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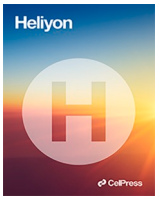

Research article

Phytochemical property and antimicrobial activity of F*icifolius A. Rich* root extract: Advancing Ethiopian indigenous wart curing medicinal plant

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ABSTRACT

Although there may be a number of disadvantages, many patients prefer the traditional medication over surgical wart therapy since it may permanently remove the abscess from the body. The roots of the *Ficifolius A. Rich* plant are one of the native plants used in Ethiopia for traditional wart treatment. Therefore, the main goal of the research was to examine the phytochemical characteristics, identify the chemical compounds, and assess the antimicrobial effectiveness of the previously described plant root extract against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* pathogenic bacteria. Consequently, phytochemical characteristics such as tannins, alkaloids, flavonoids, phenols, and saponin were perceived, which inferred the therapeutic implications of root extracts. Furthermore, gas chromatography-mass spectrometry investigations identified a number of chemical components, including the particular antiviral substance Squalene. Moreover, antibacterial test results showed that the growth of gram-positive and gram-negative bacteria was inhibited with the application of crude extract. Generally, *Ficifolius A. rich* root extract could be effectively utilized for the treatment of anal warts.

1. Introduction

Throughout human history, people have utilized plants as primary sources of medicine and natural treatments all over the world. In developing nations in East and Central Africa like Ethiopia, medicinal plants have been the main source of healthcare services [1]. The field of traditional medicine (TM) encompasses the entirety of information, abilities, and methods derived from indigenous community experiences, beliefs, and theories in many civilizations. Its applications range from the prevention, diagnosis, and treatment of physical and mental ailments to the curing of several diseases [2,3]. Plant extracts are examples of natural materials that provide new opportunities for the development of curative medicines. Approximately 80 % of the world's population relies on herbal treatments, and traditional medicine and medicinal plants are widely employed in most underdeveloped nations as a normative basis for maintaining good health. This is due to the fact that numerous chemical substances found in plants have therapeutic uses for both viral and chronic illnesses [4].

In Ethiopia, there are diverse plants that play a prominent role for traditional herbal medicine practitioners. More than 800 types of Ethiopian-grown medicinal herbs are utilized to treat more than 300 diseases [5]. For instance, Dorsternia barnimiana Schweenf,

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ficifolius A. Rich., and F. Gmel. Euphorbiaceae, Apocynaceae (Agam), Croton macrostachyus, Asteraceae, Euphorbiaceae, Salvadora persica L., Euphorbiaceae, Gladiolus schweinfurthii, and Musa sapientum L. are all indigenous plants utilized traditionally for the curing of anal warts [6,7]. The human papillomavirus (HPV) is the source of anal warts that affect the interior and exterior areas of the anus. Annually, more than 2 % of general people and 6 % of children are caught by these infections [8]. Previous literature reported that HPV is inhibited by naturally occurring plant extracts because of the presence of various bioactive compounds [9,10].

Ethiopian traditional medicines are numerous, and they are prepared and used in different manners. And it is favored by most societies over surgical therapies because of many merits, such as reduced cost, the availability of medicine, minimal risk of side effects, and works for complicated and chronic ailments, are a few examples that could show the tip of the iceberg. Even though these medicines have tremendous advantages for society, a detailed investigation is still crucial to avoid the drawbacks that come along with them, like not being effective for all conditions, dosage problems, medication interaction, the risk involved in acquiring wild herbs, and other critical issues. As a result, traditional medicine must be researched by adhering to scientific protocols for the essential components of native plants as anal wart curatives. Therefore, the purpose of this study was to investigate the traditional anal wart curing plant extract, *Ficifolius A. Rich* root, in terms of phytochemical screening, chemical composition identification, and anti-microbial activity test evaluation on both gram-positive and gram-negative bacteria.

2. Materials and methods

2.1. Materials

Ficifolius A. Rich root was collected in Mersa, commonly called Mersa-Abagetye, North Wollo, Ethiopia, with 11°40′N latitude and 39°39.5′E longitude. A pair of pathogenic bacteria was chosen for the ant-microbial activity test, namely gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*. These microorganisms were obtained from the Amhara Public Health Institute—Dessie Branch Clinical Bacteriology and Mycology Laboratory, located in Dessie City, Ethiopia. Muler-hinton agar medium (MHA), N-hexane (99.9 %) and ethanol (97 %) as an extractive solvent. Main equipment's like a Soxhlet extractor and GC-MS were used during the study. All other chemical and equipment lists are supplied in the supplementary material.

2.2. Methods

2.2.1. Sample preparation and oil extraction

The collected roots of *ficifolius A. Rich* were dried in an oven at 110 °C for 4 h to facilitate size reduction. Then, the materials were crushed with grinder and sieved to 0.5–1 mm and 1–1.8 mm, followed by oil extraction using ethanol and n-hexane solvent in a soxhlet apparatus at a temperature of 78 °C and 68 °C for 3 h, respectively. Finally, the crude extract and solvent separation were made by a rotary evaporator. The overall pictorial outlook of the crude extracts is shown in Fig. S1. The yields of the crude oil were computed with equation (1).

$$\%\ \boldsymbol{yield} = \frac{mass\ of\ oil\ extracted}{initial\ mass\ of\ the\ solid\ sample\ used} * 100 \quad (1)$$

2.2.2. Phytochemical screening

The qualitative phytochemical test of the crude extract was implemented to confirm the presence or absence of major classes of compounds applicable to anti-microbial quality as shown in Fig. S2, such as saponins, steroids, terpenoids, triterpenoids, flavonoids, phenols, tannins, and alkaloids, by following standard techniques [3,11–13].

2.2.2.1. Terpenoids. 2 mL of chloroform, 0.5 mL of crude extracts, and 3 mL of concentrated H_2SO_4 were added to a test tube. Then, the presence of terpenoids was attested with reddish brown color formation.

2.2.2.2. Steroids and triterpenoids test. Three drops of crude extracts were treated with 5 mL of $CHCl_3$ and filtered. Then, the filtrate was shaken well by adding 5 drops of concentrated H_2SO_4 and allowed to stand for 5 min. The appearance of a red color in the lower layer indicated the presence of **steroids**. The presence of triterpenoids was confirmed by the formation of a reddish-brown color at the interface while adding concentrated H_2SO_4 without any shaking.

2.2.2.3. Saponins test. The formation of stable froth was observed by mixing and shaking 0.5 g of crude extract with 5 mL of distilled water for 15 min. The appearance of foam indicates the presence of saponin.

2.2.2.4. Alkaloids test. **Hager's Test:** Three drops of crude extract were treated with five drops of Hager's reagent. The presence of alkaloids can be confirmed if a yellow precipitate is formed.

Dragidroff test: 0.5 g of crude extract and 5 g dragindroff reagents are mixed in the test tube, and then the reddish brown precipitate or turbidity assures the existence of alkaloids.

Mayer's test: Mixing 0.5 g of crude extract and 5 g of mayer reagents in the test tube would result in a cream color, opalescens, or yellowish if alkaloids are present.

2.2.2.5. Flavonoids test. **Lead acetate solution Test:** treating 0.5 mL of crude extracts with 5 drops of 10 % lead acetate solution to indicate the presence of flavonoids identified by the formation of a yellow precipitate.

2.2.2.6. Tannins test. **$FeCl_3$ test:** The presence of tannin is identified by its blue-green, bluish-black, and greenish color when 0.5 mL of crude extracts and a few drops of 5 % $FeCl_3$ are mixed.

Acid anhydride test: The existence of tannin was tested by blending 3 mL of crude extracts, 1 mL of acid anhydride, and 1 mL of concentrated H_2SO_4 to form a green colour.

2.2.2.7. Phenols. The occurrence of phenolic chemicals was confirmed by the formation of a bluish-black or dark green color when a few drops of 5 % $FeCl_3$ solution were added to 2 mL of the crude sample.

2.2.3. GC-MS analysis of extract

Identification of chemical compounds with their names present in the extract were identified using a Hewlett Packard Mass Hunter GC-MS system model 5977 equipped with a medium-polarity capillary column (BPX-5) and an MS fused silica capillary column (30 m $\times$ 0.25 mm, film thickness 0.25 μm). For spectroscopic detection, an electron ionization system with ionization energy of 70 eV was used. Pure helium gas (99.999 %) was used as a carrier gas at a constant flow rate of $\pm$1 mL/min. The mass transfer line and injector temperature were set at 200 °C and 300 °C, respectively. The oven temperature was programmed from 40 °C to 150 °C at 3 °C/min, then held isothermal for 10 min, and finally raised to 300 °C at 10 °C/min 1 μL crude extract sample was injected in split mode with a split ration of 120:1. Using electron ionization at 70 eV, mass fragments between 40 and 500 *m/z* were found using mass spectrometry. Observe that the detector activated after 5 min. The relative percentage of the chemical constituents in crude extracts from *ficifolius A. Rich* was expressed as a percentage by peak area normalization [14,15].

2.2.3.1. FT-IR. The IR spectrum of each sample was recorded in triplicate using the FTIR (Nexus 870, Thermo Nicolet) (ATR equipment running 32 scans per spectrum). The spectra were collected and processed using the Omnic software. Various indices were calculated using ratios generated from peak areas rather than peak heights.This procedure is more reliable as it takes into consideration several vibrations of the same type occurring simultaneously (Permanyer et al., 2001).Table 1 shows some of the indices calculated and the formulae used. FT-IR.The IR spectrum of each sample was recorded in triplicate using the FTIR (Nexus 870, Thermo Nicolet) (ATR equipment running 32 scans per spectrum). The spectra were collected and processed using the Omnic software. Various indices were calculated using ratios generated from peak areas rather than peak heights.

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2.2.4. In vitro antimicrobial activity tests of extract

To evaluate antibacterial activity, pathogenic bacteria, namely *Staphylococcus aureus* (1 mg/ml) as a gram-positive and *Escherichia coli* (1 mg/ml) as a gram-negative bacterium, were selected using the agar-well diffusion method at a 1 mg/mL concentration of crude extract. To make *Muller-Hinton agar medium* (MHA), their guidelines were followed. Then, the bacterial suspension was spread on MHA medium. Wells were bored on this agar medium using a sterile cork borer of 6 mm diameter. In a strategic setup, 5 μg of the standard drug (*ciprofloxacin*) was positioned at the plate's center. Subsequently, 50 μL of the 1 mg/mL extract and 50 μL of *dimethyl sulphoxide* (the negative control) were meticulously dispensed into the wells for diffusion, with a designated 30-min rest period to ensure proper diffusion. The plates were then incubated aerobically at 37 °C for 24 h, and the clear zone of growth inhibition around the well was measured the diameter of zones of inhibition (in mm), and the experiment was performed in triplicate [16].

Table 1
Phytochemical Screening tests of *ficifolius A. Rich* extracts.

Test	Test result	Sign indicates
Saponins	++	high presence
Terpenoids	–	Absent
Triterpenoids	–	Absent
Steroids	–	Absent
Alkaloids	+	Presence
Flavonoids	+	Presence
Phenols	+	Presence
Tannins	++	high presence

3. Results and DISCUSSION

The maximum yield of *ficifolius A. rich* root extract (24.45 %) was obtained at a particle size of 0.5–1 mm with n-hexane solvent, since smaller particle sizes are essential for better mass transfer and improved efficiency of the extractive solvent compared to ethanol and a larger particle size (22.15 %). The results of phytochemical test, chemical composition, and antimicrobial evaluation of the crude extract are indicated hereunder.

3.1. Phytochemical screening tests of extract

Table 1 displays the test results of the phytochemical properties of *ficifolius A. rich* root extract that confirm the presence of saponin, alkaloids, flavonoids, phenols, and tannins, which are the main phytochemical ingredients available in medicinal plants and they are mostly found in non-nutritive plants that have the ability to prevent or treat disease [3]. More pictorial illustrations are supplied in figure s2.

3.2. In vitro antimicrobial test of ficifolius A. Rich root extracts

The antibacterial activity evaluation results showed that the *ficifolius A. rich* root oil effectively inhibited the growth of gram-negative and slightly inhibited gram-positive bacteria. Table 2 showed that the diameter of inhibition zones (mm) for gram-positive and gram-negative bacteria was 6.67 and 10.67, respectively (refer to Fig. S3). In this study the *ficifolius A. rich* root crude oil have a great potential as an anti-microbial activities in the treatment of infectious disease. The plant extract's significant anti-bacterial qualities are indicated by the concentration at which it inhibits test bacteria. This provides credence to earlier research in the literature showing that crude extracts had effective antibacterial activity. Furthermore, it has been reported that crude extracts of spices contain a wide range of different chemical compounds, including phenolic compounds and their derivatives, fatty acids, terpenes, and esters of weak acids. As a result, these chemical components have the ability to affect multiple target sites against bacterial cells [17].

3.3. 3.3. GC-MS analysis

To identify the chemical compounds and their total ion chromatogram (TIC) mass spectral peaks of *ficifolius A. Rich* root extract, gas chromatography coupled to mass spectrometry analysis was implemented as shown in Table 3 and Fig. 1, respectively. Accordingly, over 17 typical chemical compounds were detected based on their peaks integral values associated with retention time. All of these compounds have their own medicinal properties for curing specific diseases, for instance, Neophytadiene (for treatment of headache, rheumatism, and skin diseases), 2-Pentadecanone, 6,10,14-trimethyl (have anti-bacterial, anti-nociceptive, and anti-inflammation activities), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-(anti-inflammatory compounds), Eicosane (have strong anti-inflammatory, analgesic and anti-pyrentic effects), and Squalene is used as anti-viral and anti-cancer therapy [18–22]. Previous reports showed that squalene is also significantly decrease the mortality rate of COVID-19 [23]. Basically, warts are caused by the human papilloma virus (HPV), and thus the particular curing anti-viral compound (squalene) was perfectly detected.

4. Conclusion

In the present research, it can be noted that *ficifolius A. rich* plant root extract has medicinal properties that can be used for wart treatment. The maximum yield of crude extract (24.45 %) was obtained with n-hexane solvent at a particle size of 0.5–1 mm. The phytochemical screening of the extract showed the presence of chemical components like saponin, alkaloids, flavonoids, phenols, and tannins, which proved the medicinal property of *ficifolius A. rich* root extract. In addition, the principal chemical compounds having medicinal activity have been identified with gas chromatography-mass spectrometry analysis. Moreover, antibacterial activity evaluations were performed on both pathogenic bacteria, namely *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative). The test results confirmed that inhibition zones on the growth of both bacterium types have been observed, which implies that the antibacterial potential of the crude extract has a substantial contribution to the treatment of human papillomavirus.

Table 2
Result of inhibition zone measurement for *ficifolius A. Rich* extracts.

Plant Extracts	Extract's Conc. (50 μL)	Type of Bacteria	Diameter of inhibition zones (mm)
			Mean ± SD
crud extract	1 mg/mL	*S. aureus*	6.67 ± 1.15
		E. coli	10.67 ± 1.15
+ve Control	(5 μg Cip.)	*S. aureus*	28.67 ± 0.57
		E. coli	38.00 ± 1.00
-ve Control	(50 μL DMSO)		0

Keys: *S. aureus* = *Staphylococcus aureus, E. coli* = *Escherichia coli,* Cip. = Ciprofloxacin, DMSO = *Dimethyl Sulphoxide*, SD = Standard Deviation.

Table 3
GC-MS analysis of chemical compounds of *ficifolius A. Rich* plant extract.

PK	Library/ID	formula	RT	Area,%
1	2,4-Di-*tert*-butylphenol	$C_{12}H_{22}O$	8.9916	1.2865
2	Neophytadiene	$C_{20}H_{38}$	10.5999	5.4431
3	9-Octadecyne	$C_{18}H_{34}$	10.8522	0.8359
4	Neophytadiene	$C_{20}H_{38}$	11.1116	1.7662
5	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{38}O$	11.5926	1.6666
6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	12.2137	8.2882
7	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	14.9745	1.6391
8	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	15.0609	4.9465
9	Methyl stearate	$C_{19}H_{38}O_2$	15.2058	2.2156
10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	15.3474	9.2726
11	Neophytadiene	$C_{20}H_{38}$	15.5845	30.5904
12	1-Tetracosene	$C_{24}H_{48}$	18.7116	1.4795
13	Eicosane	$C_{20}H_{42}$	22.4857	16.6472
14	1-Tetracosene	$C_{24}H_{48}$	22.8639	6.1367
15	Docosanoic acid, methyl ester	$C_{23}H_{46}$	23.0458	1.7613
16	Eicosane	$C_{20}H_{42}$	24.7263	3.1516
17	Squalene	$C_{30}H_{50}$	27.8691	2.8729

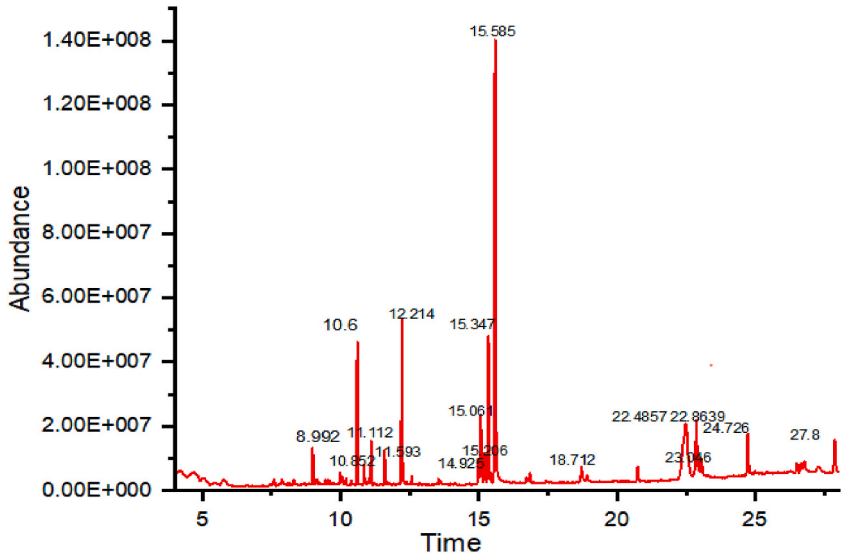

Fig. 1. *ficifolius A. Rich* root oil GC-MS chromatogram presenting the chemical components separation.

Supplementary information

It describes the details of chemical and equipment lists, photographic outlooks of phytochemical screening, Anti-microbial test, and oil yield.

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Data availability statement

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CRediT authorship contribution statement

Yassin Adem: Writing – original draft, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **kedir Yesuf:** Supervision, Resources, Methodology, Investigation, Data curation. **Solomon Getachew:** Writing – original draft, Software, Methodology, Investigation, Funding acquisition. **kedir Derbie:** Writing – review & editing, Supervision,

Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31921.

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