SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 4.
Issue: 3.
Page N. 267-272
Year: 2020

Original Article www.wjahr.com

COMPARATIVE EFFICACY OF AQUEOUS *ACALYPHA WILKESIANA* LEAVE EXTRACT AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREU*

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Received date: 28 April 2020 **Revised date:** 18 May 2020 **Accepted date:** 08 June 2020

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ABSTRACT

Overtime, medicinal plants have been shown to contain intrinsic active ingredients that can be used for curative purpose for some diseases. *Acalypha wilkesiana* is one of those ethno medicinal plants of reported health benefits. This study compared the efficacy of aqueous *A. wilkesiana* leaf extract on Methicillin Resistant Staphylococcus aureus. Powdered leaves of this plant was soaked in 100% distilled water for 3 and 7 days respectively. Both crude extracts were filtered, and concentrated distinctively to evaporate the excess solvent. This was reconstituted with distilled water in 6 dilutions (400 mg/mL, 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/L) and used against Methicillin Resistant *Staphylococcus aureus* (MRSA) which was isolated from different individuals. The mean inhibitions obtained for the aqueous extract at these varying concentrations were; 10.317 mm, 8.426 mm, 5.571 mm, 3.00 mm,1.00 mm, 0.714 mm respectively. Phytochemical analysis was also carried out on the leaf to ascertain the active ingredients present. For each concentration obtained, zone of inhibition and minimum inhibitory concentrations were each determined for *Staphylococcus aureus*. Comparisons were then made on the efficacy of *A. wilkesiana* leaf extract on *S. aureus* resistance. Results showed that the aqueous extract, had a higher antimicrobial effect against MRSA at increasing concentrations of the leaf. Phytochemical analysis also revealed the presence of Saponins, Tannins, Steroids and Terpenoids; active ingredients that may be responsible for the anti-microbial activity. Corroborative studies that consolidate on results of this study is highly recommended.

KEYWORDS: *Acalypha wilkesiana, Staphylococcus aureus,* Antimicrobial Resistance, Phytochemicals.

INTRODUCTION

The use of medicinal plants for healing is as old as mankind itself. In ancient times, people looked for drugs in nature out of instinct, in search of solution to their health problems.[1] Currently, there is a worldwide movement of evaluating the plant resources which are of medical importance and of economic value. Several species of plant used in various traditional medicines have been evaluated for distinctive pharmacological activities.[2] Indirect use simply involves the use of plants as a prototype to develop modern or orthodox medicines.[3] The substantiation of traditional knowledge in medicinal uses of plants since Inception has yielded many important drugs of modern day.[4]

Medicinal plants have shown to contain intrinsic active ingredient that can be used to relieve symptoms or cure diseases. Therefore, since herbal medicine have been reported to be safe and without any serious side effects compared to synthetic drugs, the use of medicinal plants

has gradually increased especially in developing countries.[5] The antioxidant, anti-inflammatory, antipyretic, antimicrobial etc., effect of the phytochemicals present in plant is a key factor responsible for their distinct medicinal purpose.[6]

Several medicinal plants have shown to have antimicrobial properties that are useful against different microbes.[7] Methicillin resistant Staphylococcus aureus is a significant public health problem throughout the world and has caused prominent morbidity and mortality rate due to its resistance to synthetic antimicrobial agents. This is mainly as a result of the various infections it causes such as skin infections, blood infections, bone infections etc. A. wilkesiana although has proved to be efficient against MRSA from previous studies carried out by Onuah, (2016).[8]

Acalypha wilkesiana is one of those ethno medicinal plants of proven health benefits; recently, its antifungal and antibacterial activities have been proven by the research community.[9] The presence of tannins, saponins, phenol, alkaloids, terpenes, glycosides and anthraquinous (in varying degrees) in the ethanol and aqueous extracts of *Acalypha wilkesiana* leaves proved to be responsible for its pharmacological activity. Oladunmoye, (2006) reported the presence tannis, anthraquinones and glycosides while Akinde (1983) reported the presence of Sesquiterpenes, monoterpes, triterpenoids and polyphenols. All these constituents make up its pharmacological properties. The degree and presence of these phyto-constituents varies by the type of solvent used for its extraction.[10,11]

Staphylococcus which is usually treated with antibiotics over the years, suddenly developed some strains (like Methicillin-Resistant-Staphylococcus aureus) which have become resistant to antibiotics that once destroyed it. This strain was discovered first in 1961 and has shown to be indestructible when β-lactams such as methicillin, Oxacillin among others; are used against it. This has resulted in so many difficult-to-treat infections caused by this organism. However, *Acalypha wilkesiana* has been shown to provide activity against this persistent infection caused by Methicillin - resistance- Staphylococcus aureus (MRSA).

Aim of Study

This study aimed at determining and comparing the effect of different concentrations of aqueous *Acalypha wilkesiana* leaf extracts on Methicillin - resistance-Staphylococcus aureus. Specifically, study;

1. Determined the phytochemical constituents in *Acalypha wilkesiana* aqueous extract
2. Determined and compare the effect of *Acalypha wilkesiana* aqueous extract on isolated MRSA.

MATERIALS AND METHODS

Collection of plant

Healthy and fresh *A. wilkesiana* leaves were collected from NDDC Hall at Site III, Delta state university, Abraka, Nigeria. Identification was done in the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, Delta State University Abraka.

Preparation of leaf material

Healthy and fresh leaves of *A. wilkesiana* were properly selected, while stems, stalks and infected leaves were separated. The leaves were washed with sterile water and air dried for two (2) weeks at room temperature, (Between 20-25 °C). Thereafter, the leaves were pulverized into fairly fine powder using a sterile grinding machine.

Extraction of *Acalypha wilkesiana* leaves

Four hundred grams (400g) of the pulverized *A. wilkesiana* leaf was weighed using a weighing balance, and soaked for 7 days with constant agitation. The extract was placed in a porcelain dish and stored at a temperature of 4°C until time of use. The percentage yield was calculated using;

$$Percentage\ yield = \frac{Weight\ of\ extract}{Weight\ of sample} \times 100$$

The aqueous extract of *A. wilkesiana* leaves was gotten by soaking 400g of the leave material in 3000ml of distilled water, for 3 days with constant agitation. The extract was filtered using a muslin cloth. The excess solvent was removed and concentrated for several days using a rotary evaporator, until a paste-like extract was obtained. The paste-like extract was stored at a temperature of 4°C in a porcelain dish until time of use. The weight of the aqueous extract (yield) was 169.91g. The percentage yield is 42.47g.

Phytochemical screening

Test for Saponins

Frothing test

The aqueous extract was shaken vigorously in different test tube, containing the distilled water. The presence of persistent overflowing mass of small bubbles indicates the presence of Saponin.

Emulsion test

1ml of olive oil was added to 2 mks of each extracts and shaken vigorously. Formation of two liquid layers that are immiscible (emulsion), indicates the presence of Saponin.

Test for tannin

General test

10ml of water was added to each extracts in separate test tubes. 2ml of aqueous ferric chloride was also added. A

blue black precipitate indicates the presence of hydrolysable tannins.

Confirmatory test

Modified Iron Complex Test.

About 2-3 drops of 33% acetic acid and 1g of sodium potassium tartarate was added to each extract. This was warmed and the precipitate was filtered off. The precipitate was washed and the washings were added to the filtrate. Ferric ammonium citrate solution was added to the mixture and boiled. A blackish precipitate that is insoluble in ammonia, indicates the presence of pyrogallol tannins.

Test for alkaloids

Mayer

Each extract was treated with sulphuric acid, thereafter, Mayer's reagent was added. The formation of a cream precipitate indicates a positive test.

Test for Steroids and Terpenoids

chloroform was added to each extract, and concentrated sulphuric acid was also added carefully. A reddish brown colouration at the interphase indicates a positive test.

Test for flavonoids

About 2ml of plant extract was measured and 10% sodium hydroxide was added. About 2ml of concentrated HCl was also added and agitated. The presence of flavonoids is indicated by a yellow coloured solution which slowly fades away.

Preparation of materials for antimicrobial screening

Sterilization of materials and media

Glass wares such as beakers, test tubes; measuring cylinders; were wrapped in aluminium foil and sterilized in an autoclave at 121°C fo15 minutes. The prepared culture media (Mannitol salt agar, Mueller Hinton agar and Nutrient agar) and broth was also sterilized in an autoclave at 121°C for15 minutes. The cork borer was also sterilized prior to use, by insertion into alcohol and by passing it through a flame. The inoculating wire loop was also sterilized by flaming it red hot, before and after using it. The work bench was also sterilized with disinfectant before and after each experiment.

Collection of Bacteria Isolates from Nostrils of Human Subjects

Seventy (70) swab sticks were used to take samples from the nostrils of 70 different students
in Delta state university Abraka. This was stored at a temperature of 4°C till time of use.

Isolation of the bacteria (*Staphylococcus aureus*)

Mannitol salt agar was weighed and dissolved in the appropriate volume of water according to the manufacturers specifications. (i.e. 11.3g of the agar to be dissolved in 100ml of water). The water was added bit by bit with continuous stirring to properly dissolve the agar. After proper dissolution, the mixture was transferred into a bottle. It was agitated properly without allowing it to froth. This was later sterilized in an autoclave and allowed to cool a little. The agar was thereafter poured into several labelled petri-dishes (in an aseptic condition) and allowed to solidify. After solidification, the samples collected using the swab sticks were spread on each of the solidified agar. All the petri dishes were inverted and placed in an incubator at 37°C for 24 hours. Results were taken after 24 hours of incubation.

Preparation of Subcultures

Nutrient agar was weighed and transferred into a beaker and dissolved in the appropriate volume of water according to the manufacturer's specifications (2.8g of nutrient agar to be dissolved in 100ml of water). The water was added bit by bit while mixing to cause a homogeneous and even mixture. Thereafter, the mixture was transferred into different bijou bottles. These bottles were sterilized in an autoclave at 121°C for 15mins. Thereafter, it was placed in a slanting position and allowed to cool. Thereafter, the already isolated Staphylococcus aureus was inoculated into the already labelled slant bijou bottles containing the nutrient agar; using an inoculating wire loop with subsequent flaming of the loop after each streak. This was placed in an incubator for 24hrs at 37°C.

Preparation of overnight broth

Nutrient broth was weighed into a beaker and dissolved properly in the appropriate volume of water according to the manufacturer's instructions. This was transferred into different bijou bottles and sterilized at 121°C for 15mins and allowed to cool. Using the inoculating wire loop, the growth observed from the slant cultures were picked and inoculated into the nutrient broth. This was labelled and incubated for 24hrs at 37°C.

Preparation of the antibiotics (Cloxacillin) sensitivity disc

Five (5) Labelled sterile test tubes were filled with 10ml, 9ml, 9ml, 9ml and 5ml of sterile water respectively. A ten-fold serial dilution was done by weighing 10mg of the drug and dissolved in 10ml of the sterile water in the first test tube. 1ml of this solution was collected and transferred into the 2nd test tube containing 9ml of sterile water. This process was repeated for the third and fourth test tubes. However, a two-fold dilution was done by transferring 5ml from the fourth (4) test tube into the fifth (5) test tube, that already contains 5ml of sterile water. This resulted in 10mg, 1mg, 0.1mg, 0.01mg and 0.05mg concentration of the drug in the 1st, 2nd, 3rd, 4th and 5th test tubes respectively. 5ml from the solution in the fifth (5) test tube was used in preparing the disc, while the other 5ml was discarded. The sterile Whatman filter papers obtained were transferred and soaked into a sterile beaker containing the remaining 5ml of 0.05mg cloxacillin solution, to produce the sensitivity disc.

Isolation of methicillin resistant *Staphylococcus aureus*

An overnight broth culture of the isolated Staphylococcus aureus was prepared. Mueller Hinton agar was prepared according to the manufacturer's specification and sterilized in an autoclave at 121°C for 15 mins. This was allowed to cool a little, transferred into several labelled petri dishes, and allowed to solidify. The several Staphylococcus aureus obtained from the overnight broth culture were inoculated into the culture plates, by spreading with the use of different swab sticks. The prepared antibiotic disc was picked individually, using a pair of forceps. These discs were placed at specific position in the culture plates, where inhibitions can be visibly noted. The plates were then incubated for 24hrs at 37°C and the zones of inhibitions (which indicates which strain in resistant to the antibiotics) were noted.

Test of A. wilkesiana leave extracts against methicillin resistant Staphylococcus aureus

Serial dilution of leave extract

Serial dilution of the leave extract was done, by measuring 3ml of sterile water into two (2) labelled test tubes one each for the aqueous and ethanol extract. Also, 1.5ml of sterile water was measured into the other test tubes, five (5) each for the aqueous and ethanol extract. Therefore, twelve test tubes were used in total. About 1.2g of the concentrated extract (both aqueous and ethanol), was weighed into their respective 1st test tube containing 3ml of sterile water. This was mixed properly for even distribution. Thereafter, 1.5ml from each 1st test tube was transferred into the 2nd respective test tube, that already contains 1.5ml of sterile water. This was repeated for the next four test tubes. 1.5ml of the solution was measured and discarded from the last test tube, leaving behind 1.5ml of the solution in the last test tube. This gave a concentration of 400 mg/mL, 200 mg/mL, 100 mg/mL 50 mg/mL, 25 mg/mL and 12.5 mg/mL respectively for the six (6) test tubes each, both for aqueous and ethanol extract.

RESULTS

Table 1: Phytochemical Screening of *Acalypha wilkesiana* Aqueous Leaf Extract.

Phytochemical Constituents	Aqueous Extract of *Allium Cepa*
Saponins	+
Tannins	+
Alkaloids	-
Cardiac Glycosides	-
Steroids	+
Terpenoids	+
Flavonoids	-

Key:
(+) means present
(-) means absent

Table 2: Percentage Yield of *Acalypha wilkesiana* Aqueous Leaf Extract

Extract	Sample Weight (g)	Extract Weight (g)	% Yield
Aqueous	**400**	**169.91**	**42.47**

Table 3: Zones of Inhibition from Antibiotic Sensitivity Disc of MRSA Isolation

Zone of Inhibition (mm)	Remark
7	Resistant
0	Resistant
4	Resistant
5	Resistant
9	Resistant
6	Resistant
13	Non- Resistant
10	Non- Resistant

Table 4: Zones of Inhibition for *Acalypha wilkesiana* Aqueous Leaf Extract concentrations of Isolated MRSA Strains.

S/N	Extract	Concentration (mg/mL)					
		400 (mm)	200 (mm)	100 (mm)	50 (mm)	25 (mm)	12.5 (mm)
1	Aqueous	12	8	4	Nil	Nil	Nil
2	Aqueous	10	5	3	Nil	Nil	Nil
3	Aqueous	12	9	5	3	Nil	Nil
4	Aqueous	13	10	9	4	Nil	Nil
5	Aqueous	18	14	12	10	7	5
6	Aqueous	11	6	Nil	Nil	Nil	Nil
7	Aqueous	13	7	5	Nil	Nil	Nil

Table 5: Average Concentration used from *Acalypha wilkesiana* Aqueous Leaf Extract.

	Concentration (mg/mL)					
	400 (mm)	200 (mm)	100 (mm)	50 (mm)	25 (mm)	12.5 (mm)
Solvent	Aqueous	Aqueous	Aqueous	Aqueous	Aqueous	Aqueous
Mean ± SD	10.32±2.56	8.43±2.10	5.57±3.91	3.00±3.61	1.00±2.65	0.71±1.90

Values are expressed in form of Mean ± Standard deviation (S.D)

Table 6: Minimum Inhibitory Concentration (MIC) of *Acalypha wilkesiana* Aqueous Leaf Extract for Strains of MRSA Isolates.

S/N	Concentration (mg/mL)					
	400 (mm)	200 (mm)	100 (mm)	50 (mm)	25 (mm)	12.5 (mm)
1	-	-	-	+	+	+
2	-	-	-	+	+	+
3	-	-	-	-	+	+
4	-	-	-	-	+	+
5	-	-	-	+	+	+
6	-	-	+	+	+	+
7	-	-	-	+	+	+

Key:
(+) Growth
(-) No Growth

DISCUSSION

Several reports have shown *Acalypha wilkesiana* leaves to possess several antimicrobial properties, confirming its activity against different infections that may be caused by micro-organisms.

Table 1 of the results obtained from the phytochemical screening of *Acalypha wilkesiana* aqueous leaf extract showed the absence of Tannin, reducing sugar, flavonoids, steroids and terpenoids, However, tannins, terpenoids, steroids and saponin were present. Though Alkaloids were only present in trace amount. The presence of tannin in the extract gave an intense black color, suggestive of the presence secondary metabolites in the aqueous leaf extract.

Research studies have claims that ethanolic extract of *Acalypha wilkesiana* gives better yields of phytochemical constituents when compared with that of aqueous.[12,13] This study supports the fact that the ethanolic extract of *A. wilkesenia* possesses a lighter quantity of each secondary metabolite compared to that of the aqueous extract. The presence of good amount of phytochemical constituents in the aqueous extract, may be responsible for its higher anti-microbial activity against the isolated MRSA.

Different phytochemicals have different modes or methods of action in which they exhibit their antimicrobial effect. Tannins inhibits microbial activity by depriving the organism of substrates required for their growth, by inhibiting extracellular microbial enzymes or directly disrupting oxidative phosphorylation.[12,14] Steroids causes leakage from liposomes in membrane, while terpenoids weakens the membranous tissue of microbial cells, thereby dissolving the cell walls of the organism. Flavonoids, which are extensively known antibacterial agents, inhibits cell membrane function, alters their permeability and inhibits energy metabolism thereby attenuating the pathogenic effect of bacteria.

From the result in Table 1, Flavonoids were absent in the aqueous extract, another possible factor responsible for the ethanolic extract's exhibition of a higher antibacterial activity than the aqueous extract.[15] Thus, the ethanolic extract is expected to have a better antimicrobial effect in synergy with other phytochemicals present in them.

Tables 3 and 4 compare the mean zones of inhibitions obtained at various concentrations of the solvent against methicillin resistant Staphylococcus aureus. It can be seen from the table, that the mean zones of inhibition are obtained when the aqueous extract is used against organisms. Also, Table 5 and 6 shows the minimum inhibitory concentrations of the aqueous extracts. Here, it can be inferred that the aqueous extract has the ability to inhibit methicillin resistant S. aureus at a greater concentration. It also can be inferred that the aqueous extract requires a higher concentration to inhibit MRSA.

CONCLUSION

This study compared the efficacy of both the aqueous extract of *Acalypha wilkesiana* leaves against Methicillin- Resistant Staphylococcus aureus (MRSA) at different concentrations. The showed a potent antibacterial activity against MRSA with increased concentration. Further studies are required to better evaluate the safety and effectiveness of Ethanolic extract of *A. wilkesiana* leaves when formulated (in dosage forms) to be used against Methicillin Resistant Staphylococcus aureus. Also, the extract can also be fractionated into individual constituents, and each fractions used against this organism and even other microbes.

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