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Preliminary Study on the Pharmaceutical Constituents of *Acalypha wilkesiana* and Production of Phytodrug for the Treatment of Skin Infection

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Abstract The phytochemical screening of phytocompounds of therapeutic interest were carried out on methanolic, ethanolic and aqueous extracts of *Acalypha wilkesiana.* Antimicrobial activities of the extracts were also determined using standard microbiological and chemical techniques. Five bacteria (*Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa and Streptococcus pyogenes*) and five fungi: (*Aspergillus fumigates, Trichophyton rubrum, Aspergillus niger, Aspergillus flavus and Candida albicans*) were used. The agar diffusion (disc method) was adopted in the study. The results showed that the zone of inhibition of the various extracts against the different bacteria ranged from 9mm to 17mm while that of the different fungi ranged from 0mm to 8mm. *Eschericha coli* was the most susceptible bacteria while *Aspergillus fumigates* was the most resistant bacteria. The minimum inhibitory concentration and the minimum Bactericidal/fungicidal concentration ranged from 100mg/ml to > 250mg/ml for the test micro-organisms. The extracts exhibited more antibacterial activity than antifungal. The plant extracts can therefore be used to cure diseases caused by these micro-organisms. Effective phytodrug was produced (using the plant extract) for the treatment of skin infection.

Keywords *Acalypha wilkesiana*, Bacteria, Fungi, Phytochemical screening, Inhibition zone, Minimum inhibitory concentration and Minimum bactericidal/Fungicidal concentration

Introduction

Acalypha wilkesiana is found in Africa, Asia, Australia, Kenya, Nigeria, USA, Uganda, Vietam, Thailand, Tanzania, Polyneia [1]. Other names for it include amentacea and a tricolour. *A. wilkesiana* is an evergreen shrub. It grows 3 meter high and spread 2m across. The stem is erect with many branches. The leaves are copper green with red splashes of colour. They are broad with teeth around the edge. The flowers have separate male and female flower on the same plant. The male flowers are in long spike [2]. *Acalypha wilkesiana* is a tropical and subtropical plant. It is normally propagated by stem cutting at any time of the year [3].

In Nigeria especially in the Igbo race, it is used to cure pityriasis vericolor in infants and children [4]. This is why it is called "Ogwu nra". The leaf is boiled and the aqueous extract used to bath the infected baby, and also given to the baby to drink. Oyelami et al, [5] carried out a non comparative study to evaluate the safety and efficiency of *Acalypha wilkesiana* ointment using 32 Nigerians with mycological well as chemical evidence of mycoses. The ointment successfully controlled the mycoses in 73.3% of the affected patients. Akinyemi et al [6] evaluated crude extracts from six important medicinal plants (*Phylantus discoideus, Ageratum conyzoides, Terminalia aricennoides,*

Broletta ferruginea, Acalypha wilkesiana and Ocimum gratissimum) to find activity against Methicillin resistant *Staphylococcus aureus* (MRSA). Aqueous and ethanolic extracts of these plants were obtained locally. MRSA strain obtained from patients were used. Both equeous and ethanolic extracts of these plants showed effects on MRSA. People have been discussing about the medicinal values of this flower *Acalypha wilkesiana*. In this present study, the authors tried to (a) screen the leaf extracts of *A. wilkesiana* for the presence of phytochemicals of interest (b) determine the antifungal activity of the leaf extracts of *A. wilkesiana* towards organisms that cause superficial mycoses in man and (c) develop and formulate a new phytodrug from the test plant.

Methodology

Sample Collection and Preparation

The leaves of ***Acalypha wilkesiana*** were collected from Adazi-enu in Anaocha Local Government Area of Anambra State, Nigeria. They were dried under air and mild sun-shine, for about three weeks and ground into powders. The ground sample was then kept in a clean polyethylene bottle until needed for analysis. Phytochemical and the extraction of the active components were determined by the methods outlined by Harbon [7]. The antimicrobial activity of *Acalypha wilkesiana* was determined by agar well diffusion method [8]. The zone of inhibition was recorded to the nearest size in mm [9]. After extraction of the active components using three different solvents (Ethanol, Water and Methanol), the solvent extracts were evaporated to dryness at about 67°C, 98°C and 66°C respectively in a water bath separately. 1mg of dry ethanolic, aqueous and methanolic extracts were weighed into three different labeled test tubes . Then 10ml of the corresponding solvent used for extraction was added to the dried extracts to make 0.1mg/ml concentrations of the extracts. This was used for the antimicrobial activity.

Table 1: The Result of Phytochemical screening of three solvents extracts *of A. wilkesiana*

	Ethanol	Aqueous (aq)	Methanol
Alkaloid	+	+	+
Flavonoids	+	+	+
Tannin	+	+	+
Saponin	+	+	+
Phenols	+	+	+
Glycosides	+	+	+
Cardiac Glycosides	+	+	+
Protein	+	+	+
Acidic Compound	+	+	+
Steroid & Phytosteroid	+	+	+
Reducing Sugar	+	+	+
HCN	+	+	+
Essential oil	+	+	+
Resin	+	+	+
Terpenoids	-	-	-
Fixed oil	+	+	+

+ means present

- mean absent

Table 2: Organoleptic Test Result

Parameter	Inference
Colour	Greenish brown
Texture (dried leaf)	Ductile
Texture (ground leaf)	Soft, coarse and powdery
Taste	Astringent
Odour	Nicotic smell

Table 3: Antimicrobial activity of the leaf extracts

Zone Diameter **of Inhibition** (mm),

Test Organism	Methanolic Extract	Aqueous Extract	Ethanolic Extract	Control Chloramphenical /Nystatin
Streptococcus pyogenes	10mm	9mm	11mm	7mm
Pseudomonas aeruginosa	15mm	13mm	14mm	33 mm
Escherichia Coli	17mm	15mm	13mm	35m:m
Staphylococcus aureus	16mm	14mm	12mm	3 1mm
Proteus mirabilis	11mm	13mm	14mm	23mm
Aspergillus fiimigatus	-	-	-	15mm
Trichophyton rubrum	7mm	-	6.5mm	6.5mm
Aspergillus niger	-	8mm	6.5mm	-
Aspergillus flavus	7mm	6.5mm	6.5mm	-
Candida albicans	6.5mm	6.5mm	7mm	15mm

Table 4: The result of Minimum Inhibitory Concentration (MIC) of the extracts of *Acalypha wilkesiana* (mg/ml)

Test Organism	Methanolic Extract	Aqueous Extract	Ethanolic Extract
Streptococcus pyogenes	100mg/ml	250 mg/ml	100 mg/ml
Pseudomonas aeruginosa	200 mg/ml	200 mg/ml	100 mg/ml
Escherichia coli	100mg/ml	200 mg/ml	100 mg/ml
Staphylococcus aureus	100 mg/ml	250 rag/ml	200 mg/ml
Proteus mirabilis	100 mg/ml	250 mg/ml	100 mg/ml
Aspergillus fumigatus	N.D	N.D	N.D
Trichophyton rubrum	250 mg/ml	N.D	250 mg/mi
Aspergillus niger	N.D	>250 mg/ml	250 mg/ml
Aspergillus flavus	250 mg/ml	>250 mg/ml	250 mg/ml
Candida albicans	200 mg/ml	>250 mg/ml	250 mg/ml

N.D= not Done

Key: mg/ml = milligram per millilitre

> = greater than

Table 5: The Result of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC): (mg/ml)

Test Organism	Methanolic Extract	Aqueous Extract	Ethanolic Extract
Streptococcus pyogenes	250mg/ml	250 mg/ml	250 mg/ml
Pseudomonas aeruginosa	200 mg/ml	200 mg/ml	1 00 mg/ml
Escherichia coli	100mg/ml	200 mg/ml	100 mg/ml
Staphylococcus aureus	200 mg/ml	>250 mg/ml	250 mg/ml
Proteus mirabilis	200 mg/ml	>250 mg/ml	200 mg/ml
Aspergillus fumigatus	N.D	N.D	N.D
Trichophyton rubrum	250 mg/ml	N.D	250 mg/ml
Aspergillu sniger	N.D	>250 mg/ml	250 mg/ml
Aspergilus flavus	*>2SO* mg/ml	>250 mg/ml	>250 mg/ml
Candida albicans	200 mg/ml	>250 mg/ml	>250 mg/ml

N.D = not Done

Key: mg/ml = milligram per milliliter, > = greater than

Discussion

Table 1 showed that the major phytochemical present in *Acalypha wilkesiana* includes HCN, alkaloid, phenolic compound, tanins, flavonoids, saponins, essential oil, glycosides, cardiac glycoside, protein, acidic compound, steroid and phytosteriod, reducing sugar, resins and terpeniods. The biological function of flavonoids include

protection against allergy, inflammation, platelets aggregation microbes, ulcer, vineses, and tumours [10-11]. Flavonoids are free radical scavengers, super antioxidant and potent water solute which prevent oxidative cell damage and have strong anticancer activity [12]. As antioxidant, flavonoids provide anti-inflammatory action [13-14]. This may be the reason behind the use of *Acalypha wilkesiana* in herbal medicine. The saponins constituent are responsible for the possession of heamolytic property. Alkaloid are used as basic medicinal agents because of their analgesic, antisplasmodic and bactericidal properties [15]. Tannins hastens the healing of wounds and inflammed mucous membranes. Its presence in the leave can supports its strong use for healing of wounds, varicose ulcer, hemorrhoids, frost-bite and burns in herbal medicine [16-17]. The presences of phenolic compound in the leaves of *Acalypha wilkesiana* shows that the plant may have antimicrobial potential. This explains its use in treating eczema in herbal medicine. This is because phenols and phenolic compound have been extensively used in disinfections and it remains the standard with which other bactericides are compared [18].

Tannin acts as a defence mechanism in plant against pathogens, herbivores and hostile environmental conditions. This explains the antimicrobial activities of this leaves against different strains of bacteria and fungi as shown in the Table 3. In plants terpenes are linked up with the function which include growth regulation, colour development as well as some role as photosynthesis of pigment. Saponins are steroid glycoside with detergency properties, sometimes used as foaming agents in soap and food cosmetics. Saponins may also prevent cancer by protecting DNA from damage. Saponin are antiviral in invitro studies and directly inhibit colon cancer. Saponin may be cardio protective via their ability to lower cholesterol [19], Saponins have a potential role as cancer preventive agent acting as antioxidants, anti-mutagens and even anti-retroviral in in vitro HIV studies and anti-DNA viral in Epstein Barr Virus inhibition studies [20]. This suggest a possible use of *Acalypha wilkesiana* as potential remedy for HIV infections. Table 3 brought before the sight, the result of the antimicrobial activity of three solvent leaf extracts of *A. wilkesiana* ; (methanolic, ethanolic and aqueous). *Acalypha wilkesiana* exhibited broad spectrum antimicrobial activity against the test bacteria. The zones of inhibition of the leaf extracts against Escherichia coli can be considered to be the highest with the methanolic extract: 17mm, aqueous extract: 15mm and ethanolic extract: 13mm; while the lowest zones of inhibition of the leaf extracts of *Acalypha wilkesiana* were shown on Streptococcus pyogenes with the methanolic extract: 10mm, Aqueous extract: 9mm and ethanolic exract: 11mm. However, these zones of inhibition shown on Streptococcus pyogenes were more than the zone of inhibition obtained with the commercial antibiotic (chlorophenicol). The methanolic extracts has the highest average zones of inhibition against the test bacteria, followed by the ethanolic extracts and then the aqueous extract. Table 3 also shows the antifungal activities of the three solvent extracts of *Acalypha wilkesiana.* Methanolic, ethanolic and aqueous extracts were most active against *Candida albicans* and *Aspergillus flavus* than any other fungus. *Aspergillus fumigates* was resistant against the extracts of *A. wilkesiana*. The methanolic and ethanolic extracts of *A. wilkesiana* were active against *Trichophyton rubrum* (the main causative agent of ringworm).

Haruna *et al* [21] reported the antibacterial and antifungal activity of *A. wilkesiana*. The zone of inhibition reported by the authors were lower than that obtained in this work. The author reported that methanolic and aqueous extracts had antibacterial activities against *Staphylococcus aureus* and *Pseudomonas aeroginosa* while *Proteus*spp, *Streptococcus spp and Escherichia coli* were resistant against the extracts. The authors also reported antifungal activity of the aqueous extracts of *A. wilkesiana* leaves against *Candida albicans* as also reported in this work.The antimicrobial activities of the leaf extracts of *A. wilkesiana* seems to be more of antibacterial than antifungal as reveaeled in this present work. Onocha and Olusanya [22] also reported that *Candida albicans* and *Aspergillus niger* were susceptible to the leaf extracts of *A. wilkesiana.* The fact that the extracts of *A. wilkesiana* showed antimicrobial activities against most of the test organisms is a major breakthrough in appreciating the medicinal potential of the plant especially in the management of community acquired associated infections.

Table 4, portrays the Minimum Inhibitory Concentration (MIC) of the solvent extracts .It shows that the MIC ranged from l00mg/ml to 25mg/ml for the bacterial test organisms, while MIC ranged from 250mg/ml to >250mg/ml for the fungal test organisms. The lower concentration at which the extracts inhibited the growth of the bacterial test organism when compared with that of the fungal test organism further give evidence of the higher

antibacterial activity of the extracts of *A. wilkesiana* than anti-fungal activity. The least concentration (MIC) of 200mg/ml against *C. albican* in comparison with the MIC against other fungal test organisms further proved that the extracts were more active against *C. albican* than other fungal species. The MICs of the extracts against the test bacteria are similar to the concentration of most commercial antibiotics.

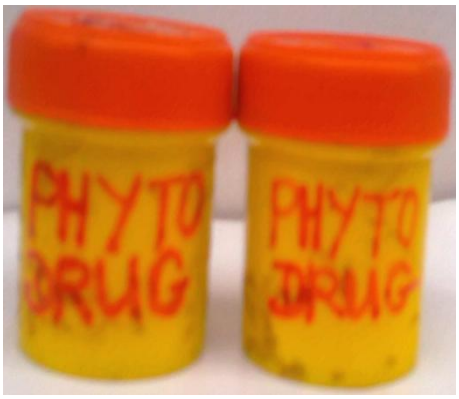
The minimum bactericidal/fungicidal concentration of the extracts of *A. wilkesiana* against the test organisms show similar pattern with that of the Minimum Inhibitory Concentration (MIC). The aqueous extracts exhibited the least bactericidal/fungicidal activities against the test organism. Most of the MBCs/MFCs for the aqueous extracts were above 250mg/ml which indicates that a higher concentration was needed to kill the test organisms other than 250mg/ml. However, the ethanolic and methanolic extracts has MBC of l00mg/ml against *Escherichia coli* and *Pseudomonas aeruginosa*. This indicates high antimicrobial efficacy against these to bacterial species.

Conclusion

This research has exposed that aqueous, ethanolic and methanolic extracts of *A. wilkesiana* posses antimicrobial activities against both bacteria and fungi. The leaf extracts of this shrub (plant) can be used for the treatment of diseases caused by these ten test micro-organisms. The diseases include skin diseases, wound infection, candidiasis and others.

Leaf

Phytodrug



A. wilkesiana (Plant)

Preparation of the phytodrug

The leaf of *A. wilkesiana* is dried at atmospheric temperature for 14 days. The dried leaf is ground into fine powder. 5g of the powdered material were loaded into a 2 liter soxhlet extractor containing the ethanol solvent. After the extraction of the plant materials with the ethanol, the extract is concentrated in the flask. The solvent is distilled off and the crude extract concentrate is put into a weighed, labeled beaker and allowed to dry completely in a dessicator. The dried extract is formulated into an ointment.

The composition of the phytodrug may preferably be shown as follows

Emulsifying white soft parafine --- 65% by weight
Liquid parafine ---------- 30% by weight
Concentration of the extract------- 5% by weight
Source: Z. Kohi and Sharma [23]

For more desirable ointment, perfume or fragrance of 2% by weight may be added. The resulting ointment is heated and stirred to mix well and is allowed to cool.

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