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# Use of *Nicotiana Tabacum, Jatropha Curcas,* and *Ficus Exasperata* for Treatment of Pus-Producing Bacteria

**Nwinyi Obinna C.*, Olawumi Damilola E. and Olubukola Oziegbe**

Biotechnology Research Cluster, Department of Biological Sciences, College of Science and Technology, Covenant University, KM 10 Idiroko Road, Canaan Land, PMB 1023 Ota, Ogun State, Nigeria.

*Corresponding author's email: nwinyiobinna@gmail.com; obinna.nwinyi@covenantuniversity.edu.ng

**Abstract**. The knowledge of orthodox medicine on the medicinal effects of plant extracts has continued to be a major booster to our modern healthcare delivery for over 80% of the world's population, especially in the developing world. In this study, we examined the leaf extracts activities of *Nicotiana tabacum*, *Jatropha curcas*, and *Ficus exasperate* on common pus – producing bacteria that occur during secondary infection of open wounds. The initial plant extracts were extracted using methanol via the cold extraction method. The obtained extracts were fractionated via solvent –solvent extraction in the following solvents ethyl acetate, hexane, and distilled water. Preliminary studies carried out revealed that only *Nicotiana tabacum* was effective at inducing inhibitions on the selected clinical isolates, namely: *Staphylococcus aureus, Streptococcus* species, *Escherichia coli*, and *Salmonella typhi*. The antimicrobial assay of the chosen plant was done by agar well diffusion method using 20.0 mg/ml and 40.0 mg/ml concentrations of the selected plant extract. The selected concentrations (40.0 mg/ml and 20.0 mg/ml) exhibited different degrees of zones of inhibition. The mean zones of inhibition ranged between ca. 6.0 -14.5 mm. From the obtained result, *Streptococcus* species were the most inhibited. In addition, the *Nicotiana tabacum* ethyl acetate fraction exhibited a significant inhibitory effect when compared to other fractions such as hexane and aqueous fractions. Thus it is evident that ethyl acetate might be the best choice for extracting the bioactive components from tobacco.

**Keywords**: pus –producing bacteria, antimicrobial activity, *Nicotiana tabacum, Jatropha curcas, Ficus exasperate*

## 1. Introduction

Nature has provided us with free sources of medicinal plants for many years [1]. Knowledge of medicinal plants have been passed down from one generation to another generation. Historically, plants have been the major source of novel drug compounds and has contributed significantly to the human well-being [2]. Medicinal plants have been proven to be highly therapeutic in the curing of various ailments [3]. Science has begun to shift its gaze from synthetic sources used in drug production towards the use of naturally occurring substances. This is because of the dietary and medicinal components from plants that prevent, reverse or delay diseases caused by oxidative stress and inflammatory processes [4, 5, 6, 7].

Plants accumulate phytochemical constituents these include Terpenes, Anthraquinones, Alkaloids, Tannins, Saponins, Flavonoids, Glycosides, Resins, Volatile and Essential oils [8]. Rios and Recio in 2005 described medicinal plants as one whose partial or whole plant organs contain substances that are of therapeutic purposes [9]. These include the leaves, flowers, roots, bark, fruits and seeds. *Ficus exasperata* Vahl. (Moraceae) has been known as a medicinal plant. Its parts have been used as animal fodder [10]. *F. exasperata* could exhibit anti-ulcer, antiparasitic agents, abortifacient, ecbolics effects. Other medicinal effects include used as a treatment for hemorrhoids and venereal diseases, hypotension,

hypoglycemic, hypolipidemic, anti-inflammatory, anxiolytic, analgesics, antiarthritic, diuretic, and wound healing conditions. It has been known to elicit oxytocin inhibiting activities, anticonvulsant, antinociceptive, antipyretic, antimicrobial, anticandidal, insecticidal and pesticidal activities [11, 12, 13, 14, 15, 16, 17, 18]. Nonetheless, there is a constant need for new and active therapeutic agents that can reduce the burgeoning rate of antibiotic-resistant microorganisms [19, 20]. Thus we examined the antimicrobial activities of some selected plants *Nicotiana tabacum, Ficus exasperata*, and *Jatropha curcas.*

## 2. Materials and Methods

### 2.1 Chemicals / Reagents

The Methanol, Hexane, Ethyl acetate, Acetone used in this study were procured from Fanor limited. Ethanol (95%) purity, 0.5% McFarland solution, buffered peptone broth was provided by the Microbiology laboratory, Covenant University. All the reagents were of analytical grade. Mueller Hinton agar was sourced from Liofilchem, Nutrient agar and Nutrient broth (Biomark laboratories).

### 2.2 Plant samples

Fresh leaf samples of *Nicotiana tabacum, Ficus exasperata*, and *Jatropha curcas* were obtained at Covenant University farm in Ota Ogun state. The identification of the leaf samples was carried out by a certified botanist in the Department of Biological Sciences. Specimens of the sample leaves were documented in the laboratory. The leave samples were air-dried at ambient temperature, milled and weighed.

### 2.3 Collection of bacterial isolates

The characterized bacterial isolates were procured from the Microbiology laboratory, Biological Sciences, Covenant University. The isolates include *Escherichia coli, Staphylococcus aureus, Salmonella,* and *Streptococcus pyogenes*. These organisms were reconstituted and the working culture prepared. The selected bacteria species are pus-producing bacteria. Each bacterial isolates were gram stained and observed under the microscope to revalidate the morphological characteristics.

### 2.4 Preparation of plant extracts

The obtained plant leaves were ground to attain uniformity using a blender. The leaf samples were weighed and about 209.30 g of *Nicotiana tabacum* were soaked in 100 % Methanol for eight days. The same amounts were also weighed out for other plant samples. The extracts were obtained by filtration using Whatman No.1 filter paper, a separating funnel and sterile cotton wool. The filtrates were concentrated by evaporating the methanol over a regulated water bath at 60 $^{o}$C. The resulting filtrate was further left to evaporate to dryness.

### 2.5 Preliminary studies/ Batch Partitioning

The preliminary studies carried out on *Jatropha curcas*, *Ficus exasperate*, and *Nicotiana tabacum* showed that the *Nicotiana tabacum* plants were more active in inhibiting the selected pathogenic organisms, because of this further work was done on the tobacco plant.

Fractionation of the crude methanolic extract into hexane, ethyl acetate, and aqueous layers via solvent-solvent extraction were carried out. The solvents and the crude extracts were shaken vigorously in the separating funnel, after which it was left until two distinct layers were observed. Each fractions were evaporated to dryness.

### 2.6 Preparation of extracts for antimicrobial studies

About 0.2g and 0.4g of dried extract fractions were weighed using the analytical weighing balance. The aqueous, hexane and ethyl acetate fractions of extracts were dissolved with 10 ml of Acetone. The concentration of the extract used are 20 mg/ml and 40mg/ml. The different proportions reconstituted

include: AqA-40 mg/ml, AqB- 20 mg/ml, HexTA- 40 mg/ml, HexTB- 20 mg/ml, EtacA-40 mg/ml and EtacB-20 mg/ml.

**2.7 Antimicrobial susceptibility**

The four organisms used for the anti-microbial assay include *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Streptococcus pyrogenes.* The selected bacteria species were tested for viability by resuscitating the microorganisms into buffered peptone broth, after which they were sub-cultured unto nutrient agar and incubated at 37°C for 24 hrs. Pure inoculum of organisms was picked and then placed in 2 ml sterile saline water (0.5 %). The suspension was mixed with a vortex mixer and compared with 0.5 McFarland solution.

Mueller Hinton (MH) Agar was prepared based on the directions of the manufacturer. About 26-28 ml of media was dispensed into sterile Petri dishes. The agar was poured to a depth of about 4 mm and left to solidify. However, when agar is too shallow in the plates, it could yield false susceptible results because the plant extracts will spread more than it should, creating wider zones of inhibition. Conversely, when plates are poured to a depth of >4 mm, it could result to false resistant results. Each dish was appropriately labeled, and sterile syringe used to seed 0.1ml of organisms unto prepared MH plate, and a glass rod was used to spread the bacterial species evenly into the dish and was left to solidify.

**2.8 Placement of extract**

After organisms had grown in the plates, holes were bored using sterile cork borer with a diameter of 6 mm. About 0.2ml of already prepared extracts of *Nicotiana tabacum* were delivered into the holes using a sterile pipette and allowed to stand so that the agar could absorb it. Gentamycin control was placed in the middle of the plate. Once all extracts were in place, the plates placed in an incubator at 37 °C for 16 -18 hours, after which plate readings were noted. MH agar was selected as the medium of choice because it shows satisfactory batch-to-batch reproducibility testing. It is also low in sulfonamide, trimethoprim, and tetracycline inhibitors.

**2.9 Minimum Inhibitory Concentration test (MIC)**

The minimum inhibitory test was done by preparing four different concentrations of *Nicotiana tabacum* 0.10, 0.15, 0.20, and 0.25 ppm. The nutrient broth was prepared, and 5ml of each broth was put in the test tubes and inoculated with the test organisms that were standardized using the McFarland standard. In each of the test tubes, 0.2ml of the concentrations prepared were introduced into each test tube. Following the incubation and measurement with a spectrophotometer, the presence of turbidity indicates the growth of the test organism.

**2.10 Qualitative Phytochemical (Alkaloid) tests:** The *Nicotiana tabacum* were tested for alkaloid using standard method. Two (2) ml of plant extract, and few drops of Mayers reagent (Potassium Mercuric Iodide) were added. The presence of creamy white precipitate indicates the presence of alkaloids [21].

**3. Results and Discussion**

The minimum inhibitory concentration (MIC) results are as shown in Table 1a –b. The MIC is the lowest concentration (in mg/ml) of the plant extract that prevents visible growth of the test organisms. Usually, MIC determination is one of the main steps to assess the antimicrobial potential of new drug candidates. The MIC using ethyl acetate showed significant activities against the growth of *E.coli.* Conversely, the MIC value does not indicate the mechanism of action (cidal or static) of the plant extract.

Table 1a: Minimum inhibitory concentration Ethyl acetate

| Concentration(ppm) | 0.10 | 0.15 | 0.20 | 0.25 |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| *Staphylococcus aureus* | 0.022 | 0.047 | 0.040 | 0.041 |
| *Streptococcus pyogenes* | 0.010 | 0.009 | 0.014 | 0.009 |
| *Salmonella typhi* | 0.025 | 0.020 | 0.035 | 0.026 |
| *Escherichia coli* | 0.003 | 0.003 | 0.007 | 0.006 |

Table 1b: Minimum inhibitory concentration hexane

| Concentrations(ppm) | 0.10 | 0.15 | 0.20 | 0.25 |
|---|---|---|---|---|
| *Staphylococcus aureus* | 0.023 | 0.047 | 0.052 | 0.047 |
| *Streptococcus pyogenes* | 0.031 | 0.026 | 0.053 | 0.023 |
| *Salmonella typhi* | 0.021 | 0.020 | 0.057 | 0.069 |
| *Escherichia coli* | 0.027 | 0.044 | 0.078 | 0.056 |

The qualitative phytochemical screening showed a creamy precipiate which indicates that alkaloid was present. Phytochemicals have been known to inhibit microbial growth via the disruption of enzymes. For the antimicrobial susceptibility assay, the aqueous, hexane, and ethyl acetate fractions of *Nicotiana tabacum* extracts were used. The strength of the extract used was 20 mg/ml and 40 mg/ml.

Table 2: Result of Antimicrobial Susceptibility Test

| Plant fractions | Test organism | Mean zones of inhibition (mm) A1 | B1 |
|---|---|---|---|
| Aqueous | *Escherichia coli* | - | - |
| | *Staphylococcus aureus* | - | - |
| | *Salmonella typhi* | - | - |
| | *Streptococcus pyogenes* | - | - |
| Hexane | *Escherichia coli* | 9 | 6.5 |
| | *Staphylococcus aureus* | 12.5 | 4.5 |
| | Salmonella *typhi* | 8.5 | 5.5 |
| | *Streptococcus pyogenes* | 5.5 | 3 |
| Ethyl acetate | *Escherichia coli* | 12.5 | 5.5 |
| | *Staphylococcus aureus* | 11.5 | 6 |
| | *Salmonella typhi* | 13.5 | 2.5 |
| | *Streptococcus pyogenes* | 14.5 | 7.5 |

A1= 40mg/ml, B1= 20mg/ml, the control used was gentamycin = 10ul

The antimicrobial susceptibility assay showed that the *Nicotiana tabacum* has antimicrobial effects against the selected microorganisms, which are known to be clinically significant antibiotic-resistant species. From Table 2, the aqueous fractions showed no reaction. The mean values of the ethyl acetate

fractions recorded the highest inhibitions. The highest inhibition was observed in *Streptococcus pyogenes*, which showed a zone of inhibition of 14.5mm. *Streptococcus pyogenes* is a gram-positive bacterium. It has been known to cause pharyngitis, skin infections, acute rheumatic fever, scarlet fever, post-streptococcal glomerulonephritis, a toxic shock-like syndrome, and necrotizing fasciitis especially in immunocompromised individuals. The selected plant *Nicotiana tabacum* was tested against species of bacteria that were both gram+ve and gram-ve and was found effective in inhibiting their growth. For the ethyl acetate fraction at the different concentrations of 20 mg/ml and 40 mg/ml *Staphylococcus aureus,* a gram-positive bacteria showed a zone of inhibition that ranged between 6-11.5 mm and *Streptococcus pyogenes* showed 7.5-14.5 mm. For the gram-negative bacteria species *Escherichia coli* and *Salmonella typhi*, their zones of inhibition were 5.5-12.5 mm and 2.5-13.5 mm, respectively. For the hexane fraction, the gram-negative bacteria *E.coli* and *Salmonella typhi* showed zones of inhibition 6.5 -9 mm, and 5.5- 8.5 mm, respectively. For the gram-positive bacteria *S.aureus* and *Streptococcus pyogenes*, their zones of inhibition recorded are 4.5 – 12.5 mm, and 3 -5.5 mm. By these results, it is evident that *N. tabacum* can be considered useful as a broad-spectrum antimicrobial agent. However, the aqueous extracts, in general, were not effective in inhibiting the growth of these bacteria species. The use of medicinal plants to heal diseases, including infectious one, has been extensively applied various researchers [11, 12, 13, 14, 15, 16].

## 4. Conclusion

From the results obtained, in this study, ethyl acetate could be the best choice for extracting the active components from tobacco when compared to the hexane fraction. The ethyl acetate fraction exhibited significant activities against the test organisms. The obtained result was in agreement with [4, 22] that reported that use of solvents of different polarity could affect not only the extraction yield but also the antimicrobial activities of the bioactive compounds. Thus polar solvents can yield higher extractions than the less polar solvents. Also, the different cell wall architectures of the gram-positive and gram-negative bacteria differ in their behavior towards various solvents. This may have accounted for the different activities experienced in this study.

In conclusion, ethyl acetate of tobacco plant extract has proven to exhibit significant antimicrobial activity. Thus, it could be explored for the development of antimicrobial agents for pus-producing bacteria species, in which some of the test organisms used in this study have been implicated.

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## References

[1]. Kaur, R., Kapoor, K. and Kaur, H (2011). Plants as a Source of Anticancer Agents. *Journal of Natural Product and Plant Resources* 1 (1): 119-124.
[2]. Nwinyi, O.C. Nwodo C. S and Ajani O.O (2008). Evaluation of antibacterial activity of *Psidium guajava* and *Gongronema Latifolium. Journal of Medicinal Plant Research* 2(8) 189-192.
[3]. Govnid, P. and Farombi, O. (2011). Antioxidant vegetables act against cancer and other diseases. *International Journal of Pharmaceutical Studies and Research* 2(1): 32-38.
[4]. Nwinyi, O.C, Smith, O. O., Olugbuyiro, J A., Rotimi S O and Njobeh, P. B (2018) Antibacterial assay and reversion of carbon tetrachloride (ccl4) induced liver damage on wistar mice by *Vernonia amygdalina Pakistan Journal of Pharmaceutical Sciences* 30 (4) 1311-1321
[5]. Farombi, E. and Owoeye, O. (2011). Antioxidative and Chemopreventive Properties of *Vernonia amygdalina* and *Garcinia biflavonoid. International Journal of Environmental Research and Public Health*, 8: 2533-2555.

[6]. Aiyegoro, O., Akinpelu, D., Afolayan, A. and Okoh, A. (2008). Antibacterial activities of crude stem bark extracts of *Distemonanthus benthamianus* Baill; *Journal of Biological Science*. 8(2): 356-361.
[7]. Iwu, M. (1993). Handbook of African Medicinal Plants. CRC Press, Boca Raton, FL. ISBN 0-8493-4266-X. pp. 201-223.
[8]. Robinson, N. (2006). Integrated traditional Chinese medicine. *Journal of Pharmaceutical studies and research.* 10: 20-24.
[9]. Rios, J.L and Recio, M.C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology* 100: 80-84.
[10]. Ahmed, F., Mueen, O., Ahmed, K., Abedin, M. and Karim, A. (2012). Traditional uses and pharmacological potential of *Ficus exasperata* vahl. *System Review of Pharmacology* 3:15-23
[11].Akah, P., Orisakwe, E., Gamaniel, S. and Shittu, A. (1998). Evaluation of Nigerian traditional medicines: II. effects of some Nigerian folk remedies on peptic ulcer. *Journal of Ethnopharmacol* 62:123-7.
[12]. Ayinde, B., Omogbai, E. and Amaechina, F. (2007). Pharmacognosy and hypotensive evaluation of *Ficus exasperata* Vahl (moraceae) Leaf. *African Journal of Traditional Complement Alternative Medicine* 64:543-6.
[13]. Adewole, S., Adenowo, T., Naicker, T. and Ojewole, J. (2011). Hypoglycaemic and hypotensive effects of *Ficus exasperata* Vahl. (Moraceae) leaf aqueous extract in rats. *African Journal of Traditional Complement Alternative Medicine* 8:275-83.
[14]. Woode, E., Poku, R., Ainooson, G., Boakye, S., Gyasi, E. and Abotsi, W. (2009). An Evaluation of the anti-inflammatory, antipyretic and antinociceptive effects of *Ficus exasperata* (Vahl) leaf extract. *Journal of Pharmacology and Toxicology* 4:138-51.
[15]. Taiwo I. A., Adebesin, O. and Funmilayo, A. (2010). Glycaemic activity of *Ficus exasperata* in fructose-induced glucose intolerance in rats. *West African Journal of Pharmalogy and Drug Research* 2:80-3.
[16]. Bafor, E., Omogbai, E. and Ozolua, R. (2011). Oxytocin inhibiting effect of the aqueous leaf extract of *Ficus exasperata* (Moraceae) on the isolated rat uterus. *Acta Pol Pharm* 68:541-7.
[17]. Odunbaku, O. and Olusanya, O. (2008). Antibacterial activity of ethanolic leaf extract of *Ficus exasperata* on *Escherichia coli* and*Staphylococcus albus. Science Research Essay* 3:562-4.
[18]. Ogunleye, R. (2011). Effectiveness of the leaf powder of *Ficus exasperata* Vahl (Moraceae) in suppressing thepopulation of two major storage insect pests. *Continental Jounal of Biological Science* 4:6-11.
[19]. Tarun, A., Rachana, S., Amar, D. and Imran, W. (2012). *Asian Journal of Plant Science and Research* 2 (1): 36-40.
[20]. Nwinyi Obinna C. Chinedu N.S, Ajani.O.O, Ikpo CO and Ogunniran K.O (2009) Antibacterial effects of *Ocimum gratissimum* and *Piper guineense* on *Staphylococcus aureus* and *Escherichia coli*. *African journal of Food science* 3 (3) 077-081
[21] Madziga, H., Sanni, S. and Sandabe K. (2010). Phytochemical and Elemental Analysis of *Acalypha wilkesiana* Leaf. *Journal of American Science* 6 (11): 510-514.
[22]. Foo, R.Q, Manogaran E and Gabriel A.A (2014) Antimicrobial and antioxidant studies of *Veronina amydalina*. J. App. Pharm., 6(4): 360-371.