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Full length paper

Isolation and Characterization of Tyrosol from the Stem Bark of *Gmelina arborea and its Antimicrobial Activities*

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ABSTRACT: The purpose of this study was to isolate and characterize the bioactive constituents of Gmelina arborea stem bark using a spectroscopic method (NMR). The crude extract was separated using silica gel column chromatography with a hexane/ethylacetate solvent system. Different fractions were obtained from column chromatography, but fraction G12 showed significant peaks on the spectra that were characteristic peaks of tyrosol when compared to the compound's literature values. The isolated compound demonstrated high sensitivity to some of the clinical pathogens tested in the study.

Keywords: Steam back, Tyrosol gmelina arorea, antimicropial activities

INTRODUCTION

Plants are known for providing some physiological, biological active ingredients, as well as main structures for the development of modified derivatives with increased activity and lower toxicity. Since the early nineteenth century, various bioactive secondary metabolites have been isolated, characterized, and identified from various parts of plants that have medicinal value for humans. The majority of these active ingredients are modern medicine precursors as well as lead compounds for new drug discovery (Uddin et al., 2011). Medicinal plants are plants that have properties that qualify them as drugs or therapeutic agents and are used for medicinal purposes. (Htay and colleagues, 2019) Medicinal plants can supply biologically active compounds that can be used to create modified derivatives with increased activity and lower toxicity (Ravikumar et al., 2014). Medicinal plants are effective, safe due to no or minor side effects, less expensive, and widely available (Joshi et al., 2017).

Gmelian arborea is a deciduous plant locally known and referred to as Ghamhar. It belongs to family Vertenaceae (Wang, 2004). *Gmelina arborea* is known to different name in different languages like Gomari in Assamese, Gamari, Gambar, Gumbar in Bengali, Shewan, Sivan in Gujarati, Gamhar, Khamara, Khumbare, Servan in Hindi and white teak in English. *Gmelina arborea* originated from Philippines and Malaysia and it is distributed all over India and South China. Bangladesh, Asia countries, Indonesia and broadly in Africa and Latin American countries (Lauridsen and Kjaer, 2002). G. arborea has a 140 cm diameter and a height of about 40 cm.

Table 1: Comparison of observed ^{l}HNMR spectrum of compound G-12 in $CDCl_3$ with reported values (□) of tyrosol.

Positions	Observed Data Hδ (ppm)	Reported Data(Falah et al., 2008) Hδ (ppm)
1	4.94 Ar-4-OH	4.94 Ar-4-OH
2	2.85 (2H, t, 5 = 7.12Hz)	2.82 (2H, t 5 = 6.6 Hz)
3	6.79 (J.8.2)	6.78 (d, J = 8.3Hz)
4	-	-
5	6.90 (J.8.2)	6.78 (J = 8.3)
6	7.03	7.01

The three form is fair to excellent, with a 6-9 m branching crown. The bark is thin and grey in color. Its leaves are 10-25 cm long and 5-18 cm wide, and are simple, opposite, and less heart-shaped. It can appear after leaf fall and has brown flowers grouped in pencilled cymes 15 to 30 cm long. The fruit measures 2.25 cm in length and contains one to four seeds. One kilogram of seeds contains 700-1400 seeds (Evans, 1982, Hossain and Fact, 1999). According to earlier research on this plant, it contains 3,4,5-trimethoxyphenol, lupeol, stigma sterol, antiarol, and beta-sitosterol (Syamsul et al., 2008). In South-South Nigeria, Gmelina arborea is a member of the family with a long history of medicinal use for the treatment of jaundice, piles, diarrhea, dysentery, and other conditions. The current investigation aimed to separate and identify any bioactive substance(s) from the hexane extract of *Gmelina arborea's* stem bark.

MATERIALS AND METHODS

The stem bark of *Gmelina arborea* was collected from Bunu Tai Local Government Area, Rivers State, Nigeria in July, 2022 and identified by David Nwisuator of the Forestry and Environment Department, Faculty of Environmental Sciences, Rivers State University, Port Harcourt, Nigeria. The voucher specimen number 2022: 48 was kept in the Herbarium. The sample was air-dried, pulverized using pestle and mortar and stored in paper bag.

Extraction procedure

400g of the pulverized stem was measured into a clean beaker and 2 litres of n-hexane was introduced and shaken intermittently and then filtered after 48hours, the same was repeated for ethyl acetate. Extracts were concentrated to one-third of the original volume in vacuum using rotary evaporator at about 40^{o}c, this yielded 1.5g (0.77%) of crude ethyl acetate.

Isolation and purification

The hexane and ethyl acetate extract of *Gmelina arborea* were dissolved in a minimum amount of ethyl acetate and hexane solvent system 7:3, preabsorbed in silica gel and subsequently loaded into a column packed with silica gel. Normal phase adsorption chromatography was carried out with gradient elution using different solvent systems of hexane-ethyl acetate (10.0, 9:1, 8:2, 7:3, 6:4, 5:5) in an increasing order of polarity. The solvent system in the ratio 7:3 was used as a basis of the TLC monitoring of the column chromatography which gave an isolate G12 and gave a single homogenous spot on TLC with the solvent system Hexane/Ethyl acetate (9:1). This compound labeled G12 appeared as white powder needle-like crystals and was subjected to spectroscopic analysis.

Spectral analysis

The HNMR Spectra of the isolated compound was obtained using Bruker Advance (400 MHz for ^{l}H) in deuterated chloroform ($CDCl_3$). The antimicrobial analysis was carried by the method according Nna *et al.,* (2019).

RESULTS AND DISCUSSION

The compound G-12 was isolated as a white powder. The ^{l}HNMR spectrum of compound G-12 showed two doublets at δ 6.91 (2H, J=8^{l}16 HZ, 3 and 5-H) and δ 7.09 for para-substituted symmetrical aromatic ring protons, a broad singlet at Ar4-OH at δ 4.94 and two triplets for an Ar-CH_2CH_2-O-moiety at δ 2.83 (2H, J=7.12 Hz, 7-H) and δ 2.85 (2H doublet). The spectrum of this compound showed signal system characteristics for a para-substituted benzene derivative and a hydroxyl ethyl residue. The compound was identified as tyrosol by comparing its spectroscopic properties with available features in the literature (Tables 1-3 and Figures 1-2). The result of the antimicrobial activity assay of the isolated compound as reported in Table 2 and 3 showed that all pathogens under investigation were sensitive to the compound isolated. The sensitivity of the human pathogens

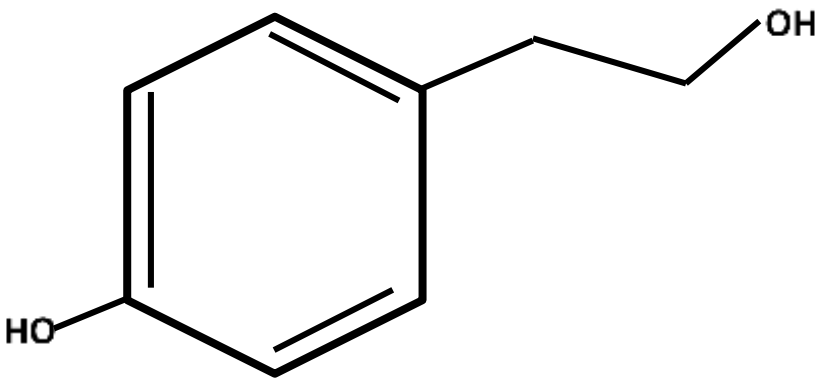

Figure 1: Structure of Compound G-12 (Tyrosol).

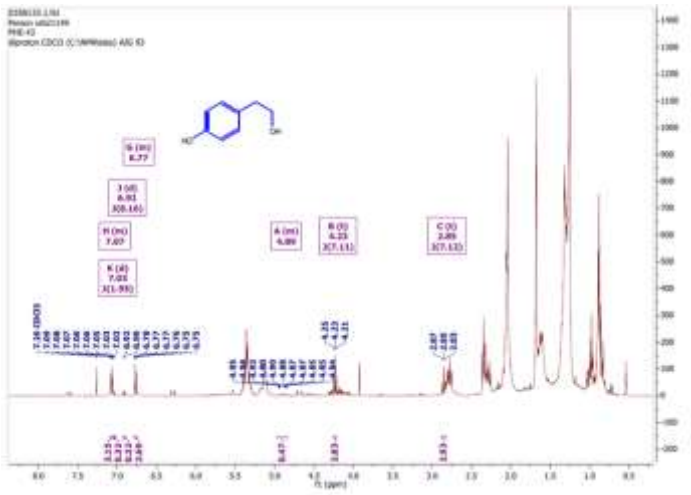

Figure 2: Spectrum of the isolated compound.

Table 2: Zone of inhibition of the isolate G-12 against test organism (mm)/

S/N	Test Organisms	Isolate G-12				Control drug
		X	Y	Z	Mean	Ciprofloxacin
1.	Methicillin resist staph aureus	24	26	26	25(s)	30
2.	Staphylococcus aureus	26	25	28	26(s)	31
3.	Vancomycin resistant enterocci	29	30	28	29(s)	37
4.	Streptococcus pneumonia	27	28	25	27(s)	28
5.	Bacillus subtilis	20	25	23	23(s)	26
6.	Escherichia coli	22	24	22	23(s)	29
7.	Klebsiella pneumonia	30	32	29	30(s)	35
8.	Salmonella typhi	27	25	29	27(s)	32
9.	Proteus mirabilis	23	22	20	22(s)	28
10.	Candida albicans	28	29	32	30(s)	33
11.	Candida krusei	26	28	26	27(s)	28
12.	Candida tropicalis	28	26	25	26(s)	29

Gram-positive (Staphylococcus aureus and methicillin-resistant Staph aureus) suggests that G-12 and the plant could be used to treat skin disorders such as acne, pimples, and seborrheic eczema.

Table 2: Zone of inhibition of the isolate G-12 against test organism (mm).

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6.	Escherichia coli	22	24	22	23(s)	29
7.	Klebsiella pneumonia	30	32	29	30(s)	35
8.	Salmonella typhi	27	25	29	27(s)	32
9.	Proteus mirabilis	23	22	20	22(s)	28
10.	Candida albicans	28	29	32	30(s)	33
11.	Candida krusei	26	28	26	27(s)	28
12.	Candida tropicalis	28	26	25	26(s)	29

Table 3: Antimicrobial activity of the isolate (G-12).

S/N	Test Organisms	Isolate (G-12)	Ciprofloxacin
1.	Methicillin resist staph aureus	S	S
2.	Staphylococcus aureus	S	S
3.	Vancomycin resistant enterocci	S	S
4.	Streptococcus pneumonia	S	S
5.	Bacillus subtilis	S	S
6.	Escherichia coli	S	S
7.	Klebsiella pneumonia	S	S
8.	Salmonella typhi	S	S
9.	Proteus mirabilis	S	S
10.	Candida albicans	S	S
11.	Candida krusei	S	S
12.	Candida tropicalis	S	S

Systemic fungal infections (fungemias), particularly those caused by Candida albicans, have become major causes of morbidity and death in individuals with compromised immune systems (such as AIDS, cancer chemotherapy, organ or bone marrow transplantation). Because pathogens are resistant to the isolated compound, it could be used in the absence of the standard synthetic drug. Candida albicans had the largest inhibition zone (32mm) against G-12, while Proteus mirabilis had the smallest (20mm). The bioassay results indicate that both the plant under study and the isolated compound could be used in place of ciprofloxacin.

Conclusion

Tyrosol was identified as compound G-12 isolated from an ethyl acetate extract of the stem bark of Gmelina arborea. The isolated compound's structure was determined using a spectroscopic approach (NMR) and compared to published literature. The isolated compound's bioassay result indicated that it could be a potential source of drug for the treatment of some diseases caused by the pathogens studied in this study. The sensitivity of Escherichia coli to the isolated compound indicates that it could be used to make anti-fever, anti-pyretic, anti-fatigue, and anti-diarrheal drugs.

The isolated compound has been reported for its anti-oxidant properties, in vitro cell protection against oxidation injury, it may be cardio-protective, and it inhibits the effects of compounds such as oxidized LDL cholesterol. This is the first study to report the isolation of tyrosol, also known as 2-(4-hydroxyphenyl) ethanol, from Gmelina arborea stem bark ethyl acetate.

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