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Antibacterial Effects of *Phyllanthus discoideus* and *Terminalia avicennioides* on Methicillin Resistant *Staphylococcus aureus* Isolated from Primary School Pupils in Ekiti State

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I. Introduction

Staphylococcus aureus has been recognized as a very virulent and frequently encountered pathogen in clinical practice (Salgado *et al*., 2003). It is an endogenous microorganism colonizing the nasal cavity, skin, gastrointestinal tract, anus, vaginal and vulvae of healthy persons (Onanuga *et al*., 2005), in this sense, about 20% of human populations are long term carriers of *Staphaureus*. Hence, *Staph aureus* has been considered to be enigmatic due to their existence in a site of infection either as significance or non-significant, significance in this sense is verified by their isolation in deep wound. However, there presence on surfaces is insignificance (Ajibade *et al*, 2010). Methicillin-resistant *S. aureus*(MRSA) was recognized as a nosocomial pathogen in the 1960s and now represents a substantial proportion of *S. aureus* infections in hospitalized (in-patients) and community (out-patient) settings (Diekema *et al*., 2001). MRSA is a specific strain of *Staph aureus* bacterium which is intrinsically resistant to methicillin and all beta lactamase (β-lactamase) antibiotics likedicloxacillin, cloxacillin, nafcillin, penicillin and oxacillin (Diekema*et al*., 2001).

The mechanism of methicillin resistance is an altered penicillin binding protein (PBP2a) in methicillin resistant *Staph aureus* that markedly reduces affinity for all available beta lactamase antibiotics, while maintaining effective cell wall binding activity. The penicillin binding protein (PBP2a) is encoded by the *mecA* gene that is carried on a mobile DNA element, the staphylococcal cassette chromosome (Katayama *et al*., 2000).

Healthy individuals may carry methicillin resistant *Staph. aureus*(MRSA) asymptomatically for periods ranging from a few weeks to many years (Hardy *et al*.,2004). The initial presentation of MRSA is red bumps that resemble pimples, spider bites or boils that may be accompanied by fever and occasionally rashes; within a few days the bumps become larger, more painful and eventually open into deep furuncles (Turnidge and Bell, 2000). Patients with compromised immune systems are at a significantly greater risk of symptomatic secondary infection (Daum *et al*., 2002). Methicillin-resistant *Staphaureus*(MRSA)can have severe public health implications because it can cause a variety of nosocomial- community acquired infections ranging from minor skin infections such as pimples, impetigo, boils (furuncles), cellulitis, osteomyelitis, bacteremia, carbuncle, scalded skin syndrome and abscesses to life threatening diseases such as necrotizing (flesh-eating) pneumonia and toxic shock syndrome (Raygada *et al*., 2009).

Methicillin-resistant *staph aureus* is a strain of *Staphylococus aureus* that is responsible for infections that are difficult to treat(Hardy *et al.*,2004). It has always been identified as one of the banes of many chronic diseases in hospitals and it has also been found to be resistant to most of the antibiotics that are commonly used nowadays. There is a need for antimicrobial agent

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from plants like *Phyllanthus discoideus and Terminalia avicennioides*. Historically, plants have provided antimicrobials that produced successful results in the treatment of notable bacterial infections. Their potency has been ascribed to possession of bioactive agents (phytochemicals) which act either singly or synergistically. *P. discoideus*is aeuphorbiaceae that has antibacterial and antihelmintic properties, extract from the leaves has been used for the treatment of bronchitis, pneumonia and gastrointestinal disorder(Akinyemi *et al*., 2006). The bark is used as a purgative, anthelmintic, toothaches and for kidney and stomach complaints to facilitate parturition. *Terminalia avicennioides* belongs to the family combretaceae and it's root has been commonly used by traditional practitioners in decoction, infusion, maceration or powder, to treat wounds and skin infections including stubborn sores, furuncles, impetigo, athletic foot, burns, bruises, toothache, conjunctivitis, leprosy, venereal diseases and respiratory tract infections including cough, pneumonia and tonsillitis(Mann *et al*.,2008). The powdered bark is taken as a purgative and emetic. Leaves are chewed to treat coughs and pulverized leaves are applied on burns and bruises. Ashes of burnt leaves together with fried bulbs of a *Crinum* species are mixed with butter and applied as ointment to parts affected by articular rheumatism or to swollen joints. This study investigated the antibacterial effects of *Phyllanthus discoideus and Terminalia avicennioides* on methicillin resistant Staphylococcus aureus MRSA isolated from primary school pupils in Ekiti- State.

II. Materials and Methods

a) Collection and treatment of plant materials

Phyllanthus discoideus and Terminalia avicennioides leaves *were* collected from farms around Erinfun in Ado-Ekiti, Southwestern Nigeria. The two plants were identified at the Department of Agriculture, Federal Polytechnic where a voucher specimen no (FPA/Ag/022/2016) was kept. The leaves were air-dried at room temperature of (28°±1°C), pulverized and kept in separate containers.

b) Extraction of Bioactive Compound

The extraction of the crude extracts from the two samples was done by using soxhlet apparatus. Each sample was evaporated to dryness by using rotary evaporator at 20° C.

c) Isolation and identification of the methicillin-resistant Staphylococcus aureus

Staph aureus were obtained from 324 samples of boils, skin and wound from 12 primary schools situated in Ekiti state. Samples collected were inoculated by streaking on to dried mannitol salt agar plates. The isolates were identified using the methods of Sadaka *et al.* (2009)and *S. aureus* was also confirmed by coagulase test(CLSI, 2005). *S. aureus* were collected and subcultured into MaCartney bottles of nutrient agar slant and stored in the refrigerator at 4˚C until required.

The methicillin resistant *Staph. aureus* was identified by agar diffusion method using methicillin disc, those found to be resistant to methicillin were used for the research.

d) Susceptibility testing of the methicillin resistant aureus

The disk diffusion method described by Bradyand Katz (1990) used in Ajibade *et al*.,(2010) was employed. Various Concentrations (10-100mg/ml) of the two extracts were used against the bacterial suspension whose innocular sizes were determined using Mcfarland standard.

III. Results and Discussion

Table 1: Number of MRSA isolates from different schools

Number of Schools	Number of samples	Number(%) positive
1	27	12(44)
2	27	6(22)
3	27	12(44)
4	27	11(41)
5	27	13(48)
6	27	15(56)
7	27	14 (52)
8	27	14(52)
9	27	15 (56)
10	27	12(44)
11	27	14(52)
12	27	13 (48)
Total	324	151(47)

Table 2: Percentage of MRSA Isolates samples susceptible and Resistant to the extract of *Phyllanthus discoideus*

Number of Schools	Number of MRSA	Resistancen(%)	Susceptibility n(%)
1	12	3 (25)	9 (75)
2	6	-(0)	6(100)
3	12	2(17)	10(83)
4	11	2(18)	9 (82)
5	13	1 (8)	12(92)
6	15	4 (27)	11(73)
7	14	3(21)	11 (79)
8	14	4 (29)	10(71)
9	15	3 (20)	12 (80)
10	12	3 (25)	9(75)
11	14	4(29)	10(71)
12	13	4(31)	9(69)
Total	151	33 (22)	118(78)

< 5mm (less than 5mm) : resistant, ≥ 5 mm (greater or equal to 5mm) : Susceptible

Table 3: Percentage of MRSA Isolates samples susceptible and Resistant to the extract of *Terminalia avicennioides*

Number of Schools	Number of MRSA	Resistance n (%)	Susceptibility n (%)
1	12	3 (25)	9 (75)
2	6	1 (17)	5 (83)
3	12	4 (33)	8(67)
4	11	3 (27)	8(73)
5	13	3 (23)	10(80)
6	15	2 (13)	13(87)
7	14	_ (0)	14(100)
8	14	1 (7)	13(93)
9	15	4 (27)	11(73)
10	12	2(17)	10(83)
11	14	4 (29)	10 (71)
12	13	3(23)	10(77)
Total	151	30(20)	121(80)

5mm = resistant; ≥5 mm = susceptible

The number of MRSA isolates from different schools are shown in table 1, 324 samples were collected out of which 151 isolates were methicillin resistant (MRSA),the highest percentage (56%) of MRSA were found in school 6 and 9. The susceptibility of MRSA to the crude extracts of *Phyllanthus discoideus and Terminalia avicennioides* leaves *were* shown in table 2 and table 3. The diameter of the zones of inhibition shown by the MRSA isolates were used to characterize their resistance and susceptibility, the diameter of zones of inhibition less than 5mm indicate resistance while the diameter of zones of inhibition greater than or equal to 5mm indicate susceptibility. In table 2, the total percentage of MRSA susceptible to *P. discoideus* was 78%.In table 3, the total percentage of MRSA susceptible to *Terminalia avicennioides* extract is 80%.

The result from above especially in table1,shows that the high rate of incidence from schools 6 and 9 is predisposed on the composition of the pupils. The pupils in these schools are exposed to environmental hazards. These hazards are poor sanitation, poor waste disposal, water sources sited near dumping site, educational background, location of the schools very close to a dumping site which expose the students to the microbe, body contact with infected pupils while playing or contact with surfaces that are contaminated with MRSA and poor personal hygiene. It could also be as a result of some risk factors of methicillin resistant *Staph. aureus* such as the status of the immune system due to malnutrition, Skin damage from conditions like eczema, insect bites or minor trauma that opens the skin, respiratory illness, burns and surgical wound.

The susceptibility of the bacterium to the crude plant extracts is due to the fact that the plants contain active biochemical that show high potency especially when used in crude form, this is due to one bioactive component potentiating the efficacy of other components. The bioactive components in *P. discoideus* leaves consist of alkaloid, flavonoids, tannins, saponin and trace amount of phenol, tannin promotes healing of wounds and inflamed mucus membranes, it's high flavonoid has antibacterial, antimalarial, anti-oxidant, anti-allergic and antiviral activity.

From the result above, *T. avicennioides* shows the highest potency, viewing the susceptibility pattern of the isolates to the crude extracts, it was shown that the resistance was predominant in *P. discoides* than *T. avicennioides* indicating that efficacy is highest in *T. avicennioides,* this result corroborate the earlier findings of Mann *et al*.,2008. The reason for the efficacy can be attributed to the concentration of bioactive components contained in *T. avicennioides* like glycoside, tannin, phenol and ellagic acid. Even though, when plants extracts are researched to possess the same bioactive component, the level of their concentration in plants and also their dispersing potentials in solvent has significant therapeutic effects.

IV. Conclusion

The susceptibility of MRSA isolates to the leave extracts of the two plants is due to the presence of bioactive components and phytochemicals are utilized for the prevention and treatment of many diseases, therefore this research provides the scientific basis for the use of these two plants as therapies for the treatment of diseases that are associated with methicillin resistant *staphylococcus aureus*.

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