

Phytochemical Screening and *in Vitro* Antimicrobial Activity of *Waltheria Indica* Linn Leaf Extracts

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Abstract: The plant, *Waltheria indica* Linn is known to possess medicinal properties according to African folklore. Scientific verification of its bioactive constituents backing its use for the treatment of upper respiratory tract infections is limited. This study aimed at evaluating the phytochemical compounds present in the crude leaf extracts as well as identify their pathogen-specific antagonistic activities. The results obtained revealed that alkaloids, cardiac glycosides, phenolic acids, saponins, steroids, tannins and terpenoids were present in the extracts. The results also revealed that the zones of inhibition from the acidic, basic, neutral polar and non-polar fractions ranged between 24-28mm against the organisms (*Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus pyrogens*, *Klebsciella pneumonia*, and *Candida krusei*). The Minimal Inhibitory Concentration (MICs) of the crude, acidic, basic, neutral polar and non-polar fractions of the leaf was recorded at 2.5mg/ml and 0.5mg/ml respectively. The results obtained suggested that the leaf of the studied plant possess strong antimicrobial activities against selected bacterial and fungal species with direct correlation to the actions of their phyto-constituents.

Keywords: *Waltheria Indica* Linn Leaf, Phytochemicals, Anti-microbial Activity

1. Introduction

Although the 21st century has witnessed a new dawn of Research and Development with regards to medical science and preventive medicine, the world, particularly developing nations, is still plagued by a blitz of infectious diseases. This occurrence stems from both the emergence and re-emergence of drug resistant pathogens, which thwart the efficacy of regular antimicrobial treatment options. Such episodes have further been intensified by the burgeoning therapeutic spectrum coupled with previously non-conceived side effects of synthetic drugs [1], [2]. Systemic failure of the body's natural defense mechanisms to mount adequate protection against invading pathogens is as a result of the consistent hijacking and modulation of crucial internal repair elements [3].

The aggravated onslaught by new breeds of drug resistant infectious organisms in conjunction with a waning assortment of therapeutic agents, has created a plethora of side-effects, invasion by other opportunistic infections that

trigger ailments like upper respiratory tract infections [4]. The scourge of such events is eminent amongst the rural populace in developing countries [5], [6]. The consequence of this includes prolonged symptoms coupled with the inability for conventional over-the-counter drugs to block the extra-/intracellular binding domains for pathogen host interaction [7].

Both the acquired resistance of pathogenic organisms to certain synthetic drugs and the indiscriminate consumption of commercial antimicrobial drugs have led to the advent of multiple drug resistant pathogens [8]. It is conceivable that incorrect use of synthetic and semi-synthetic antibiotics triggers an evolutionary adaptation thereby conferring resistance via principally plasmid exchange and activation of cascading events that block/close antibiotic binding domains which normally elicit an effective immune response [9].

New drug candidates have been sought after over the years for a variety of disease-causing pathogens; β -hemolytic *Streptococci*, *Bordetella pertusis*, *Bordetella parapertusis*, *Klebsiella* and *Mycoplasma pneumonia*, *Staphylococcus aureus*, etc, all of which elicit respiratory tract complications

[2]. This upsurge of bacterial infections accompanied by its platoon of symptoms within the respiratory system is particularly prevalent in developing countries whereby a lengthier duration of recovery is the norm. Phyto-therapy is a widely accepted alternative method of treatment for an avalanche of ailments globally [10] and the phytochemicals that have been implicated include alkaloids, cardiac glycosides, flavonoids, glycoalkaloids, glycosides, phenols, tannins, terpenes, terpenoids, etc [11].

As natural products, phyto-compounds present in certain medicinal plants are fully adept to circumvent the growth and proliferation of disease causing pathogens, particularly some multi-drug resistant variants with the added benefit of producing marginal to no adverse side effects [12]. The unique bouquet of bioactive compounds present in such plants possess dual functionality whereby it is consumed as an effective antimicrobial agent for infectious diseases as well as a valuable nutritional supplement [13], [14], [15].

A report has indicated that such plant derived antimicrobial therapeutic agents are intriguing in that they do not prompt any adverse effects nor bear expensive production cost in comparison to their synthetic and semi-synthetic counterparts [16].

The application of whole herbal plants, stem bark, roots, leaves, etc for the treatment of many diseased conditions like respiratory complications has been common practice within Africa and the Middle East for a long time [11]. The earliest plant used as a natural deterrent of infectious diseases was that of Hollyhock (*Alcea rosea* L.) [17].

In vitro and *in vivo* studies have revealed that certain plants functioned just as well as synthetic antibiotics owing to their rich pharmacological arsenal which are antagonistic to the proliferation of bacteria and viruses within a suitable host. Such studies included but are not limited to the menthol compounds found in *Mentha piperita* (peppermint) which also possessed additional pulmonary enhancing functions [18], thymol and carvacrol, both phenolic terpenoid compounds, isolated from *Origanum syriacum* [19], [20], the latter of which was identified as a potential bronchodilator using animal models [21]. The phyto-compound; cineole, found within the essential oils of *Eucalyptus globulus* and *Rosmarinus officinalis* [22] has been implicated and documented as an anti-inflammatory agent which ameliorates chronic bronchitis, a condition triggered by either bacteria (*Mycoplasma pneumonia*, *Bordetella pertussis*, *Moraxella catarrharis*, *Haemophilus influenza*) or induced by rodents [22].

With the success in isolating and providing commercially available new drug candidates from plant origin like Atropine, Ephedrine, Digoxin, Morphine, Quinine, Reserpine and Tubocurarine [23], one would consider the evaluation of several other indigenous plants grown in the wild that can be exploited as drug candidates. Studies have shown that many phenolic compounds present in the form of crassinervic acid, aduncumene, hostmaniane and gaudichaudanic acids obtainable from species of Piper (*Piper crassinervium*, *Piper aduncum* L., *Piper hostmannianum* and *Piper*

gaudichaudianum) as well as gingerol, capsaicin, ellagic acid exhibit antimicrobial and anti-cancerous effects [24].

Some drugs of aminoglycosides origin, including kanamycin and streptomycin have been used to treat an assortment of extreme upper respiratory tract infections like pneumonia, brucellosis and tuberculosis [25]. Since plants possess an arsenal of phyto-compounds, downstream activities of individual constituents or a synergy of sorts would form a logical next step in the screening for new drug candidates of plant origin.

In Nigeria, every community and culture practices its own version of phyto-medical therapy based on the diversity of available herbal plants. The underlying scientific principle for the use of certain plants needs to be established. *Waltheria indica* Linn (*hankufah* in Hausa, *korikodi* in Yoruba), belonging to the Sterculiaceae family, is one of such plants indigenous to the north central region of Nigeria (Figure 1). The plant is reported to possess therapeutic potential in the treatment of inflammations, malaria and upper respiratory infections in addition to the prevention of oxidative stress [26]. Its roots have been reportedly used in the treatment of diarrhea, wounds and stomach ache [27]. Different parts of the plant have been used to treat coughs, haemorrhages, fever, gonorrhea etc [28]. Amongst traditional practitioners, it is believed that treatment of a myriad of infections previously unconceived is possible. Like other selected indigenous plants, the leaves and roots of *Waltheria indica* Linn display pharmacological properties against selected disease causing bacterial strains. The success of other plant extracts from species including *Alpinia galangal*, *Adhatoda vasica*, *Aegle marmelos*, *Tectona grandis*, *Solanum trilobatum*, in treating upper respiratory tract infections suggest that it could be a potential agent [29], [30]. The present study was undertaken to confirm the antimicrobial activity of the leaves of *Waltheria indica* against selected pathogens that trigger upper respiratory tract infections.



Figure 1. *Waltheria indica* Linn plant.

2. Materials and Methods

The fresh leaves of *Waltheria indica* Linn were collected from the Kwali local government of Abuja, Nigeria. The

plant was authenticated at the National Institute for Pharmaceutical Research and Development, Abuja, voucher number NIPRD/H/6738. It was then air-dried and kept in airtight containers until required for further laboratory analysis.

The air-dried crispy leaf samples (3,200g) were ground to powder with a mortar and pestle and then extracted via the soxhlet extraction method for a duration of 6-8 hours with 5.0L of 95% ethanol. It was then filtered [31]. The extract was later concentrated using rotary evaporator at 40°C and then air-dried to give the crude extract. The extract was weighed and the weight recorded (400g).

2.1. Phytochemical Screening

Phytochemical screening was carried out on the crude extract for the qualitative determination of major constituents using methods previously described [32].

2.2. Fractionation

The crude extract (345.03g, 10.6%) was subjected to bioassay-guided fractionation (Figure 2) to give the basic, acidic, non-polar neutral and polar neutral fractions [33].

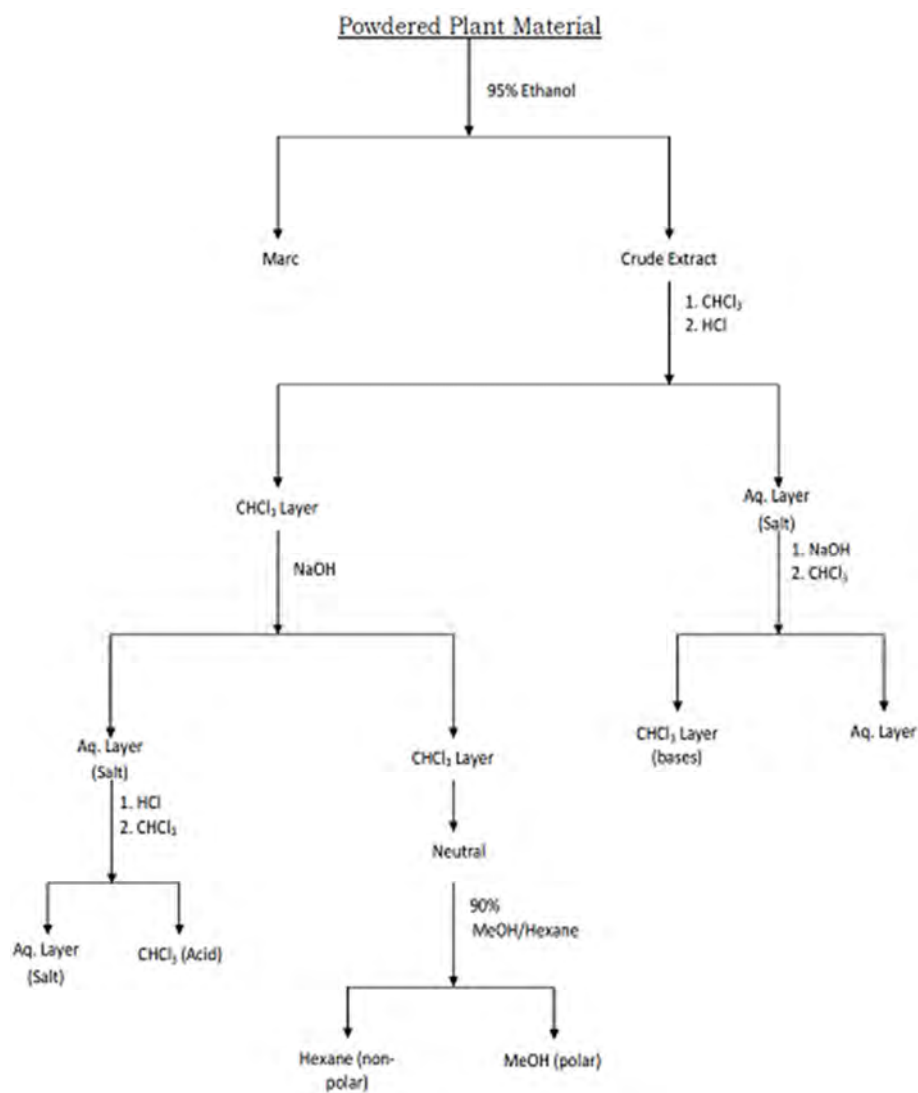


Figure 2. Flowchart of key fractionation steps of leaf.

2.3. Antimicrobial Screening

Mueller Hinton and Sabouraud dextrose agars (Sigma-Aldrich) were the media used as the growth media for the microbes.

Pathogenic bacterial and fungal isolates were obtained from the 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 [34]. Agar diffusion method was employed [35].

The crude extract (0.1g) was weighed and dissolved in 10mls of DMSO to obtain a concentration of 10mg/ml. Each of the acidic (A), basic (B), polar neutral (D) and non-polar (E) of the leaf extracts (0.02g) was dissolved in 10ml of DMSO to obtain a concentration of 2mg/ml. The sterilized

Mueller Hinton agar was seeded with 0.1ml of the standard inoculum of the test bacteria and the Sabouraud dextrose agar was seeded with fungi. The inoculum of the test microorganism was swabbed onto the surface of the medium. The solution (0.1ml) of the crude extract (10mg/ml) and 2mg/ml of each of the fraction was each then introduced into the respective wells, bored with a sterile 6mm cork borer, on the inoculated medium. The samples were then incubated for 24 hours at 37°C for the bacteria and at 30°C for 168 hours for the fungi, after which each plate of the media was observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and the result was recorded in millimeters.

2.4. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined on the test organisms that were sensitive to the extracts by broth dilution method [36]. Mueller Hinton broth was prepared and dispersed into test tubes and the broth was sterilized at 121°C for 15mins and the broth was allowed to cool. Normal saline (10ml) was dispersed into sterile test tubes and the test microbes were inoculated and incubated at 39°C for 6hrs. Dilution of the test microbes was done in the normal saline until the turbidity matched that of the McFarland's standard scale by visual comparison at this point. The test microbes had concentration of about 1.5×10^8 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, 0.625mg/ml and 0.313mg/ml. The initial concentration was obtained by dissolving 0.05g of the crude extract in 10mls of DMSO to obtain a concentration of 5mg/ml from which subsequent dilutions were made. Having obtained the different concentrations of the extracts the sterile broth was observed for turbidity (growth). The lowest concentration of the extract in the broth which showed no turbidity was recorded as the MIC.

2.5. Determination of Minimum Bactericidal and Fungicidal Concentrations

Minimum bactericidal concentration (MBC) and Minimum

fungicidal 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 [36]. 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°C for 24 hours (bacteria) and 30°C for 1-7 days (fungi) after which the plates were observed for colony growth. The MBC and MFC were the plates with the lowest concentrations of the extracts without colony growth.

3. Results

Table 1 reflects the phytochemical compounds detected from the alcoholic extracts from the crude leaf extracts of *Waltheria indica*. Analysis revealed that the bioactive agents present included tannins, saponins, steroids, phenolic acids, alkaloids, cardiac glycosides and terpenoids.

Table 1. Phytochemical screening of *Waltheria indica* Linn crude leaf extract.

Phyto-constituents	Leaf extract
Tannins	+
Saponins	+
Flavonoids	-
Steroids	+
Phenolic acid	+
Alkaloids	+
Cardiac glycosides	+
Terpenoids	+
Carbohydrates	+

(+) – Present (-) – Absent

Antimicrobial screening of the crude leaf extracts tested against gram positive and gram negative clinical bacterial isolates as well as against clinical fungal isolates was captured (Table 2). The data revealed that *Staphylococcus aureus*, *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Candida krusei* and *Candida tropicalis* were all susceptible to the antagonistic effects of the crude leaf extracts towards microbial growth.

Table 2. Antimicrobial activity of *Waltheria indica* Linn crude extracts against test microorganisms Zone of inhibition (mm).

Test microorganisms	Crude Leaves	Fluconazole (30 µg/ml)	Ciprofloxacin (10 µg/ml)
<i>Staphylococcus aureus</i>	23 ± 0.15 (S)	0 (R)	32 ± 0.45 (S)
<i>Streptococcus pneumonia</i>	22 ± 0.80 (S)	0 (R)	30 ± 0.25 (S)
<i>Streptococcus pyogenes</i>	0 (R)	0 (R)	32 ± 0.30 (S)
<i>Klebsiella pneumonia</i>	22 ± 0.60 (S)	0 (R)	35 ± 0.10 (S)
<i>Corynebacterium ulcerans</i>	0 (R)	0 (R)	0 (R)
<i>Candida albicans</i>	0 (R)	35 ± 0.95 (S)	0 (R)
<i>Candida krusei</i>	23 ± 0.40 (S)	35 ± 0.55 (S)	0 (R)
<i>Candida tropicalis</i>	24 ± 0.35 (S)	34 ± 0.60 (S)	0 (R)

R= Resistant, S= Sensitive

Analysis of the phytochemical extracts using the bio-assay guided protocol on the leaves of *Waltheria indica* Linn, displayed the respective inhibitory activities of the acidic, basic, polar neutral and non-polar neutral phyto-compounds

against the selected clinical isolates of bacteria and fungi in comparison to the effects of Ciprofloxacin and Fluconazole used as controls (Table 3).

Table 3. Zones of inhibition of the leaf fractions against the test microorganism.

Test microorganisms	A (Acidic)	B (Basic)	D (Non Polar)	E (Polar)	Control
<i>Staphylococcus aureus</i>	25 ±0.45 (S)	25 ±0.55 (S)	26 ±0.15 (S)	24 ±1.10 (S)	32 ±0.15 (C)
<i>Streptococcus pneumonia</i>	27 ±0.30 (S)	29 ±0.60 (S)	27 ±0.80 (S)	26 ±0.10 (S)	31 ±0.65 (C)
<i>Streptococcus pyogenes</i>	0 (R)	0 (R)	0 (R)	0 (R)	32 ±0.75 (C)
<i>Klebsiella pneumonia</i>	26 ±0.30 (S)	25 ±0.75 (S)	26 ±0.35 (S)	28 ±0.90 (S)	34 ±0.80 (C)
<i>Corynebacterium ulcerans</i>	0 (R)	0 (R)	0 (R)	0 (R)	0 (C)
<i>Candida albicans</i>	0 (R)	0 (R)	0 (R)	0 (R)	36 ±0.35 (F)
<i>Candida Krusei</i>	24 ±0.10 (S)	26 ±0.60 (S)	28 ±1.20 (S)	24 ±0.15 (S)	34 ±0.90 (F)
<i>Candida tropicalis</i>	26 ±0.25 (S)	24 ±0.55 (S)	26 ±0.95 (S)	24 ±0.80 (S)	33 ±0.10 (F)

R= Resistant, S= Sensitive, C = Ciprofloxacin, F = Fluconazole

Table 4 data displays the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the crude, acidic, basic, polar neutral and non-polar neutral

extracts of *Waltheria indica* Linn. The bioassay guided fractions gave overall lower MIC, MBC and MFC values than that of the crude against each selected microorganism for which activity was shown (Table 4).

Table 4. Minimum inhibitory, bactericidal and fungicidal Concentrations (MIC, MBC, MFC) of *Waltheria indica* Linn leaf fractions against test organisms [Zone of inhibition (mg/ml ± SD)].

Microorganisms	Minimum Inhibitory Concentration (mg/ml)					Minimum Bacterial/Fungal Concentration (mg/ml)				
	Crude	Acidic	Basic	Polar	Non Polar	Crude	Acidic	Basic	Polar	Non Polar
<i>Staphylococcus aureus</i>	2.5	0.5	0.5	0.5	0.5	5.0	1.0	1.0	1.0	1.0
<i>Streptococcus pneumonia</i>	2.5	0.25	0.25	0.25	0.5	10.0	0.5	0.5	0.5	1.0
<i>Streptococcus pyogenes</i>	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumonia</i>	2.5	0.5	0.5	0.5	0.25	10.0	1.0	1.0	1.0	0.5
<i>Corynebacterium ulcerans</i>	2.5	-	-	-	-	-	-	-	-	-
<i>Candida albicans</i>	2.5	-	-	-	-	-	-	-	-	-
<i>Candida krusei</i>	2.5	0.5	0.5	0.5	0.5	5.0	1.0	0.5	0.5	1.0
<i>Candida tropicalis</i>	2.5	0.5	0.5	0.5	0.5	5.0	1.0	1.0	1.0	1.0

Each value represents mean (n = 3).

4. Discussion

Findings from Table 1 suggest that the leaves contain a collection of secondary metabolites, namely the alkaloids, cardiac glycosides, phenols, saponins, steroids, tannins and terpenoids in both plant extracts are known individually or collectively for their defensive or signaling functions [37]. The herpato-stimulatory properties of plant glycosides, particularly the cardiac glycosides are well documented and have been recently implicated in the treatment of heart muscle complications as well as in the treatment of many forms of cancer [38], [39]. The therapeutic uses of phenols in particular cannot be over emphasized as from this group, antibiotics like griseofulvin, natamycin and nystatin have been obtained [40]. Steroids play a vital role in enhancing the well-being of animals and humans as they function in resisting infiltration by many pathogens owing to their antimicrobial properties [41]. Tannins are generally known for their use as a prophylactic against diarrhea as plants rich in this phyto-compound alongside others like steroids and saponins have shown activity against disease causing pathogens like *S. aureus* and *S. thypi* which are responsible for diarrhea and stomach upsets [42]. Another interesting group of phyto-compounds are the alkaloids, revered for their neuro-stimulatory, analgesic and upper respiratory protective functions amongst others [43], [44]. In addition, the presence of both alkaloids and steroids in the leaf extracts creates the allusion that the plant possesses sufficient activity against

disease causing pathogens like *S. aureus* and *S. thypi* which are responsible for diarrhea and stomach upsets [42]. Phenolics, which also comprise of flavonoids, phenolic acids and tannins are one of the most abundant phyto-constituents in plants and as a result are not only an essential part of our diet but have also been associated with good mental performance through the consumption of items such as berries, chocolate, red wine and tea [45]. Some studies have reported on the various applications and healthcare benefits of individual phenolics owing to the wide range of biological and pharmacological activities they possess, ranging from anti-allergic, anti-inflammatory, antioxidant, antimicrobial, anticancer and antidiarrheal properties [46], [47].

The mean zones of inhibition of the crude extract against different bacterial and fungal species revealed an overall higher antimicrobial activity against selected bacteria implicated in several upper respiratory tract infections (Table 2). The antifungal activity was highest against only *Candida tropicalis* (24 mm) as the extract also displayed susceptibility towards *Candida albicans* and *Streptococcus pyogenes* in a manner that suggests a preference for particular bacteria than fungi. Individual comparisons revealed an almost similar range of inhibition against *Staphylococcus aureus* (23 mm), *Streptococcus pneumonia* (22 mm), *Klebsiella pneumonia* (22 mm) and *Candida krusei* (23 mm). The antimicrobial spectrum of the crude leaf could be linked to the bouquet of phytochemicals present. The presence of alkaloids alone is highly suggestive of a broad antimicrobial spectrum as alkaloids are reportedly extensively used as pharmaceuticals,

psycho-stimulants, narcotics and poisons due to their renowned biologic activities [48]. The activity of alkaloids have been investigated in plants like *Callistemon citrinus* and *Vernonia adoensis* whereby at concentrations of 1.7mg/ml, it inhibited the growth of *Staphylococcus aureus* with comparable effects to ampicillin [49]. Other possibilities include the synergies between phenolic acids and tannins, both of which have been studied and found to be active against *Staphylococcus aureus* alongside a host of bacterial pathogens linked with respiratory infections [50].

Separation of the leaf extractions into different solvents fractions revealed that the different phytochemicals present are active within the acidic, basic, polar and non-polar fractions (Table 3). The data showed that the fractions of the leaf are ineffective against *S. pyrogens*, *C. ulcerans* and *C. albicans*. The polar and non-polar fraction of the leaf is best suited for treating *S. aureus*, *C. krusei* and *C. tropicalis* infections (26mm, 28mm and 26mm respectively), while the basic fraction is active against *S. pneumonia* (29mm) and the polar fraction is strongly against *K. pneumonia* (28mm).

Table 4 data show the MIC, MBC and MFC of the different fractions of *Waltheria indica* Linn. All fractions had the same MIC value (0.5mg/ml) against the tested clinical strains with the exception of *S. pneumonia* for which the non-polar fractions exhibited a MIC value of 0.5(mg/ml) while other fractions gave an MIC value of 0.25(mg/ml). These results in comparison to that of the crude extract (2.5mg/ml) suggest that individual fractions of the leaf were more active, thereby supporting data obtained in table 3. The lowest MBC values obtained was 0.5(mg/ml) against *S. pneumonia* for all but the non-polar neutral fraction which exhibited a 1.0(mg/ml) value. The lowest recorded MFC value (0.5mg/ml) was against *C. krusei* by the basic and polar neutral fractions (Table 4).

5. Conclusion

The present study has shown the phytochemical composition and anti-microbial activity of *Waltheria indica* Linn leaf. The results supports the indigenous claim for the ethnomedical applications of the leaf extracts of *Waltheria indica* Linn. The data obtained revealed that the solvent-based fractions exhibited a higher antimicrobial activity than its crude counterpart due to enhanced concentration of its constituents. The bioactive compounds identified revealed that the included alkaloids, cardiac glycosides, carbohydrates, phenols, saponins, tannins and terpenoids. Thus, the activity of the plant may be ascribed to the presence of these phytochemicals.

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