INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

ANTIBACTERIAL ACTIVITY OF THREE PLANT EXTRACTS USED IN NIGERIA FOLKLORIC MEDICINE AGAINST HOSPITAL ISOLATES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AND METHICILLIN-SENSITIVE *STAPHYLOCOCCUS AUREUS* (MSSA)

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Article Received on: 11/01/2011 Revised on: 09/02/2011 Approved for publication: 02/03/2011

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ABSTRACT
Staphylococcus aureus is a species of bacterium commonly found on the skin and/or in the noses of healthy people. Although it is usually harmless at these sites, it may occasionally get into the body (eg through breaks in the skin such as abrasions, cuts, wounds, surgical incisions or indwelling catheters) and cause infections. These infections may be mild (eg pimples or boils) or serious (eg infection of the bloodstream, bones or joints). It is one of the important bacteria as a potential pathogen specifically for nosocomial infections. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics.Hexane, ethylacetate, methanol and water extracts from 3 different plant species, *Jatropha curcas*, *Piliostigma thonningii* and *Hyptis suaveolens* used in Nigeria as popular medicine for the treatment of several ailments of microbial and non-microbial origin were evaluated for potential antimicrobial activity against methicillin-resistant *Staphylococcus aureus (*MRSA) and methicillin-sensitive *Staphylococcus aureus (*MSSA) using agar dilution method. Results revealed that there were no significant differences in the % susceptibility to MRSA and MSSA between the standard drugs and the different plant extracts using different extracting solvents (P>0.05). All the extracts of the 3 plants were effective on MRSA except water extract of *Jatropha curcas* and *Piliostigma thonningii*. Hexane extract from *P. thonningii* was inhibitory to 100% of both MRSA and MSSA isolates followed by ethyl acetate extract of *J. curcas* 61% of MSSA, ethyl acetate extract of *P. thonningii* on 38% of MRSA, methanol extract of *J. curcas* on 33% of both MSSA and MRSA and the least activity was with water extract of *H. suaveolens* on 17% of both MSSA and MRSA; no activity was observed with water extract of *J. curcas*. Hexane extract of *P.thonningii* was the only extract found in this study to inhibit the growth of both MRSA and MSSA. The phytochemical screening of crude extracts revealed the presence of tannins, phenols, sterol, and saponin in *J curcas*; saponin, tannin, flavonoid, phenols and alkaloid was observed in *H. suaveolens*, while *P. thonningii* extracts consist of flavonoid, phenol, sterol, tannin, saponin and cardiac glycosides. The presence of these bioactive components in the present study may be responsible for inhibition of the isolates. The results provide a scientific basis for the centuries-old usage of these medicinal plants. This study may help to suggest an alternative possible leading compounds for development of new antimicrobial agents against MRSA and MSSA resistant *S. aureus*

KEY WORDS: Antibacterial activities, Plant extracts, Methicillin-resistant *Staphylococcus aureus,* Methicillin-sensitive *Staphylococcus aureus*

INTRODUCTION

The use of plant compounds to treat infections is an age-old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases[1,2]. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics[3,4].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is now common in many areas of the world. The frequencies of infections and outbreaks due to MRSA have continued to increase. MRSA is often multidrug resistant and therapeutic options are limited[5-8]. MRSA is a major cause of nosocomial infection in UK hospitals and throughout the world. MRSA infections account for one fifth of all hospital-acquired infections, costing the UK National Health Service approximately £1 billion per year[9]. The problem has been aggravated by the rapid spread and high incidence of MRSA in intensive-care units[9]. The continuing rise in MRSA infection rates and its spread worldwide has led to calls for action to control infection and develop novel anti-MRSA agents[10,11] and vaccines.

In Nigeria, antimicrobial resistance has clearly emerged as a serious problem with MRSA. In this study, 3 medicinal plants in popular use in Nigeria for the

treatment of several ailments of microbial and non-microbial origins were tested for in vitro MRSA activity.

MATERIALS AND METHODS

Collection and Authentication of Plant material

Three plant samples were collected from Minna and Bida environment in Niger State. The taxonomic identity of the plants were confirmed with the assistance of the taxonomist of the Department of Soil Science, School of Agriculture and Agricultural Technology, Federal University of Technology, Minna (Prof. M. I. S Ezenwa) and authenticated by a botanist, Mrs G. E. Ugbabe, of Herbarium Department, National Institute for Pharmaceutical Research and Development, Idu, Abuja. Table 1 shows the botanical name, local name and plant part used under study.

Preparation of extracts

Dried and milled plant materials were extracted successively with Soxhlet extractor. Each of the solvent, hexane, ethyl acetate, methanol and water were replaced 3 times with fresh solvent and was allowed to remain in contact with the plant material for 48 h. After filtration of total extracts, the extracts were evaporated to dryness in vacuum and weighed.

Microorganisms used

MRSA and MSSA strains used in this study were clinical isolates from patients presenting with symptoms of *S. aureus*-associated diseases. The isolates were identified as *S. aureus* according to colonial and microscopic morphology, positive catalase, and coagulase production. All *S. aureus* isolates were tested for methicillin resistance. The disk diffusion method outlined by the National Committee for Clinical Laboratory Standards (NCCLS)[12,13] was used with a 1 µg oxacillin disk (Oxoid). Zone sizes were read after incubation at 35 ^{0}C for 24 h.

Phytochemical screening

Phytochemical screening of the extracts was carried out according to the methods described by Odebiyi and Sofowora[14] and Trease and Evans[15] for the detection of active components like saponins, tannins, alkaloids, phlobatannins, glycosides and e.t.c. To detect the presence of tannins- 1cm3 of freshly prepared 10%KOH was added to 1ml of the extract. A dirty white precipitate showed the presence of tannins. The presence of Glycosides was observed by the appearance of a brick-red precipitate. For saponin, Frothing test: 2ml of the extract was vigorously shaken in the test tube for 2 minutes. Presence of frothing indicates saponins. Yellow colouration is indicative of the presence of flavonoids. For Steroids, 5 drops of concentrated H_2SO_4 was added to 1cm3 of the extract in a test tube. Red colouration indicates the presence of steroids. Blue-green colour indicates the presence of triterpenes. For phenolics, two drops of 5%$Fecl_3$ was added to 1cm^3 of the extract in a test tube. Presence of greenish precipitate indicates the presence of phenolics. The presence of carbohydrate was observed by boiling 3g of the powdered sample in 50ml of distilled water on a hot plate for three minutes. The mixture was filtered while hot and the resulting filtrate was cooled. A few drops of Molisch's reagent was added to 2mls of the warm extract, then a small quantity of concentrated sulphuric acid was added to form a lower layer. A purple ring at the interface indicates the presence of carbohydrates

Antibacterial activity

Agar dilution method was used to screen all the isolates. This was done by dispensing 1ml (2mg/ml) of the plant extract into 19mls of Mueller Hinton Agar. After mixing, this was poured in to a plate and allowed to set. The organism equivalent to 0.5x10^6 (0.5 MacFalard Standard) was then streak on the medium; this was then incubated at 35^{o}C for 24 hours.

Interpretation of Results

Growth after 24 hours indicates resistance; no growth after 24 hours indicates susceptibility.

RESULTS

Profile of some medicinal plants that are used in the treatment of MRSA from literature are shown in table 2

Phytochemical screening

Phytochemical screening of these plants revealed the presence of tannins, saponins, phenols, terpenes, steroid, alkaloids and flavonoids.

The invitro susceptibility of both methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) to extracts of the plants using agar dilution method.

Kruskai-Wallis ranking test revealed that there were no significant differences in the % susceptibility to MRSA and MSSA between the standard drugs and the different plant extracts using different extracting solvents (P>0.05). For the activity of ethylacetate extract on MRSA, both *H. suaveolens* and *J. curcas* and oxacillin had 0% susceptibility to MRSA followed by *P. thonningii* with 29.6% susceptibility to MRSA. Both fusidic acid and trimethoprim had the same % susceptibility of 33.3% which by observation were approximately the same as that of *P. thonningii* . Vancomycin had the highest % susceptibility on MRSA. This showed that *P. thonningii* extract was as effective as vancomycin (Fig.1.1). For the activity of ethylacetate extract on MSSA, the effect of both *J. curcas* and *P. thonningii* are low with *J. curcas* much lower than *P. thonningii* . *P. thonningii* had approximately the same

effect as fusidic acid, trimethoprim and vancomycin. Oxacillin had 100% effect on MSSA (Fig.4.1).

The effect of methanolic extract on MRSA was high for *J. curcas* (29.6%) followed by *P. thonningii* (14.8%) and the least was *H. suaveolens* (3.7%) (Fig.1.2). *J. curcas* extract had approximately the same % susceptibility as fusidic acid and trimethoprim. Both *J. curcas* and *P. thonningii* had approximately the same effect as fusidic acid, trimethoprim and vancomycin on MSSA, the effect of *H. suaveolensi* (6.3%) on MSSA is very low (Fig.1.2). Hexane extract of .*P. thonningii* had 100% susceptibility on MRSA. The activity was higher than fusidic acid, trimethoprim and oxacillin. Vancomycin compared with hexane extract had 66.7% activity on MRSA (Fig.1.3). The activity of both *H. suaveolens* and *J. curcas* were low 18.5% and 7.4% respectively. 100% susceptibility of hexane extract was observed on MSSA, the activity was same as oxacillin but lower with fusidic acid, trimethoprim and vancomycin. *H. suaveolens* had approximately similar activity with on MSSA as both trimethoprim and vancomycin. *J. curcas* extract had no activity on MSSA (Fig.1.3).

Both *J. curcas* and *P. thonningii* water extract and oxacillin had no effect on MRSA. The susceptibility of *H. suaveolens* on MRSA was approximately the same as fusidic acid and trimethoprim. Vancomycin had the highest activity of 66.7% (Fig. 1.4). Both *J. curcas* and *P. thonningii* water extract had no effect on MSSA (Fig. 1.4).. The effect of *H. suaveolens* was low 12.5%. The effect of water extract was very low and it is not comparable with with the standard antibiotics. Alhough, the standard antibiotics fusidic acid, trimethoprim and vancomycin had low effect oxacillin had 100% susceptibility on MSSA. This implied that oxacillin was 100% effective on MSSA but the water extracts were not effective on MSSA (Fig. 1.4).

DISCUSSION

The increasing occurrence, particularly in hospitals, of *S. aureus* resistant not only to methicillin but to a wide range of antimicrobial agents, including all kinds of β-lactams, has made therapy more difficult[5-8]. Although strategies have been proposed in an attempt to control the spread[19]. The search for new ways to treat MRSA infections stimulates the investigation of natural compounds as an alternative treatment of these infections. In the present study, the analysis of the growth inhibition activity by the agar dilution method showed that all the 3 medicinal plants ((*J. curcas, H. suaveolens* and *P. thonningii*) commonly used by traditional medical practitioners in Nigeria were active against hospital strains of MRSA under test conditions with crude extract concentrations as high as 2mg/ml. Our results agree with the previous antibacterial studies related to these 3 botanical families[4,16,17]. The extract with the greatest antimicrobial activity against both MRSA and MSSA was that of *P. thonningii*. This may be due to the presence of some secondary metabolites present in the extract. The extracts from *Cassia occidentalis* of the same family were active against clinical strains of MRSA ; the greatest activity was from an ethanolic extract of roots of *Cassia occidentalis*[20]. Antibacterial activity of species of Euphorbiaceae, Lamiaceae and Caesalpiniaceae has previously been reported (Table 2).

In the present study, the range of bacteria (MRSA and MSSA) against which activity is attributed, is amplified and in addition aqueous extracts, often excluded in previous studies, were tested. We also tested the activities of extracts against clinical isolates of MRSA and in many cases highlighted the potential for these extracts to be used against such multiple antibiotic resistant organisms. Voss and Doebbeling[21] pointed out that MRSA is a problem for the world and some hospitals in Africa have reported an increase in MRSA isolation from 2% in 1985 to 50% in 1987. MRSA is now believed to have caused infections responsible for the deaths of nearly 19,000 hospital and nursing home patients in 2005. This number suggests that MRSA infections may be twice as common as previously believed and that deaths due to the superbug would exceed those due to HIV-AIDS, Parkison's disease, empysema or homicide for each year since 2005. It is the view of us and others[11], that novel sources of agents active against MRSA, and other drug resistant micro-organisms, should be actively sought. This is the reason we highlighted the activity of these extracts against MRSA in this study. In many cases our findings agreed with, or showed greater antimicrobial activity, to that of earlier workers. In some cases however our findings showed less activity. Such variations were possibly due to the different extraction methods employed, the sources of plant materials and/or the different strains of bacteria tested. The fact that different extraction methods can affect antibacterial activity has been reported in earlier studies[22]

CONCLUSIONS

Multiple drug resistance in bacterial pathogens is a continuing problem throughout the world. There is an established need to develop new antimicrobial agents to combat these pathogens.

In our study, we found that all the 3 crude plant extracts with a history of ethnobotanical use in Nigeria for the treatment of wounds, contain antibacterial compounds

that we have demonstrated have a potential use against both MRSA and MSSA. Further work is ongoing to identify the exact nature of these antimicrobials.

ACKNOWLEDGEMENT

We are grateful to laboratory personnel of General Hospital, Minna and National Hospital Abuja for supplying us with the isolates. We appreciate the assistance of the technologists of Microbiology Department, National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria.

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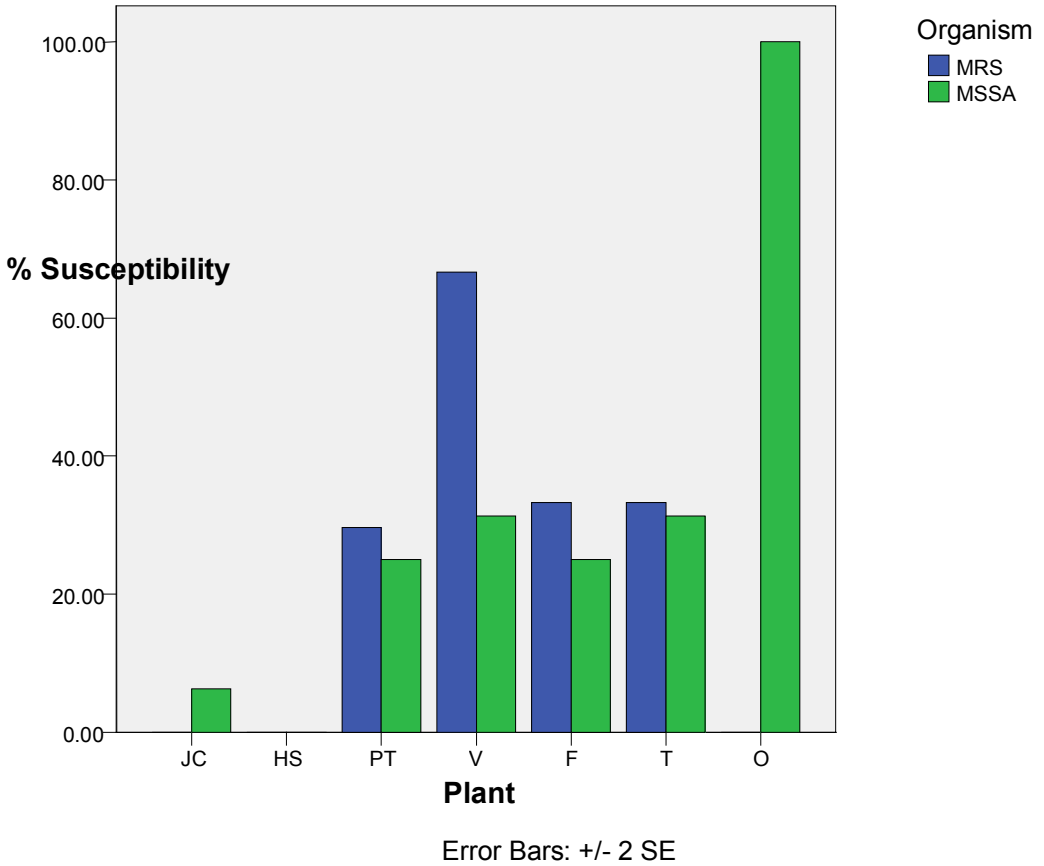
Table 1: List of medicinal plants used in the antimicrobial assay

Botanical Name	Family	Common name	Plant part used
Jatropha curcas	Euphorbiaceae.	Physic nut	Leaf
Hyptis suaveolens	Lamiaceae	Bush tea	Leaf
Piliostigma thonningii	Caesalpiniaceae	Monkey bread	Leaf

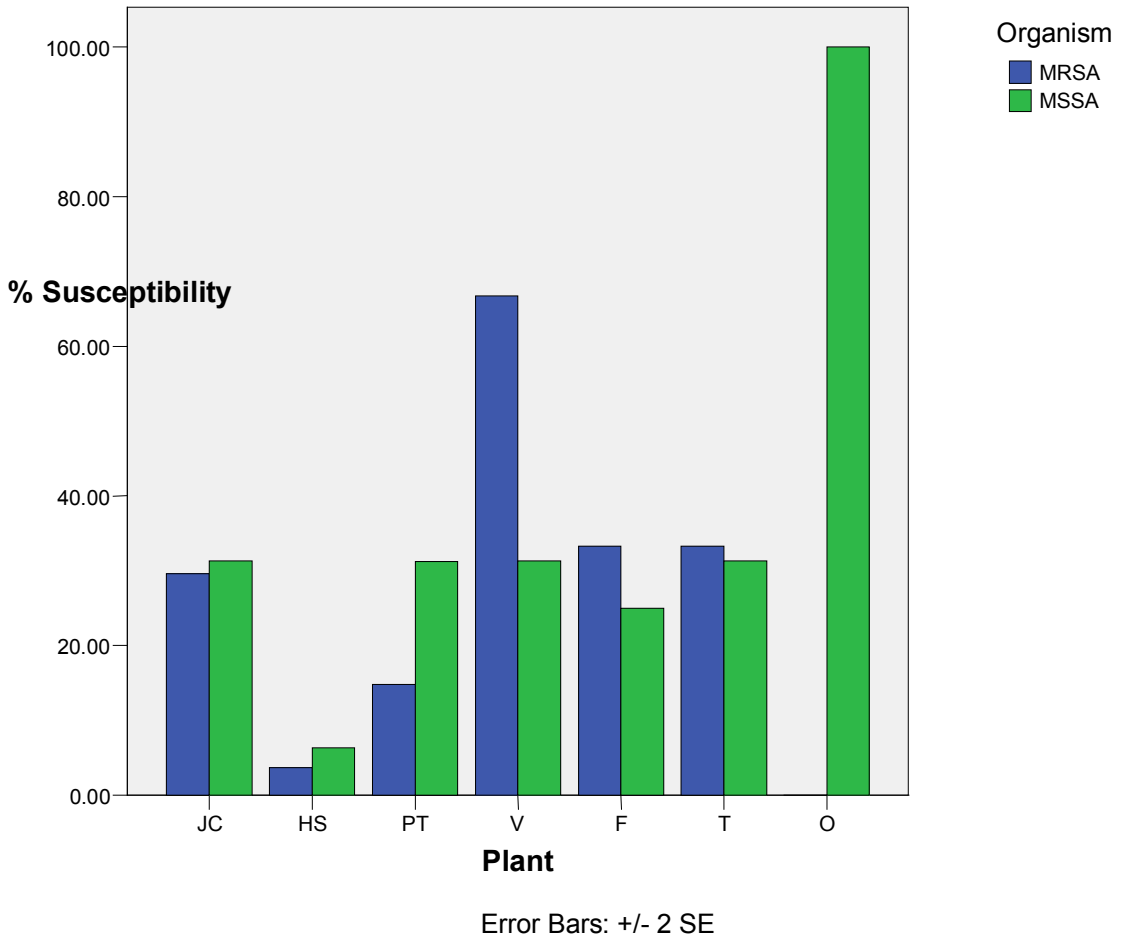
Table 2: Profile of some medicinal plants used in the treatment of MRSA from literature

Botanical Name	Family	Plant part used
[b]*Cassia occidentalis*	Caesalpiniaceae	Leaf
[b]*Cassia alata*	Caesalpiniaceae	Leaf
[a]*Bridella ferruginea* Engl.	Euphorbiaceae	Leaf
[a]*Phylantus discoideus* muel. Muel-Arg.	Euphorbiaceae	Bark
[a]*Acalypha wilkesiana* Muell-Arg.	Euphorbiaceae	Leaf
[b]*Elaeophorbia drupifera*	Euphorbiaceae	Leaf
[c]Mentha longifolia	Lamiaceae	Aerial part
[c]Melissa officinalis	Lamiaceae	Aerial part
[a]*Ocimum gratissimum* Linn.	Lamiaceae	Leaf



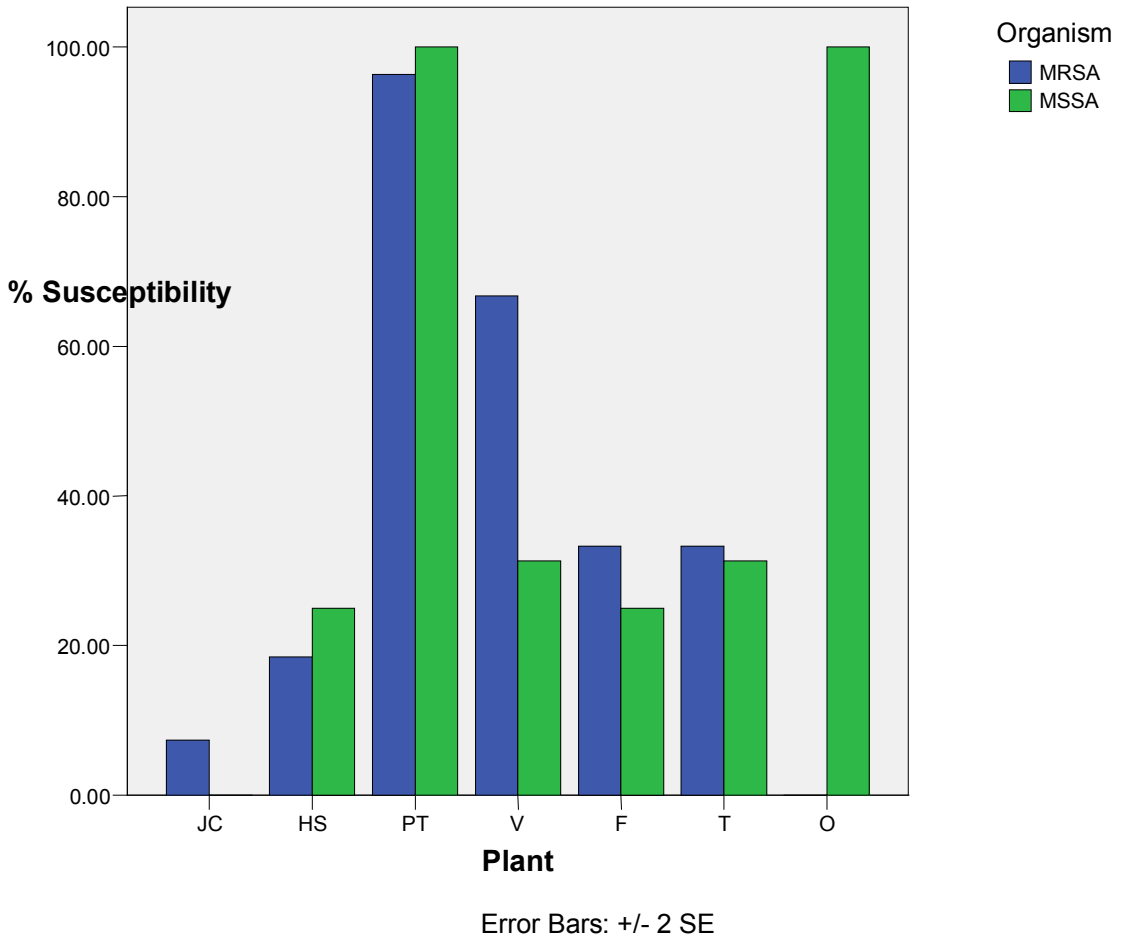
KEY: JC=*J. curcas* , HS= *H. suaveolens*, PT= *P. thonningii* , V= Vancomycin, F= Fusidic acid, T= Teicoplanin, O= Oxacillin, MSSA= Methicillin-sensitive *S. aureus*, MRSA= Methicillin-resistant *S. aureus*.

Figure 1. 1: Susceptibility of MRSA and MSSA to different ethyl acetate plant extract and standard control drugs



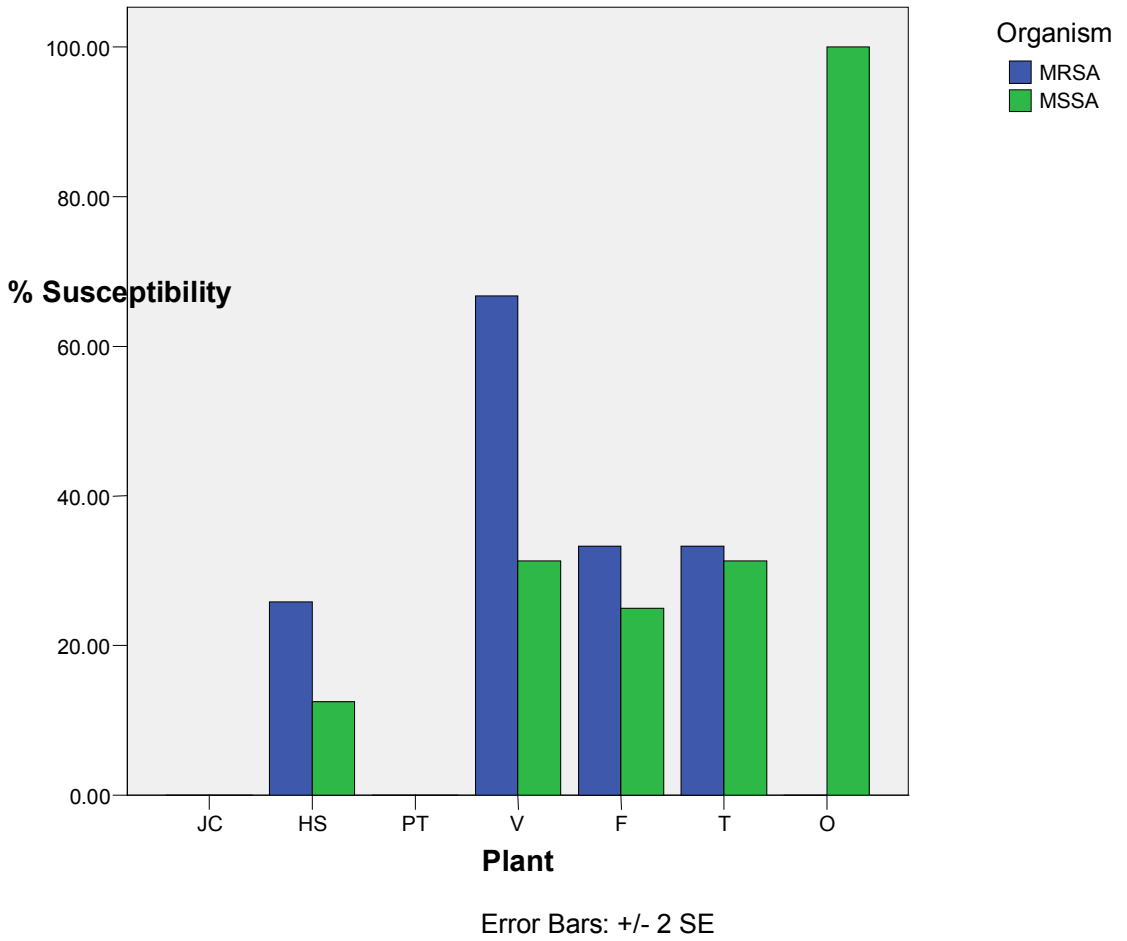
KEY: JC=*J. curcas*, HS= *H. suaveolens*, PT= *P. thonningii* , V= Vancomycin, F= Fusidic acid, T= Teicoplanin, O= Oxacillin, MSSA= Methicillin-sensitive *S. aureus*, MRSA= Methicillin-resistant *S. aureus*.

Figure 1. 2: Susceptibility of MRSA and MSSA to different MeOH plant extract and standard control drugs



KEY: JC=*J. curcas*, HS= *H. suaveolens*, PT= *P. thonningii* , V= Vancomycin, F= Fusidic acid, T= Teicoplanin, O= Oxacillin, MSSA= Methicillin-sensitive *S. aureus*, MRSA= Methicillin-resistant *S. aureus*.

Figure 1. 3: Susceptibility of MRSA and MSSA to different Hexane plant extract and standard control drugs



KEY: JC=*J. curcas*, HS= *H. suaveolens*, PT= *P. thonningii* , V= Vancomycin, F= Fusidic acid, T= Teicoplanin, O= Oxacillin, MSSA= Methicillin-sensitive *S. aureus*, MRSA= Methicillin-resistant *S. aureus*.

Figure 1.4: Susceptibility of MRSA and MSSA to different Water plant extract and standard control drug

Source of support: Nil, Conflict of interest: None Declared