



Research/Technical Note

Phytochemical Constituents, Thin Layer Chromatography and Antimicrobial Activity of Methanol Extract of the Stem and Leave of *Citrus Limon* (L)

Ayuba Yohanna Musa^{1,*}, Okoro Benedict Chidiogor², Abubakar Muhammad Nazif³, Ilesanmi Esther⁴

¹Department of Chemistry, Abubakar Tafawa Balewa University Bauchi, Bauchi, Nigeria

²Department of Microbiology, Godfrey Okoye University Emene, Enugu, Nigeria

³Department of BioTechnology, Modibbo Adama University of Technology Yola, Adamawa, Nigeria

⁴Department of Chemistry, University of Benin, Benin, Nigeria

Email address:

ayubayohanna688@gmail.com (A. Y. Musa)

*Corresponding author

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Abstract: *Citrus limon* (L) leaf and stem used traditionally for the treatment of diseases and infections was phytochemically and antimicrobially screened. And also Thin layer Chromatography (TLC) was carried out on the crude methanol extracts. The phytoconstituents were qualitatively determined. The phytochemical screening of the crude methanol extract revealed the presence of saponins, terpenes, flavonoids, steroids, tannins, carbohydrates, anthraquinones, alkaloids, volatile oils and glycosides. The result of the antimicrobial screening of the crude methanol extract of the leaf showed potent activity against *Staphylococcus aureus*, *candida albicans* and *bacillus subtilis*. However, the crude methanol extract of the stem only showed activity against *Staphylococcus aureus*. TLC analysis of the of the crude methanol extract of the leaf showed three (3) spots while that of the stem revealed two (2) spots.

Keywords: Phytochemical, Antimicrobial, TLC, *Staphylococcus Aureus*, *Candida Albicans*, *Bacillus Subtillis*

1. Introduction

Medicinal plants have always been associated with cultural behaviors and traditional knowledge. The renaissance of interest in plant products has been stimulated by the use of plant extracts in chronic conditions for which conventional drugs is perceived to offer very little specificity in its target [1]. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases [2]. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts [3]. The blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Also, the development of adverse effects

and high microbial resistance to the chemically synthesized drugs, has forced men into ethnopharmacognosy. More so, in our local situation, degree of ignorance and illiteracy had forced many to abandon or neglect pharmaceutically formulated drugs in favor of locally prepared herbal remedies coupled with the fact that pharmaceutical products are increasingly being faked. Thus, the herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment [4].

Herbs are staging a comeback, herbal renaissance 'is happening all over the globe and people returning to the naturals with hope of safety and security. By and large, the public is gradually drifting towards acceptance and usage of herbal preparations. In Africa, traditional healers and remedies made from plants play important role in the health of millions

of people [5]. The users of these remedies, found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effects. The Pharmacognosy Society of Nigeria supports the acceptance of herbal remedies or treatment of ethnomedicinal practice along with conventional orthodox health care system [6]. This is largely due to the fact that plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases [7]. Biological organisms particularly plants produce two distinctly different types of chemical products. The first types of primary metabolites which consist of compounds such as sugars and proteins those are common to most organisms and are essential for functional metabolism. Secondary metabolites, on the other hand, are chemicals unique to a single species or related group of organisms. Not until the 1990s that scientists fully realize that these secondary metabolites are more than mere leftovers from an organisms metabolic processes. These chemicals can function as communications tools, defense mechanisms or sensory devices. The biological activity of these chemicals is beneficial to the organism that produces them, but it is often harmful to other species, including humans [8]. This toxicity can adversely affect the functions of the entire human body or only a specific biological process, such as the growth of cancer cells [9]. Also, many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity of plants have been reported. In this way, certain foreign, naturally produced chemicals can act as powerful drugs when administered at the proper concentration [9].

Natural products are important in health care. They can be used as starting materials for semisynthetic drugs. The main examples are plant steroids, which led to the manufacture of oral contraceptives and other steroidal hormones. Today, almost every pharmacological class of drugs contains a natural product or natural product analog [10]. Similarly, it represents an excellent resource for the identification of new lead structures [11].

It is estimated that 25% of all prescriptions dispensed in the USA contained a plant extract or active ingredients derived from plants. It is also estimated that 74% of the 119 currently most important drugs contain active ingredients from plants used in traditional medicine for health care [12], these traditional medicines are primarily plant-based [13]. Another study of the most prescribed drugs in the USA indicated that a majority contained either a natural product or a natural product was used in the synthesis or design of the drug [14]. Similarly, about 121 drugs prescribed in USA today come from natural sources, 90 of which come either directly or indirectly from plant sources [15]. Forty-seven percent of the anticancer drugs in the market come from natural products or natural product mimics [11].

Tropical and subtropical Africa contains between 40,000 – 45,000 species of plants with a potential for development and out of which 5,000 species are used medicinally [16]. Still there is a paradox that in spite of this huge potential and diversity, the African continent has only contributed 83 out of the 1100 classic drugs globally [16].

2. Materials and Methods

2.1. Plant Collection, Identification and Preparation

Leaves and stem of *Citrus limon (L)* were used as the sample under investigation, plant leaf and stem were collected from a garden in Abuja, Nigeria. The plant was identified and Authenticated by Mr. Akeem Lateef of the herbarium department of National Institute of Pharmaceutical Research and development (NIPRD), Idu Abuja. The leaves and stem bark were air-dried separately under shade for three weeks and pulverize using a wooden mortar and pestle to obtain a fine powdered-like texture. This was done to enhance the penetration of the extracting solvents into the plant cells, thus facilitating the release of the active principles. The pulverized plant samples were then stored in amber bottles and kept in a cool and dried environment under room temperature until it is required for usage.

2.2. Extraction

Cold maceration was adopted for 24 hours and the extraction was carried out using Methanol (polarity index = 5.2P'). This was achieved by dissolving 300 grams of the dried plant material (Stem & leaves) separately in 700 ml of the extracting solvent. After 24 hours, the mixture was filtered through whattman No.1 filter paper with pore size of 0.7 µm to obtain a clear filtrate which was concentrated to a semisolid substance with the use of rotary evaporator at 50°C. The concentrate was then freeze dried using the water bath.

The percentage yield of crude extracts was determined using the equation below.

$$\text{Percentage yield} = \left[\frac{\text{weight of extracts}}{\text{Weight of dry plant material}} \times 100/1 \right]$$

2.3. Phytochemical Screening

The Phytochemicals: Alkaloids, Carbohydrates, Saponins, Tannins, Steroids & Terpenes, Anthraquinones, Volatile oils, Flavonoids, Phenols and Glycosides were determine using standard procedures [17] [18].

2.4. Antimicrobial Screening

The antimicrobial activities of this extract were determined using some human pathogens which were obtained from the Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. All the pure culture of the test organism was subculture for 24 hours and 2 hours respectively in broth agar.

The extracts (0.4 g) was weighed and dissolved in 5ml of sterile water to obtain a concentration of 80 mg/ml and were serially diluted to 40mg/ml, 20mg/ml and 10mg/ml of the different concentrations of the extract was aseptically dispensed in each of the well using pasteurized pipette [19].

Nutrient agar was the medium used as the growth medium for the test microbes. The medium was prepared according to the manufacturer's instruction, sterilized at 121°C for 15 minutes in the autoclave, poured into 12 (10 dishes for the five

duplicate organism, 1 dish for media sterility control MSC and the other for organism validity control OVC) sterile petri dishes and was allowed to cool and solidify. The inoculated medium was incubated at 37°C for 24 hours after which, each plate was observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and the result was recorded in millimeters.

2.5. Thin Layer Chromatography (TLC)

Thin layer chromatography was carried on the two extract of *Citrus limon (L)* using a glass TLC plate whose surface was coated with silica gel. The TLC plate was cut into a size of 10cm by 5cm; a mark was made in pencil about 1.5cm from the lower edge of the end of the plate which marks the origin. The dissolved extracts were spotted on the line creating a distance of about 0.5 to 1cm between each spot, the distance prevent the spots from overlapping or mixing while separating. Solvent was prepared in the development chamber which makes the solvent system in which the spotted plate was placed in such that the liquid solvent does not touch the origin (line marked in pencil) and spotted with dissolved extract. The solvent which is the eluent moves through the plate and goes up by capillary action of the plate carrying the compounds present in the extract which separates and appears as spots on the plate. The compounds that are closer to the origin shows less movement and are polar than the compounds that move further from the origin. The distance travelled by solvent was marked in pencil which marks the solvent front. The spots were seen by day light and with the help of iodine crystal vapor kept in a closed chamber. This procedure was repeated with different ratio and volume of solvent until a perfect separation was obtained. Then the retention factor was calculated using the formula

$$R_f = \frac{\text{Distance travelled by solute}}{\text{distance travelled by solvent}}$$

The retention factor (Rf) value essentially describes the distance travelled by the individual component.

3. Result

Table 1. Yield of extract the result of the yield of extract for both the stem and leave is summarize in the table below.

	Stem	Leave
Weight of empty bottle (g)	15.1700	15.4088
Weight of bottle + extract (g)	25.8158	25.7863
Weight of extract yield (g)	10.6458	10.3775
Weight of dry sample (g)	300	300
% yield (%)	3.55	3.46

Key: weight of extract yield = weight of bottle + extract (g) – weight of empty bottle (g)

%yield = [(weight of extracts yield/ Weight of dry sample) x 100/1]

Table 2. Phytochemical Screening Result.

Test	Stem	Leave
Saponins	+	+
Carbohydrates	+	+
Tannins	+	+
Cardiac Glycoside	-	+
Alkaloids	+	+
Flavonoids	+	+
Anthraquinones	+	-
Terpenes & Sterols	+	+
Volatile oil	-	+
Phenols	+	+

Key: + = Present and - = Absent

Table 3. Thin layer chromatography.

No. of spots	Stem		Leave	
	Rf value	Visualization	Rf value	Visualization
1	0.76	Iodine tank	0.45	Iodine tank
2	0.89	Iodine tank	0.66	Iodine tank
3		Iodine tank	0.79	Iodine tank

Key: Rf = Retention factor Solvent system - ethyl acetate: Methanol Ratio: - 3: 2

Table 4. Antimicrobial activity result (mean zone of inhibition) leaf extract.

Test organism/Conc.	Mean Zone of Inhibition (mm)			
	80mg/ml	40mg/ml	20mg/ml	10mg/ml
<i>Pseudomonas aeruginosa</i>	0	0	0	0
<i>Escherichia coli.</i>	0	0	0	0
<i>Candida albicans</i>	9	0	0	0
<i>Bacillus subtilis</i>	4	0	0	0
<i>Staphylococcus aureus</i>	4	2	0	0

Table 5. Antimicrobial activity result (mean zone of inhibition) stem extract.

Test organism/Conc.	Mean Zone of Inhibition (mm)			
	80mg/ml	40mg/ml	20mg/ml	10mg/ml
<i>Pseudomonas aeruginosa</i>	0	0	0	0
<i>Escherichia coli.</i>	0	0	0	0
<i>Candida albicans</i>	0	0	0	0
<i>Bacillus subtilis</i>	0	0	0	0
<i>Staphylococcus aureus</i>	10.5	0	0	0

4. Discussion

This report offers a guide to the extraction, phytochemical screening, TLC and antimicrobial activity of the constituent of *citrus limon* (*L*) stem and leave. The percentage yield for the methanol extract of the stem and leave gave 3.55 and 3.46 percent recovery respectively. While the phytochemical screening in table 2 shows the presence of secondary metabolites such as saponins, tannins, steroids, alkaloids, anthraquinones etc. Table 3 shows the thin layer chromatography of both the stem and leave, with a good separation from the first spot of the stem having a retention factor of 0.76 while the leave 0.66. The bioassay shows inhibition against staphylococcus aureus, candida albicans and bacillus subtilis. This organism are gram positive and and some are gram negative bacteria. The report of the antimicrobial of the leave is consistent with that of [20]. The gram negative bacterial (*Escherichia coli*) are the organism that cause opportunistic infections and are found in the intestinal tract, causing urinary tract infection, infection of wound, sepsis, diarrhea disease, dysentery, abdominal wound. While staphylococcus aureus are gram positive bacteria that causes boils, styes, infections of wounds, ulcers, burns, pneumonia etc.

5. Conclusion

This preliminary work shows that *citrus limon* (*L*) contains many classes of natural products such as alkaloids, flavonoids, tannins, saponins etc. the fact that the extracts have some antimicrobial activities showed that the extract could be useful chemotherapeutic agents against infections arising from the activities of this microorganisms. The research is still on-going to isolate the individual phytochemicals, test their antimicrobial activities against the aforementioned microorganism and from microanalysis be able to carry out their synthesis using simple and available starting material.

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