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Aderaw Anteneh (aderaw1234org@gmail.com)
Jigjiga University
Getachew G/Mariam W/Hana
University of Gondar
Desta Shumuye Meshesha
University of Gondar

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Preliminary Phytochemical Screening, Isolation, Characterization, Structural Elucidation and Antibacterial Activities of Leaves Extracts *Rhus vulgaris* (Kimmo)

Aderaw Anteneh Belew[1†], Getachew G/Mariam W/Hana[2], Desta Shumuye Meshesha[2]*

[1]Department of Chemistry, College of Natural and Computational Sciences, University of Gondar, P.O. Box 196, Gondar, Ethiopia

[1†]Department of Chemistry, College of Natural and Computational Sciences, Jigjiga University, P.O. Box 1020, Jigjiga, Ethiopia

[†]Current address

**Corresponding author email: aderaw1234org@gmail.com*

ABSTRACT

Background: *Rhus vulgaris* commonly known as sumac, a plant that is known to possess different therapeutic values including antioxidant and antibacterial activities. Medicines from plants contributed largely to human health. The aim of this study was to screen the phytochemical constituents, isolate, elucidate the structure and antibacterial activity of methanol extract from the leaves of *Rhus vulgaris*.

Methods: The methanolic extract of *Rhus vulgaris* was subjected to column chromatography and eluted with solvent mixture of methanol: chloroform (1:8) ratio. The eluted fractions were run in the TLC mobile phase with the different solvent ratio. Based on the TLC profile the fractions with similar Rf values were pooled together. The structure of the isolated compound was characterized based on the spectral data (IR, ^{1}H NMR, ^{13}C NMR, and DEPT) and extracts from *Rhus vulgaris* has been shown to have antibacterial activity were tested against four strains bacteria

*Streptococcus aureus(*gram-positive*)* and *Escherichia coli, Salmonella typhimurium*, *and K. pneumoniae (*gram-negative) using Agar well diffusion method.

Result: The results showed that the methanol extracts were active against all the tested bacteria. The structure of this compound 1-p-tolyl pentadeca-7,9-dien-1-ol was characterized by means of 1H NMR, 13C NMR, and IR spectral data.

Conclusion: Therefore, it is concluded that the use of herbal plants and their recipes are the major source of drugs in a traditional medicinal system to cure different diseases.

Key word: Isolation, Structural Elucidation, Phytochemical screening, Antibacterial activities, *Rhus vulgaris*

1. Introduction

Plants have been used to treat a wide range of diseases throughout the history of human beings and this practice continues to date. This is mainly because most of these herbals are accessible, affordable and the extracted chemicals have little or no side effects as compared to drugs synthesized in the laboratory. Plants comprise the largest component of the diverse therapeutic elements of traditional health care practices both in humans and animals. The medicinal values of plants are due to the chemical substances that produce a definite physiological action on the human body and are called phytochemicals[1,2].

Natural products as the term imply 'naturally occurring compounds that are the end products of secondary metabolism and they are unique compounds for particular organisms or classes of organisms[3]. Natural products therefore continue to play a crucial role in drug development as they account for almost 50% of new chemical entities in drug discovery and hence providing a

starting point for new synthetic drugs[4]. The value of medicinal plants in drug discovery is known to us well and human being have used them for various purposes from the beginning of human history. Traditional folk remedies from plants have always guided scientists to search for new medications in order to maintain and promote healthy life for humans and animals [5].

Sumac is the common name for a genus (*Rhus*) that contains over 250 individual species of flowering plants in the family Anacardiaceae [6], which occur mainly in the tropics, subtropics and temperate areas of the world. The sumac name is derived from "sumaga", meaning red in Syriac .In general, Rhus species can grow in non-agricultural regions and various species have been used by indigenous people for medicinal and other purposes, suggesting a potential for commercializing the bioactivity of these plants without competing for food production land uses [7].

Previous studies on phytochemical investigation of the stem bark of *Rhus vulgaris* revealed the presence of secondary metabolites such as tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids and phenol. *R. vulgaris* methanolic extract (1000 mg/kg) showed greater anti-inflammatory activity compared to indomethacin (10 mg/kg), the standard anti-inflammatory drug, with a decrease in inflammation for up to 90 min. The dichloromethane, ethyl acetate and aqueous extracts of *R. vulgaris* stem bark, root and leaves have exhibited moderate to toxic toxicity against brine shrimp with LC50 values ranging from 3.55 μg/ml to 734.06 μg/ml while cyclophosphamide, the positive control, demonstrated an LC50 value of 15.28 μg/ml [8].

Several reports describe isolations of new biflavonoids on the genus *Rhus*. Such as agathisflavone, amentoflavone, hinokiflavone, *rhus* flavanone and succedanea flavone has been sourced from *Rhus* species and evaluated for activity against a range of pathologically significant viruses. In

another study, hinokiflavone was found as the most active among 65 natural flavonoids to inhibit the pro-coagulant activity of adherent human monocytes stimulated by endotoxin and interleukin-1-β in vitro. Other *Rhus* biflavonoids have also shown cytotoxic and antimalarial activities[10,11].

In traditional medicine, extracts of *Rhus* species are used to manage several ailments including influenza, wounds, diarrhea, abdominal pain, indigestion, diabetes, malaria, rheumatism, aching gums, toothaches, swollen legs, dog bites, peptic ulcer, kidney stones, skin eruptions, bruises and boils[9,10]. The leaves of R. *vulgaris* are chewing, decoction, eating and steaming, used to treat diarrhea, malaria, highly diuretic, yellow fever, cough, gastro-intestinal disorder, toothache, immunity booster, smallpox swollen lymph, gonorrhea. The roots of this species are used to treat measles, hemorrhoids, syphilis, sterility and antidotes[11–14].

Traditionally, people in Ethiopia have used medicinal plants to treat different diseases and this has made a great contribution in primary health care systems. *Rhus vulgaris* is one of those ethnomedicinal plants that have been commonly visited by traditional healers in most parts of Ethiopia. The Leaves of *Rhus vulgaris* have been widely used by the local people for the treatment of different alignments including wound , lung TB , evil eye diarrhea, gonorrhea and inflammation in Ethiopia [15].

Further, the paper aims to isolate possible biological active compounds from the leaf of Rhus vulgaris by using various chromatography techniques such as column chromatography and preparative thin layer chromatography. The structure of the active component was elucidated by means of spectroscopic techniques (IR and NMR). Beyond this, the bioactivity of Rhus vulgaris leaves against different pathogens was checked.

1.1. Description

Rhus vulgaris is found in all parts of Tanzania; Uganda and Kenya and from Cameroon to Ethiopia and south to Mozambique, Malawi, Zambia and Zimbabwe[16]. It is a shrub or small tree that occasionally reaches 1-9 m; bark smooth, dark brown, branches yellow-red-brown, often densely hairy. Leaves are 3 leaflets, dull green, softly hairy, the central leaflet larger, 4.11 cm long x 2.6.5 cm wide, the two laterals smaller, shortly stalked, edge entire or soft toothed towards the tip, which is blunt or pointed, leaflets dark above, paler below. Flowers are small cream-green-yellow, parts in fives, in terminal loose heads or from upper leaf axils, 5.20 cm long, all densely hairy. Fruits are drupes, with thin flesh, flat and round, red-brown, only 3.5 mm across[17].

1.2. Taxonomic Classification

According to the international code of botanical nomenclature, the present taxonomic classification of *Rhus vulgaris*:

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Anacardiaceae
Genus	*Rhus*
Species	*Rhus vulgaris* Meikle

Synonyms

: - *Searsia pyroide* (Burch.) Moffett

: - *Rhus pyroide* (Burch.) [15]

2. Material and Methods

2.1. Reagents used

The chemicals and reagents that were used in the study include: Sulphuric acid, acetic acid ,methanol, ethanol, n-hexane, chloroform, acetone, ethyl acetate, DMSO, Vanillin solution (15g of vanillin in 250 ml of ethanol and add 2.5 ml of conc. H_2SO_4), acetic anhydride, ferric chloride solution, NaOH, concentrated hydrochloric acid, silica gel (60-200 Mesh size), Sodium bicarbonate, ammonia, distilled water, I_2/KI, Mayer's reagent, Benedict's reagent, Ninhydrin, Molisch's reagent, Millon's reagent, and lead acetate are more relevant to make this experiment.

2.2. Apparatus/Equipments

The apparatus and instruments that were used for the study include

- Test tubes
- Measuring cylinders
- Glass stirrer
- Pyrex beakers
- Conical flasks
- Volumetric flasks
- Fisher Scientific heating mantle
- Rotary evaporator
- Inoculating loop
- Autoclave
- Separating funnel
- Buchner's funnel
- Whatman`s funnel
- Thin Layer Chromatographic plates

2.3. General experimental procedures

The isolate was mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FT-IR spectra were recorded in the range 400- 4000 cm^{-1} in FT-IR spectroscopy (Bruker FT-IR Spectrometer, USA).

Nuclear Magnetic Resonance Spectroscopy: The ^1H, ^{13}C-NMR and 2D NMR spectra of base degradation impurities were recorded in DMSO-d6 solvent on Bruker 400 MHz Avance -III HD

NMR spectrometer equipped with broadband observe (BBO) probe. The 1H and 13C chemical shifts are reported on the δ scale in ppm, relative to tetramethyl silane (TMS) as an internal standard. The spectra were set to δ 0.00 ppm in ^{1}H NMR (TMS) and δ 39.50 ppm in ^{13}C NMR (DMSO-d6).

2.4. Collection of plant:

The fresh and healthy leaves of *R. vulgaris* were collected from a local farm in the Region of Amara; Central Gondar Zone in Takusa Woreda Kebele 12 in February 2020. The plant was taxonomically identified by botanist Mr. Getenet Chekol (MSc) at the Department of Biology, University of Gondar.

The plant materials were washed thoroughly with tap water to remove dust particles from the leaves. The collected plant material was dried under a shade placed at room temperature after collection for 10 days. The shaded dried health leaves were powdered using an electrical grinder in uniform powdered size, weighted and then the powdered samples were kept in a sealed container /bag/ for extraction purpose.

Figure 1:The photograph of Rhus vulgaris Leaves.

2.5. Preparation of extracts:

Extraction and isolation of organic compounds found in the leaves of *Rhus vulgaris* were done by using maceration method. The maceration method is more preferable for exhaustive extraction of *R.vulgaris* leaves to reduce any possibility of thermal decomposition of any thermolabile compounds that may be present [18].

The powdered leaves (800g) were macerated sequentially with solvents of increasing polarity starting with n-hexane (C_6H_{14}) 2000mL, chloroform ($CHCl_3$) 2000mL and finally methanol (CH_3OH) 2000mL. Each extraction involved maceration for about 72 hours. Occasional shaking was done to ensure thorough extraction. The extracts were decanted and filtered using Whatman filter paper. The extracts were concentrated using a rotary evaporator at temperatures n-hexane (69℃), chloroform (61℃) and methanol (65℃). The crude extracts were weighed by using the digital balance and obtained n-hexane (4.35 g), chloroform (14.7 g) and methanol (100.7g) crude extracts. Out of the three crude extracts, the methanol extract showed better antibacterial activities and was further subjected to column chromatography, mixture of suitable solvents methanol: chloroform (1:8) which chosen by a number of trials with the help of TLC to isolate the major component as indicated in **Table 1** below.

2.6. Column Chromatographic

The methanol extract was subjected to silica gel (60-120 mesh ASTM, Merck) glass column chromatography (20-25 mm diameter). Briefly, silica gel (150 g) was mixed with chloroform to form a homogenous suspension/slurry and stirred using a glass-stirring rod to remove bubbles. The silica gel slurry was then poured into a glass column. The sample to load on the column was prepared by dissolving 6g of the extract in 40 ml of methanol. To the solution, 10 g of silica was

added and mixed by stirring with a glass rod. The mixture was allowed to dry at room temperature. The dried silica extract mixture was layered on the column layer bed. The column was first eluted with methanol: chloroform in a ratio of 1:8 as the mobile phase and allowed to run until it reached a consistent flow.

A total of 59 fractions were collected. The column fraction's profiles were monitored by TLC to confirm the similarities of elutes based on the number and the spots on the plate were visualized by using UV-lamp, vanillin solution. Hence the eluted compound was crystallized and subjected to Nuclear Magnetic Resonance (NMR) analysis (^{1}H-NMR, ^{13}C-NMR, DEPT- 135) and Infra-Red spectroscopy (IR) studies to elucidate the structure of the compound isolated from this fraction of column eluted sample. dark green gummy.

Table 1:Solvent system used to separate AA1.

No.	Solvent	Solvent Ratio	No. fractions	Code of Fraction	Rf value
1	$MeOH:CHCl_3$	1:8	1-3	A_1	0.9
2	$MeOH:CHCl_3$	1:8	4-16	A_2	0.85
3	$MeOH:CHCl_3$	1:8	17-45	A_3	0.70
4	$MeOH:CHCl_3$	1:8	46-56	A_4	0.75
5	$MeOH:CHCl_3$	1:8	57-59	A_5	0.8

2.7. Preliminary Phytochemical Screening

Phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and coloration to identify the major natural chemical groups such as alkaloids, phenolic compounds, glycosides, carbohydrates, flavonoids, saponins, terpenoids, anthraquinones, tannins, steroids, amino acids, coumarins, and proteins. General reactions in these analyses revealed the presence or absence of these compounds in the

crude extracts tested. Crude extracts of the plants previously prepared and stored in a refrigerator were used for the phytochemical tests.[19–23].

2.8. Antibacterial Activities of the Leaves extracts of *Rhus vulgaris*

The test organisms that were used to check the antimicrobial activity of the crude extract were *Streptococcus aureus(*gram-positive*)* and *Escherichia coli, Salmonella typhimurium*, *and K. pneumoniae (*gram-negative) bacteria. The antibacterial activity of crude /purified extracts was determined by using agar well diffusion assay. The media used was Mueller Hinton Agar (MHA), it was prepared according to the manufacturer's instruction, where 19 g of media powder was dissolved in 500ml of distilled water and enclosed in a container and autoclaved at 121℃ for about 15 minutes to sterilize the media. The media were allowed to cool and pour in twelve plates and put on the leveled surface. The media were allowed to solidify, kept in the upright position in the incubator avoiding contamination from the hood.

All the cultures were growing in Mueller Hinton Agar (MHA). The inoculums were used for antibacterial assay. Colonies of fresh cultures of the well separated from overnight growth was picked with sterile inoculating loop and suspend in 3-4 ml physiological saline contain in sterile test tubes, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard giving a bacterial load about 1x108 CFU [45].

Antibacterial activities of *R. vulgaris* extracts were tested against four strains of bacteria using the agar well diffusion method. The test cultured bacteria were swabbed on the top of the pre-leveled media (about 45-50ml) and allowed to dry for 10 minutes. The sterilized well borer (6mm diameter) was used to bore holes on the plates and 100 mg/ml of each extract (n-hexane, chloroform and methanol) were dissolved by DMSO. Gentamicin disc (30 mcg/disc) will be used

as positive antibiotic control. The Petri dishes were then incubated at 37°C for 24 hrs. After incubation the zone of inhibition was measured and recorded the average inhibition zone in millimeters.

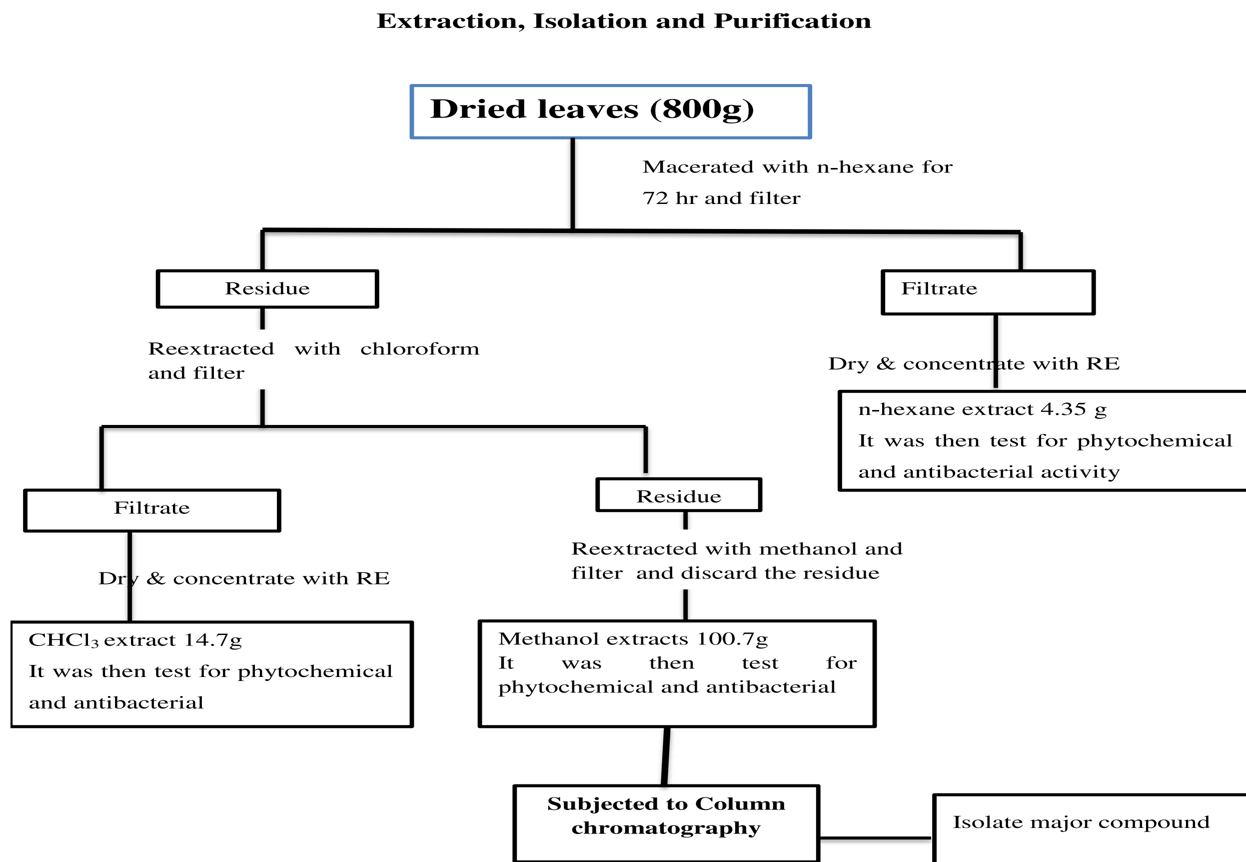

Figure 2:Method of extraction of the plant material.

3. Results and Discussion

3.1. Yield of solvent extract and isolation of Leaves of *Rhus vulgaris*

The dried and powdered Roots (800 g) of *Rhus vulgaris* subjected to exhaustive extraction successively with n-hexane, chloroform and methanol. The solvent from each extract was recovered under reduced pressure using a rotary evaporator to obtain a *n-hexane (*4.35g),

chloroform (14.7g) methanol extract (100.7g). Chromatographic purification of the methanol extract (7g) yielded a compound coded; AA_1. The structure of this compound has been elucidated on the basis of spectroscopic evidence as described in the following section.

Table 2:Percentage Yields of the Extracts.

Extracts	Weight	Yield (%)
n-hexane	4.35g	0.54
Chloroform	14.7g	1.84
Methanol	100.7g	12.6

3.2. Phytochemical screening of the Leaves Extracts of *Rhus vulgaris*

The phytochemical analysis of each crude extract of *Rhus vulgaris* revealed the presence of pharmacologically useful classes of secondary metabolites. Alkaloids, Glycosides, Steroids, Anthraquinones, and Carbohydrates were present in all extracts. Phenols, Flavonoids, Tannins, Coumarins, and Proteins were present only in methanol extracts while Terpenoids, Saponins and Amino acid were absent in all the extracts were summarized in **Table 3**.

Table 3: Phytochemical constituents of the leaves extract of Rhus vulgaris.

Phytochemical constituent	Hexane	Chloroform	Methanol
Alkaloids	+	+	++
Phenols	-	-	++
Glycosides	+	+	++
Flavonoids	-	-	++
Terpenoids	-	-	-
Steroids	+	+	+

Carbohydrates	+	+	+
Anthraquinones	+	+	+
Proteins	-	-	+
Coumarins	-	-	+
Tannins	-	-	+
Saponins	-	-	-
Amino acid	-	-	-

Key: + = Present, - = Absent

3.3. Antibacterial Activity of the Crude Extracts against Bacterial Strain

The antibacterial property of *Rhus vulgaris* extract using different solvents showed varying degree of response towards the selected pathogens (**Table 4**). In this work three samples were tested by the above method. The results showed the crude methanol extract was strongly active while the pure n-hexane and chloroform extract was not active at all against the *S.typhi* and *K.pneum* pathogen.

Table 4: Antibacterial efficacy of extracts against pathogens

Extract	Inhibition zone(mm) against			
	S.aureus	*S.typhi*	*K.pneum*	*E.coli*
MeOH	15	14	11	13
$CHCl_3$	7	0	0	7
n-hexane	3	0	0	2
Ge	18	17	14	15
DMSO	0	0	0	0

In this work two samples were tested by the above method. The results showed the crude methanol extract was strongly active while the pure n-hexane and chloroform extract were not active at all against the *S. typhi* and *K. pneum* pathogen. The chloroform and n-hexane

extract of *S. aureus and E. coli* are active against. The outcome of this study has shown that the leaves extract of *Rhus vulgaris* possesses inhibitory potential against *S. aureus* while *S. typhi* and *K. pneum* were partially resistant to their activity.

Generally, the extracts showed greater antibacterial activity against gram-positive as compared to gram-negative bacteria. With respect to individual pathogens, the methanol extract showed greater inhibition than chloroform and n-hexane extract.

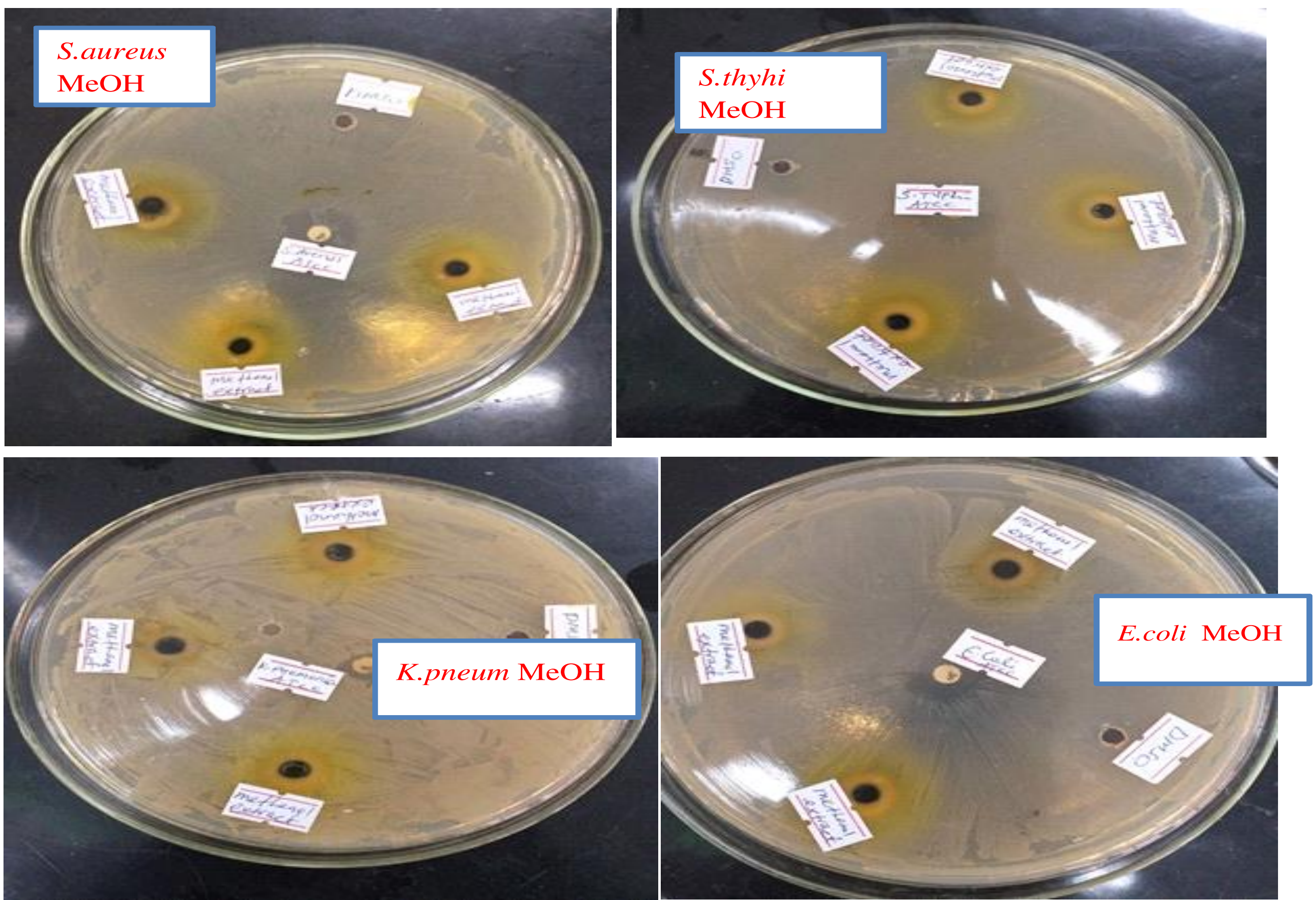

Figure 3: Zone of inhibition of MeOH compared with an inhibition zone of standard antibiotic Gentamicin on S.aureus, S.typhi, K. pneumoniae and E.coli.

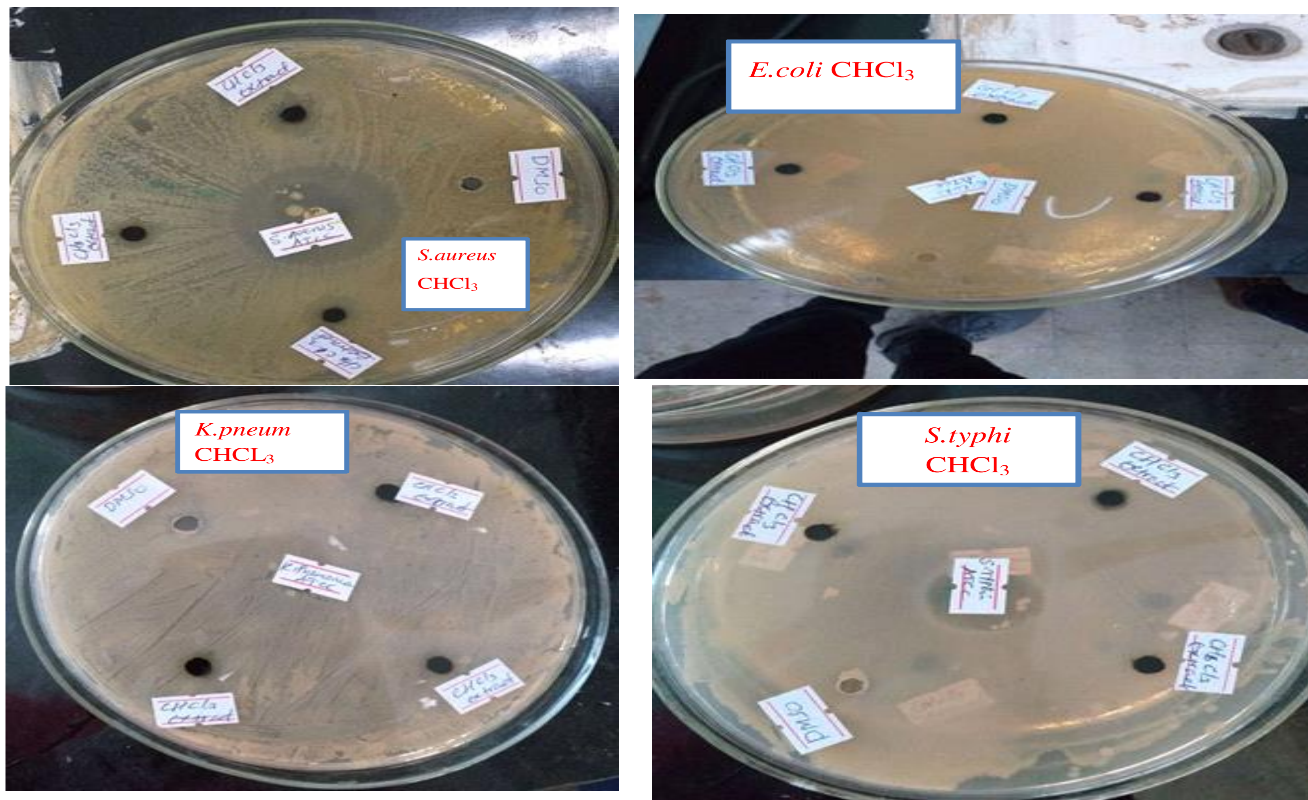

Figure 4: Zone of inhibition of chloroform compared with an inhibition zone of standard antibiotic Gentamicin on S. aureus, S. typhi, K. pneumoniae and E. coli.

3.4. Characterization of Compounds

In order to characterize the compounds isolated from the leaves of *Rhus vulgaris* the R_F value, and spectroscopic data of the compounds were utilized.

Partial Characterization of AA_1

Compound AA1 was dark green gummy material isolated from the leaves of *Rhus vulgaris*. It was detected as a pink spot under a UV lamp and became yellow when sprayed with 4% vanillin H_2SO_4. Its R_f value was 0.70 in Methanol/ Chloroform (1:8).

FTIR Spectral Analysis

The FT-IR spectrum of compound AA_1 (**Fig.5**) afforded absorption band at 3454 cm^{-1} indicating the presence of -OH group. On the other hand, the bands 3000 cm^{-1}, 2912 cm^{-1} ,2882 cm^{-1},1637 cm^{-1} show the stretching of aliphatic =C-H, CH_2, CH_3 and C=C bending respectively. The band that raises at 1464 cm^{-1}, 1355 cm^{-1}, and 1078 cm^{-1} can be assigned to CH_2 bending, CH_3 bending and C-O stretching vibrations respectively.

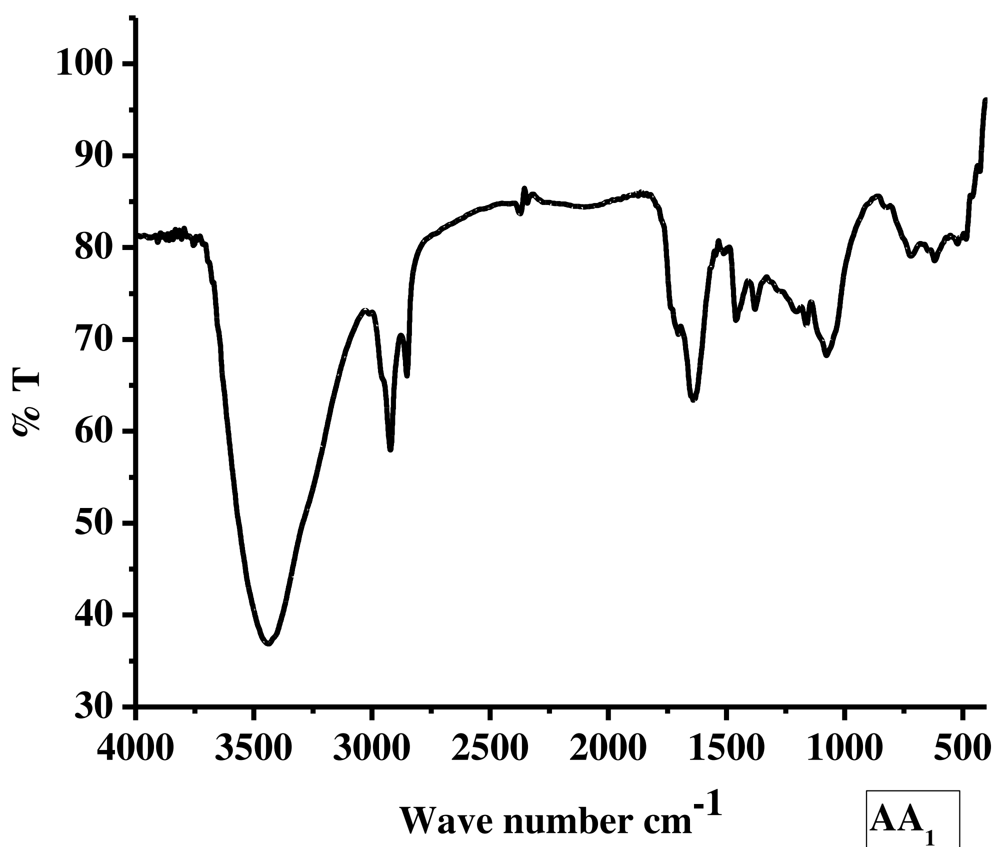

Figure 5: FT-IR Spectrum of compound AA1.

^{1}H-NMR Spectrum

The ^{1}H-NMR spectrum in **Fig.6** shows the peak at δ 1.25-2.07(m) correspond to the protons of the nine methylene groups, which appeared as multiplet due to overlap of signals, the peaks from δ

0.84(t) and δ 2.35(d) indicates the signals of the protons of the methyl groups. The peak at δ 4.50-6.03(t) indicates the protons of the five methine groups and δ 6.99-7.07(d) indicate the protons of the four methine groups.

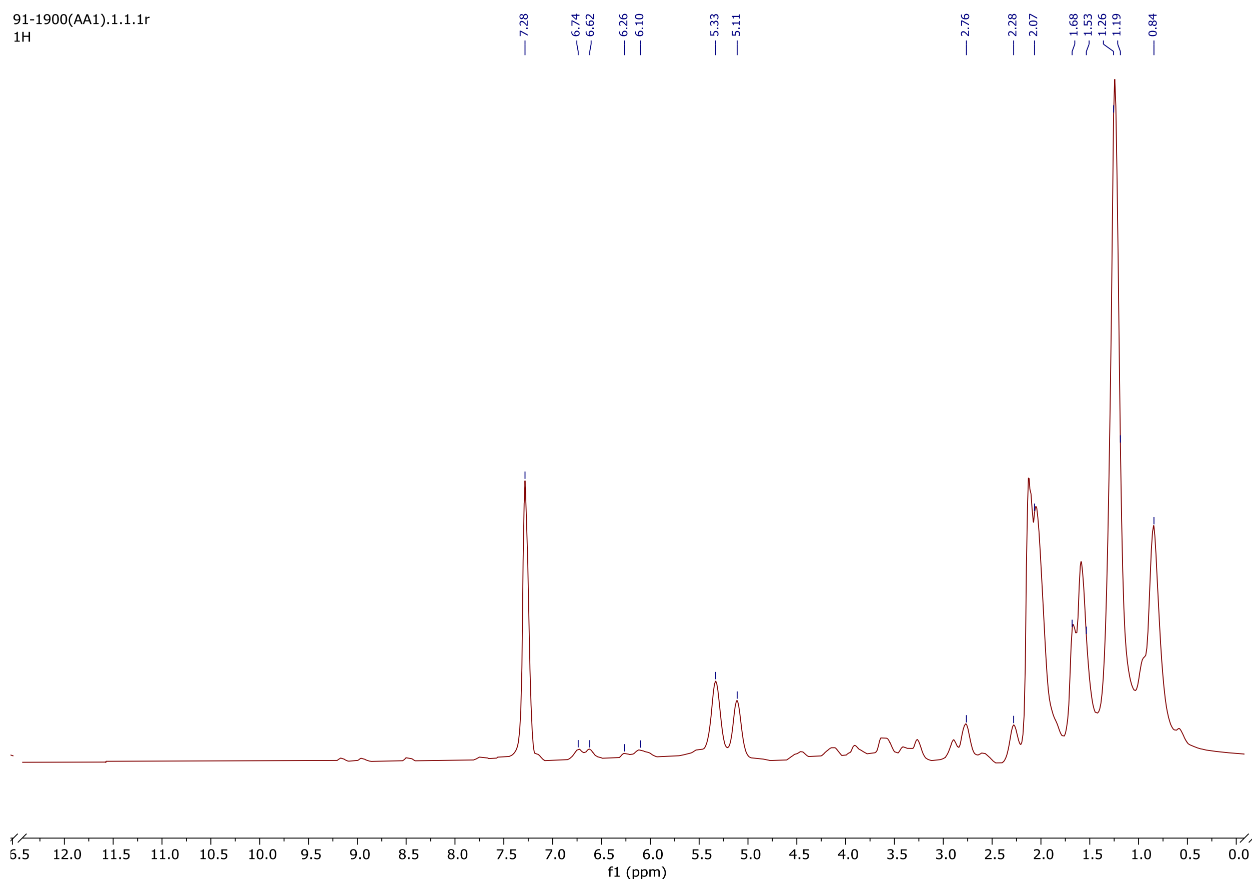

Figure 6: ^{1}H-NMR spectrum of compound AA1.

^{13}C-NMR Spectrum

The proton decoupled ^{13}C-NMR spectrum in **Fig.7** showed signals of 17 carbon atoms. But in the structure (AA1) proposed there are 22 carbon atoms. The difference in the number of carbon atoms between the proposed structure and the signals obtained from the ^{13}C NMR spectrum might be due to the presence of chemically/magnetically equivalent carbon atoms. As indicated in **Fig. 9**, carbon atoms 4,5& 12, 6 & 11, 8,9,10 &3' 1'&4'and 2'&6' might be chemically equivalent.

The (DEPT) spectrum in **Fig.8** showed signals for 11 carbon atoms. Out of these 4 signals indicate the presence of 9 methylene groups and the rest 7 signals for CH and CH_3 groups. In DEPT spectrum data are collected in such a way that the resulting signal is either up field (CH & CH3) or downfield (CH_2) depending on the number of protons attached. In the proton decoupled ^{13}C NMR spectrum shown in **Fig.7** there are signals for 22 carbon atoms, while in the DEPT spectrum overlap of signals were observed for 20 carbon atoms.

The difference in signals between the two spectra indicated the presence of 2 quaternary carbon atoms that are not normally observed in the DEPT spectrum. The $_{13}C$ and DEPT chemical shifts of the proposed structure are summarized in **Table 5** below.

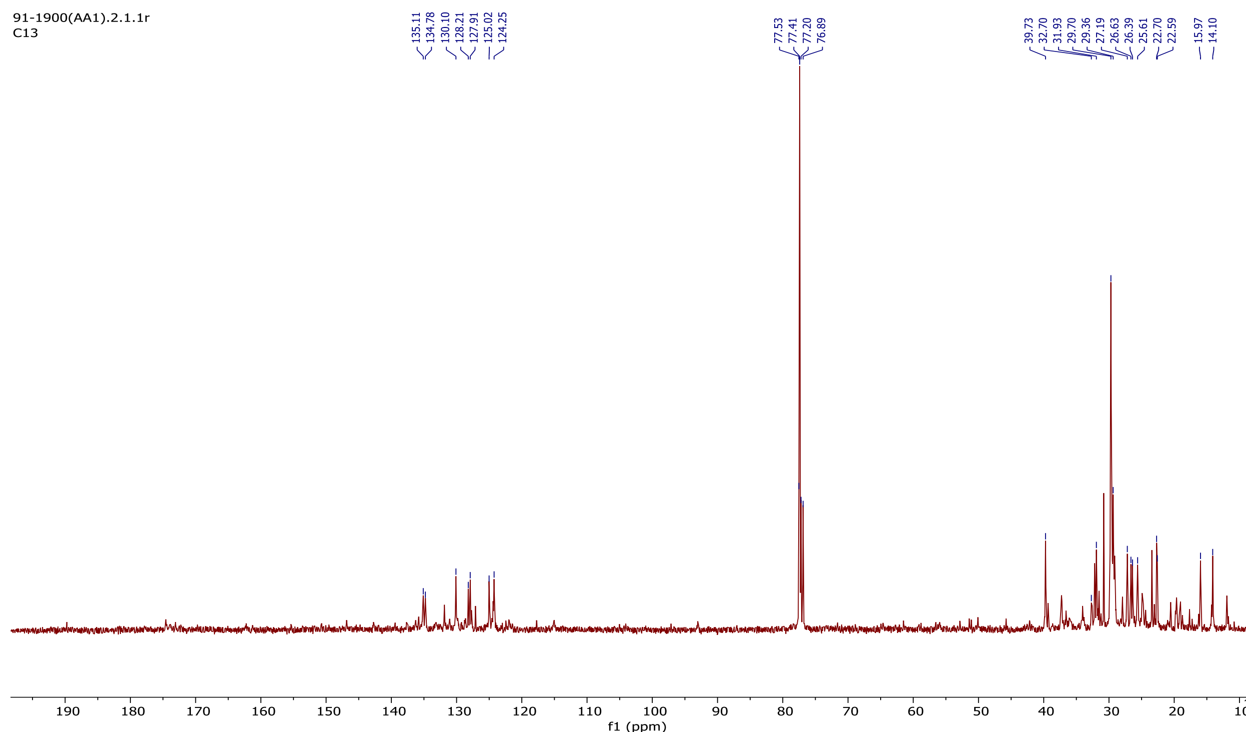

Figure 7: ^{13}C NMR spectrum of compound AA1.

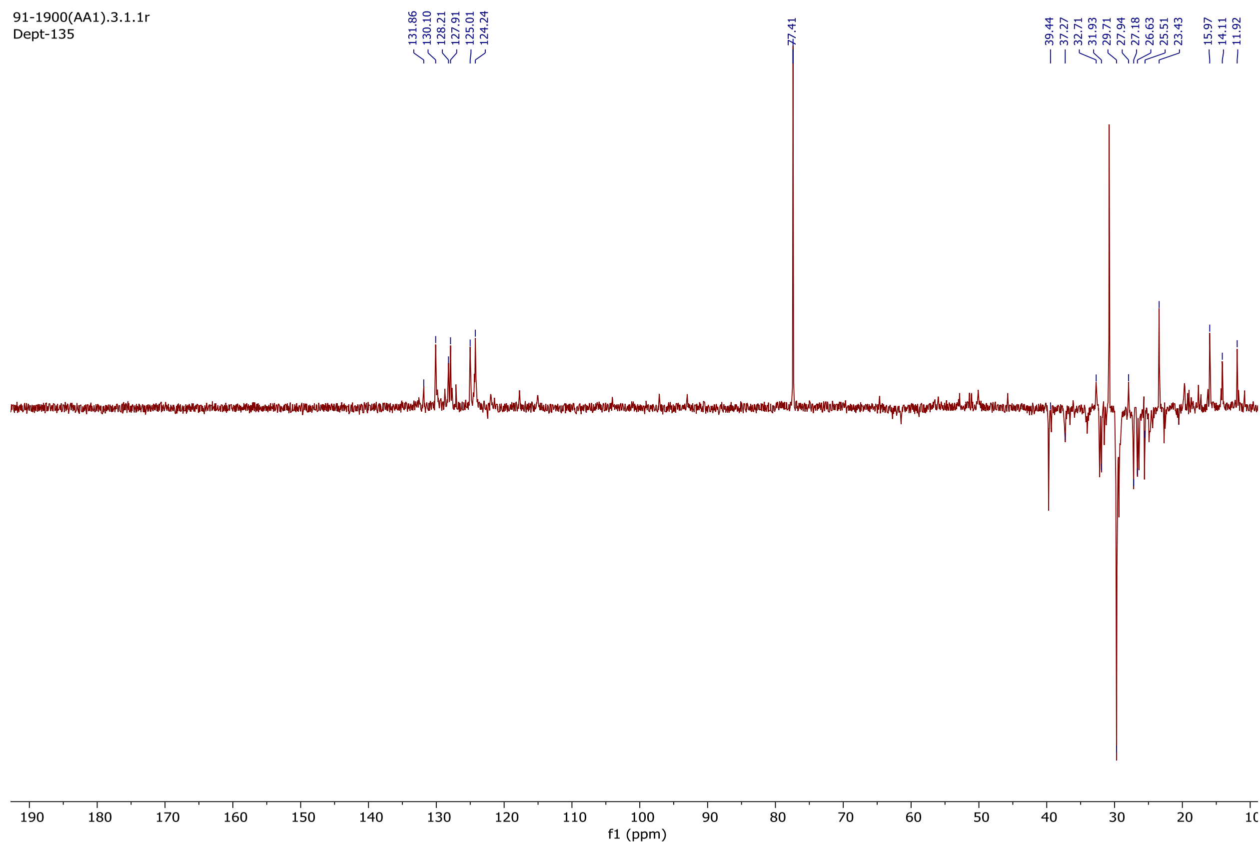

Figure 8: DEPT-135 spectrum of compound AA1.

Table 5: ^{13}C NMR and DEPT-135 spectra data of compound AA1

NO.	^{13}C NMR of AA1 δ (ppm)	DEPT	Remark
1	76.89	CH	-
2	39.73	CH_2	-
3	22.70	CH_2	-
4	29.70	CH_2	-
5	29.70	CH_2	-
6	32.70	CH_2	-
7	135.70	CH	-
8	128.21	CH	-
9	128.21	CH	-
10	128.21	CH	-

11	32.70	CH_2	-
12	29.70	CH_2	-
13	32.70	CH_2	-
14	22.59	CH_2	-
15	14.10	CH3	-
1’	135.11	-	Quaternary
2’	127.91	CH	-
3’	128.21	CH	-
4’	135.11	-	Quaternary
5’	128.71	CH	-
6’	127.91	CH	-
7’	25.61	CH3	-

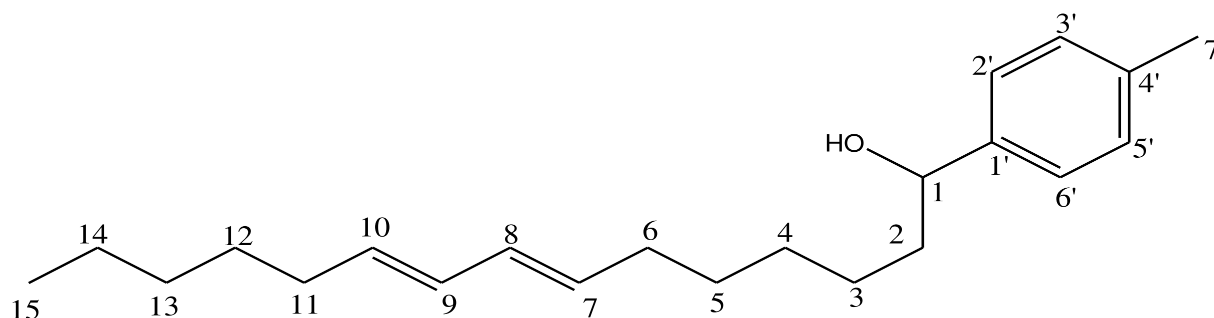

Figure 9: The proposed structure of AA1 (1-p-tolyl pentadeca-7,9-dien-1-ol).

Conclusion

Qualitative phytochemical screening tests were done on the crude extracts of the leaves of *R. vulgaris,* and the results showed the presence of Alkaloids, glycosides, steroids, anthraquinones, and carbohydrates. The analyses of the results from bioactivity tests confirm the presence of active compounds which were extracted by methanol that has a high inhibition zone.

In this study the extraction, isolation and structure elucidation of compound AA1 (1-p-tolyl pentadeca-7,9-dien-1-ol) was accomplished using chromatographic, FTIR and ^{1}D NMR spectroscopic techniques.

The researcher recommended that advanced chromatographic techniques such as HPLC and GS-MS should be used to isolate more compounds from different extracts of the plants. 2D-NMR, and MS techniques are also required to elucidate structures of novel compounds isolated from the plant. Additionally, bioassay tests should be conducted on crude extracts fractions and isolated compounds from the plant.

Ethics approval and consent to participate

Approval to carry out this work was obtained from the University of Gondar Scientific and Research Center of University of Gondar.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AA - Study design, Literature search, data collection, data analysis, data interpretation, writing manuscript, GG - Research supervision, Study design, editing manuscript, DS – Research supervision, editing manuscript. All authors read and approved the final manuscript.

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Author details

[2]*Department of Chemistry, College of Natural and Computational Sciences, University of Gondar, P.O. Box 196, Gondar, Ethiopia*

[1†]*Department of Chemistry, College of Natural and Computational Sciences, Jigjiga University, P.O. Box 1020, Jigjiga, Ethiopia*

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