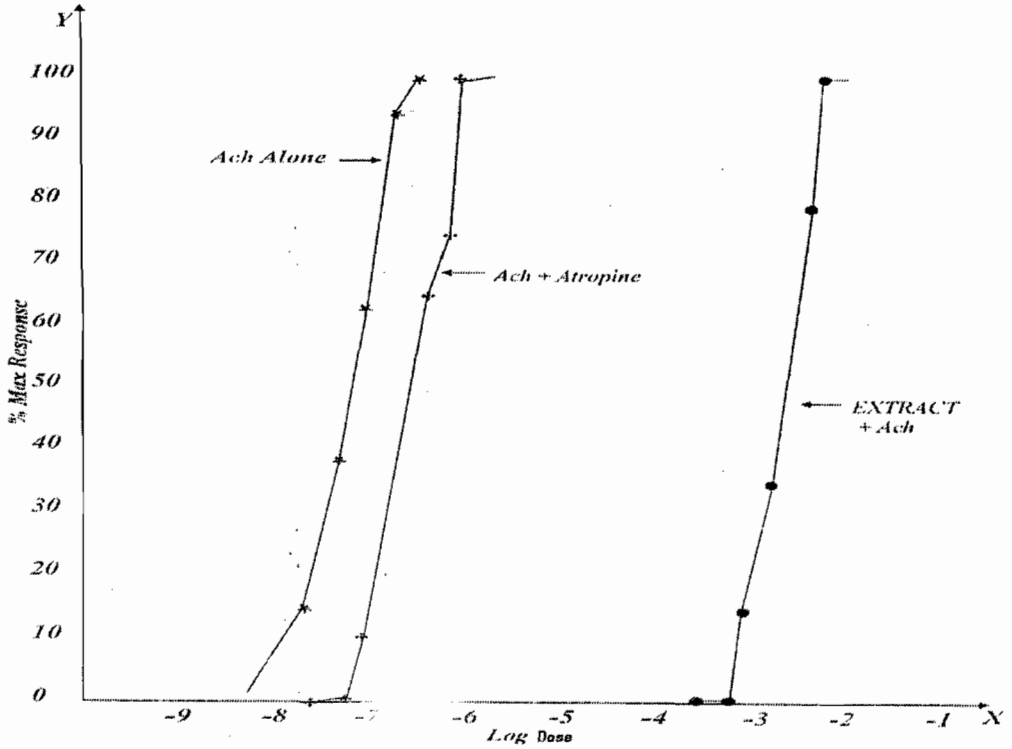

Fig 2. Effect of Atropine (1 x 10^{-1}g/ml) and *H. floribunda* extract (1 x 10^{-1}g/ml) of on Acetylcholine (1 x 10^{-5} g/ml) induced contraction of guinea pig ileum

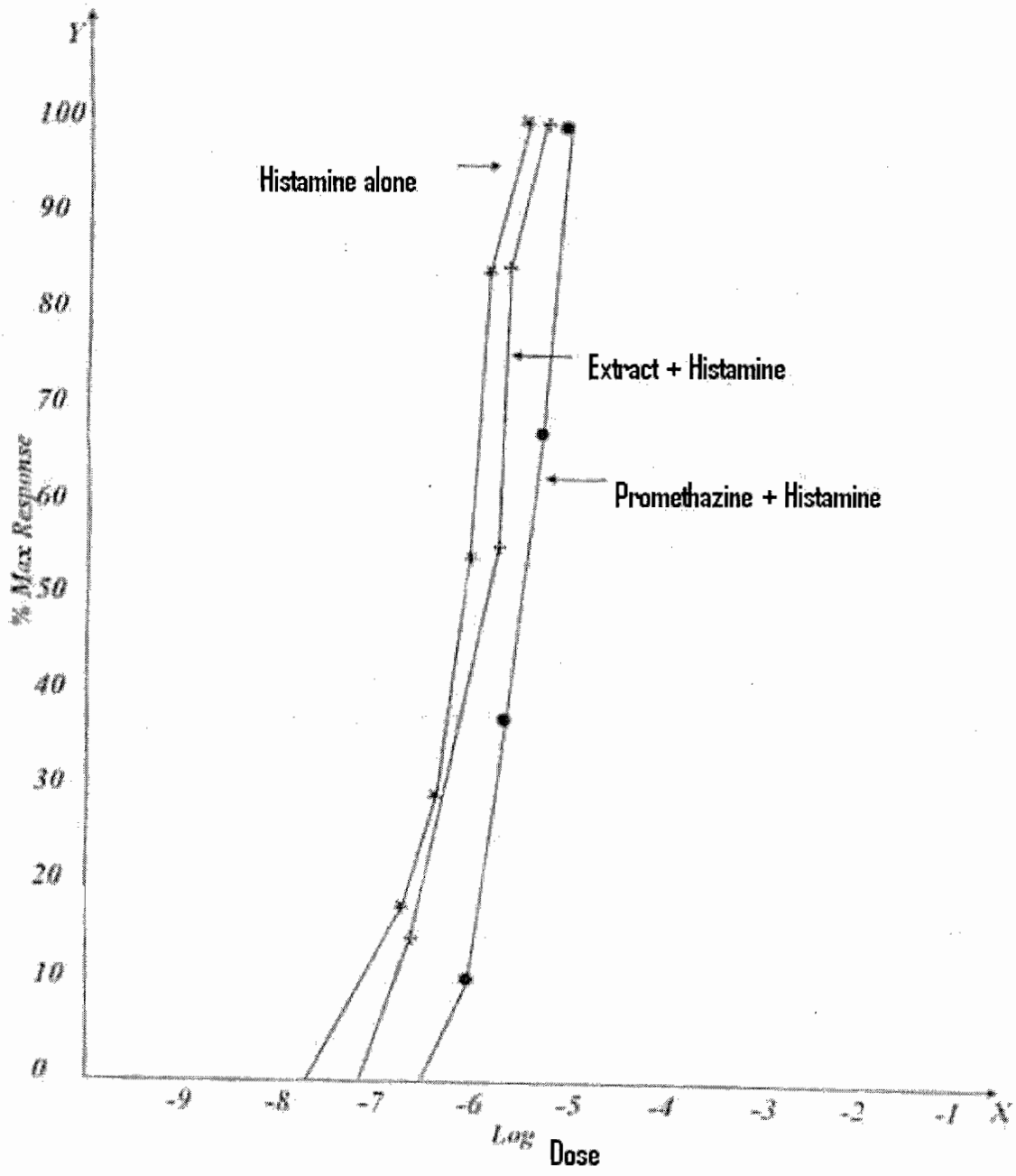

Fig 3. Effects of Promethazine (1x 10^{-2} g/ml) and *H. floribunda* extract (1 x 10^{-1} g/ml) on Histamine (1 X10^{-5} g/ml) induced contraction of the guinea pig ileum.

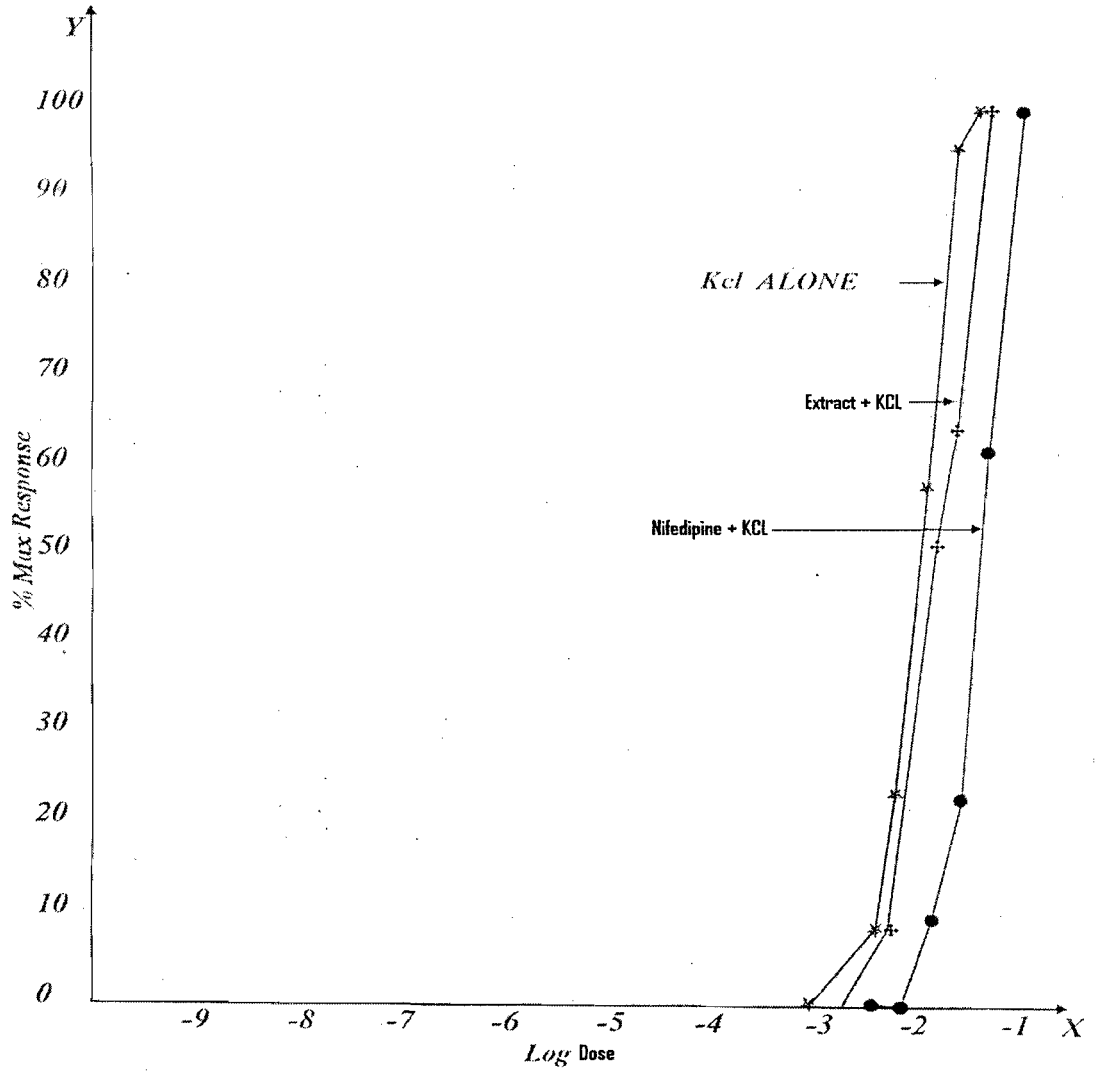

Fig 4. Effects of Nifedipine (1x 10^{-1} g/ml) and *H. floribunda* extract (1 x 10^{-1}) on KCL (1 X10^{-2} g/ml) induced contraction of the guinea pig ileum.

Discussion

The importance of Plants in the management of disease has been known to man from time immemorial[6,11,12]. In the last decades, the basis for the use of herbs for the management of disease conditions is being evaluated. Research findings have shown that quiet a number of these plants have the claimed activity[1,3,4]. Most communities in the world have a kind of folk medicine. In Nigeria, each tribe has her form of folk medicine and traditional methods of managing disease conditions. Plants are used to complement orthodox medicines and to treat incurable diseases. These methods though not fully developed as Ayorveda of India, is in wild use by different tribes in the country. Scientific research into some of this method of providing health care has yielded useful results. For example, eserine (Calabar bean, which was once used as ordeal poison to assess the guilt or innocence of suspected criminals and heretics), first found in Calabar (in Nigeria) has been found to contain physostigmine, a useful drug in the treatment of glaucoma, (Rand and Dale, 1991). Medicinal Plants used for the management of diseases are administered by experts (trained traditional healers) in the field. These people are normally called native doctors by the community. They acquire the knowledge from their father or guardian who trains them from an early age. *H. floribunda* healing potentials have been passed from generation to generation by this method. Scientifically, *H. floribunda* has been proven to be effective in the management of

several disease conditions. The results obtained from this study showed that methanolic extract of *H. floribunda* leaves is devoid of anti-microbial activity against *Pseudomonas aeroginosa, Staphylococcus aures, Bacillus subtilis and Proteus mirabis.* (Table 1). Gentamycin was shows to be effective against these organisms. *H. floribunda* can be said from the result above not to be effective in the management of infectious diseases. This result is contrary to the claims by Traditional Healers.

Methanolic extract of *H. floribunda* leaves was shown from our results to have no significant contractile or relaxing effects on the guinea pig ileum (Fig. 1). *H. floribunda* extract inhibited Acetylcholine, (Fig.2) Histamine (Fig.3) and Potassium (Fig.4) induced contraction of the guinea pig ileum. The effect of *H. floribunda* on contraction induced by acetylcholine was more pronounced than that produced by Atropine (Fig 2). The effect of *H. floribunda* on contraction induced by Histamine was less pronounced than that produced by promethazine (Fig 3). This indicates that Promethazine in a more effective anti-histamine than the extract (Fig 3). Similarly, nifedipine was more effective in blocking contraction induced by KCL than that induced by the methanoilic extract of *H. floribunda* (Fig 4). From the results, it can be concluded from the results that *H. floribunda* has Atropine like activity (Fig 2) on the guinea pig ileum. The blockade of contractile effect of Acetylcholine, Histamine, and Potassium Chloride revealed that the extract contains anti-muscarinic, anti-histamine and Potassium channel blocking activities respectively.

In conclusion, the methanolic extract of *H. floribunda* is not suitable for the management of infection caused by *Pseudomonas aeroginosa, Staphylococcus aures, Bacillus subtilis* and *Proteus mirabis*. These results negate its use by traditional healer in the management of infection wounds. The effect of *Holerrhena floribunda* on guinea pig ileum suggest that it has anti-muscarinic , anti-histamine and potassium channel blocking activities.

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