PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (7) 2077-2082 July 2024

Phytochemical, Proximate and Anti-Microbial Screening of Methanol Extract of *Populus grandidentata* (big-tooth aspen) collected from Uvwie Local Government Area in Delta State, Nigeria

***OGWUCHE, CE; PERE, C**

Department of Chemistry, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria

**Corresponding Authors Email: ogwuche.christiana@fupre.edu.ng*
**ORCID: https://orcid.org/0000-0002-7030-550X*
**Tel: +2347037166612*

Co-Author Email: pere.charlesfupre.edu.ng

ABSTRACT: Throughout antiquity till the modern age, medicinal plants have been used worldwide in traditional and ethnomedical medicine. Hence, the objective of this paper is to present the phytochemical, proximate and anti- microbial screening of methanol extract of Populus grandidentata (big-tooth aspen) collected from Uvwie Local Government Area in Delta State, Nigeria using. Proximate analysis, antimicrobial analysis, and phytochemical screening were performed on the obtained extract. The findings demonstrate the presence of saponin, flavonoids, alkaloids, glycosides, tannins, and steroids in the phytochemical screening of the investigated plant. The elemental analysis revealed the following percentages to be present: nitrogen (5.64%), carbon (37.46%), hydrogen (3.50%), and sulfur (0.68%). The proximate analysis resulted in low moisture content of 1.9%, low lipid content of 20.9%, and low ash content of 15.6%. Antimicrobial efficacy of *Populus grandidentata* leaf was examined using standard laboratory bacterial isolates. With a zone of inhibition ranging between 22 and 27 mm as compared with standard dugs, the isolates of bacteria and fungi include Methicillin Resistant Staphaureus, Vanomycin Resistant Enterococci, Staphylococcus, Escherichia coli, Helicobacter pyroli, Campylobacter jejuni, Proteus mirabilis, Candida albicans, Candida tropicalis, and Candida krusei. All of the microbes had minimum inhibitory concentrations of 25 ug/ml and minimum fungicidal and bactericidal concentrations of 50 ug/ml, respectively.

DOI: https://dx.doi.org/10.4314/jasem.v28i7.20

Cite this Article as: OGWUCHE, C. E; PERE, C. (2024). Phytochemical, Proximate and Anti- Microbial Screening of Methanol Extract of *Populus grandidentata* (big-tooth aspen) collected from Uvwie Local Government Area in Delta State, Nigeria. *J. Appl. Sci. Environ. Manage.* 28 (7) 2077-2082

Dates: Received: 21 February 2024; Revised: 22 March 2024; Accepted: 26 June 2024 Published: 02 July 2024

Keywords: *Populus grandidentata*; Antimicrobial activity; proximate analysis; Ethnomedical medicine; Big-tooth aspen

Throughout history, medicinal plants have been used worldwide in traditional and ethnomedical medicine. These dynamic fields lie at the intersection of phytochemistry and plant biology and are concerned with the evolution mechanisms and systematics of medicinal plant genomes, the origin and evolution of plant genotype and metabolic phenotypte, the interaction between medicinal plant genomes and the environment, and the relationship between genomic diversity and metabolite diversity, (Cheng *et al.*, 2019). It is necessary to identify and research the wide range of biological characteristics found in African medicinal plants. Africa only has a few volumes with basic information on medicinal plants, compared to other countries that have large pharmacopoeias to use as a guide. The principal medicinal plants are source of numerous beneficial chemicals and medications. Approximately 1300 medicinal plants are used in Europe; of these, 90% are derived from natural sources. According to the World Wildlife Fund and the

**Corresponding Authors Email: ogwuche.christiana@fupre.edu.ng*
**ORCID: https://orcid.org/0000-0002-7030-550X*
**Tel: +2347037166612*

International Union for Conservation of Nature, between 50,000 and 80,000 blooming plants are used medicinally. With rates of extinction 100–1000 times higher than those of natural extinction, the decline of medicinal plant species has been accelerated by the growth in human population and the desire for natural remedies, Gusain *et al.*, 2021. Even in less nutrient-rich soil and on uneven, hilly terrain, the plant grows quickly and readily. Another way it can spread is through cutting (Khurana and Khosla, 1991). Additionally, it is said that the plant is employed in phytoremediation (Balatinecz *et al*., 2001). However, there is no prior research that has been published on this specific specie of the Populus family. Hence, the objective of this paper is to present the phytochemical, proximate and anti- microbial screening of methanol extract of Populus grandidentata (big-tooth aspen) collected from Uvwie Local Government Area in Delta State, Nigeria.

MATERIALS AND METHODS

Gathering of vegetation: The local government of Uvwie in Delta State provided the *Populus grandidentata* plant. Getting the plant extract ready. After being harvested, the vegetable was cleaned to get rid of any debris, allowed to air dry for 14 days in the lab, and then ground.

*Technique for extraction***:** The plant material was extracted using the soxhlet extracting method, which involves filling a thimble holder with condensed fresh solvent from a distillation flask and then aspirating the solution when the liquid reaches the overflow level. Thimble-holder, transferring the extracted solutes into the bulk liquid as it is unloaded back into the distillation flask. Distillation is used in the solvent flask to separate the solute from the solvent. Fresh solvent returns to the plant solid bed, leaving the solute in the flask. Until full extraction is accomplished, the process is repeated. The leaves that had been powdered were extracted for eight hours at 80°C using the right amount of solvent. Methanol was the extraction solvent used.

The phytochemical screening: Following established protocols, the extracts were examined for the presence of phenolic compounds, flavonoids, alkaloids, cardiac glycosides, terpenoids, saponins, and reducing sugar.

The Folth Test is a saponin test: 20ml of *Populus grandidentata* extract was created by diluting 0.2g of the extract with distilled water. For fifteen minutes, the suspension was shaken inside a graduated cylinder. The absence of two-layer foam development suggests that saponins are not present.

Flavonoid detection (Alkaline Reagent Test): To add an intense yellow color to the Popoulus grandidentata extract, a few drops of sodium hydroxide were added. The presence of flavonoids is indicated by the color remaining visible even after diluted hydrochloric acid is added.

Alkaloids test (Wagner's Test): After being mixed with a few milliliters of diluted hydrochloric acid, 0.2 grams of Populus grandidentata extract was filtered. The Wagner's reagent was then added to it in a few drops. Alkaloids are present when a reddish-brown precipitate forms.

Salkowski's Test, a terpeniod detection method: To create a layer, 0.2g of the extract, a few milliliters of chloroform, and concentrated sulfuric acid were added. The interface did not develop a reddish-brown hue, which would have indicated the absence of terpeniods.

Glycoside test (Keller-Kilian's test): Two milliliters of glacial acetic acid with a single drop of 5% ferric chloride were used to treat a small amount of extract. 1 milliliter of concentrated sulfuric acid was then added. A brown ring is indicative of cardinolideoxy sugar at the interface. The absence of cardiac glycosides was indicated by the absence of a violet ring in the acetic acid layer.

Check for phenol: A few drops of 5% ferric chloride were added after dissolving 0.2g of the extract in 5ml of distilled water. The extract's bluish-black color suggests the presence of phenolic compounds.

Check for tannins: After soaking in 2% hydrochloric acid, the Gold Beater's Skin was cleaned with distilled water. After that, it spent five minutes in an extract solution before being cleaned with distilled water. Lastly, 1% ferrous sulfate solution was added to it. If the skin of the gold beater turned brown or black, tannins are still there.

Proximate evaluation: In accordance with (AOAC. 2005), standard procedures found in the literature were used to carry out this analysis. Ash, moisture, protein, fiber, fats, and carbohydrates are found in the analysis. Furthermore, it enables us to legitimately compare extracts based on particular nutrients, allowing us to determine how much more superior one extract is to another in terms of nutrients.

Determining the moisture content: After being weighed, 2g of the sample (leaf) was baked for two hours to dry it out. The weight of the leaf sample was added to the weight of the crucible. Samples are dried

in an oven set at 105 degrees Celsius for two hours. Tannins have caused Beater's skin to turn brown or black. Were given a 10-minute cooling period in the desiccators. After that, the crucibles and the dried sample were weighed and put back in the oven for an additional half-hour at the same temperature. After removing the samples from the oven and letting them cool, they were once more weighed using the crucible.

Determination of ash content: One gram of dried leaf sample was ash-treated in a muffle furnace for three hours at 500 degrees Celsius. The sample was then allowed to cool in desiccators before the weight was measured using a weighing balance.

The Elemental Analysis Method: A tin capsule is used to weigh the sample that is being tested. The necessary quantity of organic material is 2 to 3 mg, and if inorganic matter with low carbon content is examined, the amount can scarcely surpass 10 mg. The sample is put into the auto sampler after the capsule, which resembles wrapped tin foil, has been folded. The sample and its tin capsule fall into the reactor chamber, which has previously been filled with excess oxygen. The substance becomes "mineralized" at 990 °C. At this temperature, carbon monoxide formation is likely to occur even in the presence of excess oxygen. The gaseous reaction products pass through a tungsten trioxide catalyst, where the full oxidation occurs. Therefore, CO2, H2O, and NOx should be the components of the final mixture. Additionally, some extra O2 gets past the catalyst. A silica tube filled with copper granules is passed through by the mixture of product gas. Remaining oxygen is bound and nitric and nitrous oxides are reduced in this zone, which is maintained at roughly 500 °C. The analytically significant species CO2, H2O, and N2 are present in the departing gas stream. Eventually, hydro-halogenides or included SO2 are absorbed at the proper traps. As a carrier gas, high purity helium (Quality 5.0) is utilized. After the gas mixture has reached a certain pressure/volume state, it is transferred to a gas chromatographic apparatus. Zone chromatography is the method used to separate the species. Using this method, a signal of the staircase type is Antimicrobial analysis:

The Inhibition Zone: Pathogenic microbes from the ABU Teaching Hospital in Zaria's Department of Medical Microbiology were used to test the plant extract's antimicrobial properties. To achieve a concentration of 100µg/ml, 0.001 mg of the complex was weighed and dissolved in 10 mmls of DMSO. This was the complex's starting concentration, which was used to measure its antibacterial properties. The complex was screened using the diffusion method. The microbes were grown on Mueller Hinton agar as their growth medium. The medium was made as directed by the manufacturer, sterilized for 15 minutes at 121 degrees Celsius, and then poured into the sterile petri dishes. It was then left to cool and solidify. One milliliter of the test microbe's standard inoculum was added to the sterilized medium, and a sterile swab was used to evenly distribute the inoculum across the medium's surface. A standard cork-borer with a 6 mm diameter was used to create a well in the middle of each inoculation medium. Next, 0.1 milliliter of the complex solution at a concentration of 100 micrograms per milliliter was added to the well on the inoculation medium. Following a 24-hour incubation period at 37°C, the medium plates were examined for the zone of growth inhibition. The zone was then measured using a transparent ruler, and the result was noted in millimeters.

Lowest concentration of inhibition: The broth dilution method was used to find the complex's minimum inhibitory concentration. After making the Mueller Hilton broth, 10 milliliters were filled test tubes, which were then sterilized for 15 minutes at 121 degrees Celsius. The broth was then left to cool. To provide a solution, MC-Farland's turbidity standard scale number 0.5 was created. After making normal saline, 10 ml was added to a sterile test tube, the test microbe was inoculated, and the mixture was then incubated for six hours at 30 oC. The microbe was diluted in regular saline until, when compared visually, the turbidity marched toward the MC-Farland scale.

Minimum concentrations of fungicidal and bactericidal agents: To find out if the test microbes were killed or merely had their growth inhibited, MFC was used. Mueller Hilton agar was made, sterilized, and then poured into sterile petri dishes. It was then left to cool and solidify at 121 oC for 15 minutes. Following a 24-hour incubation period at 37 oC, the contents of the MIC in the serial dilutions were subcultured onto the prepared medium. The medium plates were then examined for colony growth; the MFC plates had the lowest concentration of the complex without any colony growth.

RESULTS AND DISCUSSION

Popoulus grandidentata crude leaf extracts are subjected to phytochemical screening. A phytochemical screening of *Populus grandidentata* methanol extract as presented in on Table 1, reveals the presence of tannins, steroids, alkaloids, glycosides, flavonoids, and saponins. This demonstrates that the extract can be used in the food, cosmetic, and pharmaceutical industries due to its versatility and relatively high concentration of active ingredients.

Kabera *et al*. (2014) state that these phytochemicals are very helpful in the search for new drugs to cure a wide range of illnesses. Alkaloids, phenols, glycosides, flavonoids, steroids, saponins, and tannin compounds are among the phytochemicals used in medicine to combat various pathogens and treat illnesses (Palombo, 2006). The proximate analysis results of *Populus grandidentata* as presented in Table 2 to 3 has a relatively low moisture content of 1.9%. This plant extract won't quickly go rancid because of its low moisture content, such has been found as reported by Omorogi *et al*., 2011

Table 1: Qualitative phytochemical screening of *Popoulus grandidentata* leaf extracts.

Test performed	**Result for methanol**
Appearance	Liquid
Colour Descriptionn	Black
Saponins	+
Flavonoids	+
Alkaloids	+
Terpenoids	-
Tannins	+
Glycosides	+
Phenol	-
Steroids	+

Table 2: Moisture content

Weight of crucible (g)	Weight of sample + crucible before drying	Weight of sample + crucible after drying for 2 hours (g)	Weight of sample + crucible after drying for 30 minutes (g)	Weight loss (g)	%moisture
42.449	44.499	46.706	46.699	0.038	1.9

Table 3: Ash Content

Weight of crucible	Weight of sample + crucible before ashing	Weight of sample + crucible after ashing	Weight of ashed sample	% ash
34.402	35.402	34.558	0.156	15.6

Table 4: Zone of inhibition

Test organism	**Sensitivity**	**Zone of inhibition**
Methicillin Resist Staphaureus	S	27
Vanomycin resistant enterococci	S	24
Staphylococcus aureus	S	22
Escherichia coli	R	0
Helicobacter pylori	S	25
Campylobacter jejuni	S	27
Proteus mirabilis	R	0
Candida albicans	R	0
Candida krusei	S	24
Candida tropicalis	S	23

Key: S => Sensitive, R => Resistance

Table 5: Minimum inhibition concentration

Test organism	100µg/mL	50µg/mL	25µg/mL	12.5µg/mL	6.5µg/mL
Methicillin Resist Staphaureus	-	-	-	0*	+
Vanomycin resistant enterococci	-	-	0*	+	++
Staphylococcus aureus	-	-	0*	+	++
Escherichia coli					
Helicobacter pylori	-	-	0*	+	++
Campylobacter jejuni	-	-	-	0*	+
Proteus mirabilis					
Candida albicans					
Candida krusei	-	-	0*	+	++
Candida tropicalis	-	-	0*	+	++

Key: - => No turbidity (no growth), o => MIC, + => Turbid (light growth), ++ => Moderate turbidity, +++ => High turbidity*

Table 6: Minimum bactericidal/fungicidal concentration

Test organism	100µg/mL	50µg/mL	25µg/mL	12.5µg/mL	6.5µg/mL
Methicillin Resist Staphaureus	-	0*	+	++	++
Vanomycin resistant enterococci	-	0*	+	++	++
Staphylococcus aureus					
Escherichia coli					
Helicobacter pylori	-	0*	+	++	++
Campylobacter jejuni	-	0*	+	++	++
Proteus mirabilis					
Candida albicans					
Candida krusei	-	0*	+	++	++
Candida tropicalis	-	0*	+	++	++

Key - => No turbidity (no growth), o* => MIC, + => Turbid (light growth), ++ => Moderate turbidity, +++ => High turbidity

A 15.6% was found to be the ash content, which is also comparatively low when considering the other proximate analyses that were done. This analysis demonstrates the plant extract's high mineral content. (Igile *et al*., 2013). Lipid content in the plant extract is a respectable 20.9%. This essentially indicates that the

fatty acid content of the plant extract is average. *Populus grandidentata*'s antimicrobial screening demonstrates the plant extract's sensitivity and resistance to microorganisms.

Compared to resistivity, this plant extract demonstrated greater sensitivity to antimicrobial activities as presented in Tables 4, 5 and 6. With a zone of inhibition ranging between 22 and 27 mm as compared with standard dugs, the isolates of bacteria and fungi include *Methicillin Resistant Staphaureus, Vanomycin Resistant Enterococci, Staphylococcus, Escherichia coli, Helicobacter pyroli, Campylobacter jejuni, Proteus mirabilis, Candida albicans, Candida tropicalis,* and *Candida krusei.* All of the microbes had minimum inhibitory concentrations of 25 ug/ml and minimum fungicidal and bactericidal concentrations of 50 ug/ml, respectively. *P. grandidentata* has not been the subject of any prior antibacterial research that has been documented in the literature to substantiate its medicinal status. However, studies have documented the antimicrobial properties of various other *Populus* species. Findings indicated that minimal concentrations of inhibition (MIC) and minimal. Water extracts exhibited the strongest antimicrobial activity, as evidenced by the bactericidal/fungicidal concentration (MBC/MFC) of three of the eight tested microorganisms and a weak to moderate inhibition of their growth. Although less so than the water extract, the quaking aspen methanol extract also showed antimicrobial activity (Al-Hussaini and Mahasneh, 2011; St-Pierre, 2019; Hafeez *et al.,* 2019; Kumari, 2020).

Table 7: Result of Elemental analysis,

Elements	Percentage composition
Nitrogen	5.64%
Carbon	37.46%,
Hydrogen	3.50%
Sulphur	0.68%

Conclusion Numerous physiologically active substances, such as flavonoids, phenolic acids, tannins, and alkaloids were found in *Populus grandidentata* through phytochemical screening. Numerous pharmacological activities, including anti-inflammatory, antioxidant, and antimicrobial effects, have been demonstrated for these compounds.

REFERENCE

Al-Hussaini, R; Mahasneh, AM (2011). Antibacterial and antifungal activity of ethanol extract of different parts of medicinal plants in Jordan. *Jordan J. Pharm. Sci*., 4: 57-69

AOAC (2005). Official Method of Analysis (18th ed). Association of Official Analytical Chemists Washington D.C. p. 106.

Balatinecz, JJ; Kretschmann, DE; Leclercq, A (2001).Achievements in the utilization of poplar wood-guide-posts for the future. *Forest Chron*. 77: 265–269.

Chen, SL; Wu, WG; Wang, CX; Xiang, L; Shi, YH; Zhang, D (2019). Molecular genetics research of medicinal plants. *Zhongguo Zhong Yao Za Zhi* 44 2421–2432. 10.19540/j.cnki.cjcmm.20190514.102

Gusain, P; Uniyal, D; Joga, R (2021). Conservation and Sustainable Use of Medicinal Plants. In Preparation of Phytopharmaceuticals for the Management of Disorders; Apple Academic Press, Elsevier: Amsterdam, The Netherlands, pp. 409–427.

Hafeez, M; Arshad, R; Hameed, MU; Akram, B; Ahmed, MN; Kazmi, SA; Ahmad, I; Ali, S (2019). *Populus ciliata* leaves extract mediated synthesis of zinc oxide nanoparticles and investigation of their anti-bacterial activities. *Mater. Res. Express.*6:075064. doi: 10.1088/2053-1591/ab19c8

Igile, GO; Iwara, IA; Mgbeje, BI; Uboh, FE; Ebong, PE (2013). Phytochemical, Proximate and Nutrient composition of Vernonia calvaona Hook (Asterecea): A Green-leafy vegetable in Nigeria. *J. Food Res*. 2 (6): 111-122.

Kabera, JN; Semana, E; Mussa, AR; He, X (2014). Plant secondarymetabolites, biosynthesis, classification, function and pharmacological properties. *J. Phar. Pharmacol*. 2:377–392

Kumari, A; Guleria, I; Pathania, DN; Verma, R (2020). Phytochemicals analysis and antibacterial activity of leaf extracts of Populus ciliate. *Med. Plants*. 12 (1): 120-129

Ogwuche, CE; Patience, O; Rakesh, KJ; (2020). Phytochemical, antimicrobial and proximate analysis of the leaves of *mirabilis jalapa* from Uvwie, Delta State, Nigeria. *Int. J. Herb Med*. 8(1): 39-45

Omoregie, ES; Osagie, AU (2011). Effect of Jatropha tanjorensis leaves supplement on the activities of some antioxidant enzymes, vitamins and lipid peroxidation in rats. *J. Food Biochem.* 35 (2): 409-424.

Palombo, EA (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytother. Res*. 20: 717–724

Rishi, AS; Nelson, ND; Goyal, A (2001).Genetic modification for improvement of *Populus. Physiol*. 7: 7–21.

St-Pierre, A; Blondeau, D; Lajeunesse, A; Bley, J; Bourdeau, N; Desgagné-Penix, I; (2018). Phytochemical Screening of Quaking Aspen (*Populus tremuloides*). Extracts by UPLC-QTOF-MS and Evaluation of their Antimicrobial Activity. *Mole*. 16: 23(7):1739.