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Phytochemical Screening and Antibacterial Activity of Stem Bark Extract of Maesa Lanceolate and Isolated Compounds

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Abstract

Maesa lanceolate locally kmown as "Kowwaada" in Hadiya, and "Yekalaha zaf" in Amharic is one of the species in Primulaceae family whoseaerial and axial parts have been in wide use for the treatment of a variety of diseases in different parts of the world. Its stem bark in association with leaves or alone is employed for medicinal value among the Hadiya community of Southern Ethiopia. The stem bark of Maesa lanceolata was collected, chopped into pieces, air-dried, ground and extracted successively with dichloromethane (DCM) and methanol (MeOH) to afford 0.8% DCM and 12.26% methanol crude extracts, respectively. The methanol crude extract which showed clear TLC profile and yield, was subjected to silica gel column chromatography in n-hexane to ethyl acetate solvent system with increasing polarity and two compounds namely, 2, 5-dihydroxy-3-methyl-6-(nonadec-14-enyl)-1, 4-benzoquinone and isopropyl oleate were isolated. The methanol extract as well as the isolated compounds were investigated for their in vitro antibacterial activities against two gram-negative bacterial strains (P. aeruginosa (ATCC27853), E. coli (ATCC25922), and two gram-positive bacterial strains (S. pyogen (ATCC19615), S. aureus (ATCC25923) using ampicillin as a positive control. The results showed that the methanol crude extract and isolated compounds were all active against tested bacterial strain but 2,5-dihydroxy-3-methyl-6-(nonadec-14-enyl)-1, 4-benzoquinone was the most active among them. The later exhibited 8mm, 8.5mm, 8 mm, and 9 mm zones of inhibitions at concentrations of 350 µg/ml and 9.5mm, 10mm, 9mm, and 11mm at (400 µg/ml) against E. coli, S. aureus, P. aeruginosa and S. pyogen, respectively. The results further indicated the increase in the inhibition zone with concentration. This study supports the traditional medicinal uses of Maesa lanceolata for the treatment of infectious diseases.

1. Introduction

Plants have been used for centuries to try to cure diseases, relieve physical pain, and provide spices, dyes, poisons and drugs. People in the past knew a lot about the chemical compounds that plants produce to help them heal. This information is often called traditional medicine [1]. Currently, most of the 170 member states of the World Health Organization (WHO) recognize the use and regulation of traditional medicine, including herbal medicines, for primary health care [2]. This is much higher than the number of member states as of 2000 [3]. This could indicate the growing popularity and use of traditional medicine among the people.[4] In Ethiopia, the use of traditional medicine is widely practiced. The widespread use of traditional medicine in Ethiopia could be attributed to cultural acceptability, efficacy against certain types of diseases, physical accessibility and economic affordability as compared to modern medicines. [5] Natural products from plants remain vital in drug discovery where they can be used directly as drugs or serve as clues to new drugs by providing chemical entities. [6] Antibiotics are one of our most important weapons against bacterial infections and have greatly improved people's health-related quality of life since their introduction.[7] However, in recent years, the emergence of drug resistance as well as unfavourable side effects from some antibiotics has prompted researchers to search for new antimicrobial agents, primarily among plant extracts, to discover new chemical structures that overcome the aforementioned drawbacks.[8] Plants are the primary subject of current research on natural molecules and products since they are easier to get and may be chosen for their ethanol-medicinal applications.[9] Therefore, the increase in failures due to chemotherapy and antibiotic resistance leads to the screening of several medicinal plants for antimicrobial activity [10]

Maesa lanceolata locally known as Kowwaada" in Hadiya and "Yekalaha zaf" in Amharic. Forsk is commonly known as 'false assegai' and belongs to Myrsinaceae family. It is one of the species under Maesa spp. and native to most of southern and eastern African countries including Ethiopia. It is a sprawling shrub, 2 to 3m tall, or a small tree with a single stem up to 9m tall, or a rounded bushy shrub with branches almost at ground level. It thrives on stream banks, cliff tops in both midland and coastal areas to about 1500m above sea level [11]. Traditionally, crushed stem bark is mixed with

water and administered orally since early times among Hadiya people of Ethiopia against various bacterial infections and it also shows anthelmintic activity against helminthes such as ascaris [12]. In view of the therapeutic relevance of Maesa lanceolate in the indigenous system, it was determined to focus on the phytochemistry and antibacterial research on stem bark extract of Maesa lanceolate and isolated compounds.

2. Materials and methods

2.1. Plant material collection area

The stem bark of Maesa lanceolata was collected from Ya-bukuna Kebele, Mirab Badawacho Woreda, Hadiya Zone, SNNPR, Ethiopia. Ya-bukuna Kebele is located 353 km South of Addis Ababa and 139 km west of Hawassa as shown in (Fig. 1)

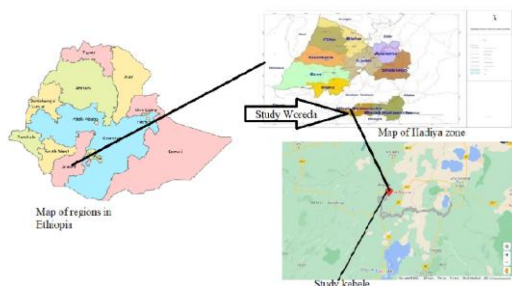


Fig. 1. Map of the study area. Source: Adapted from Google Maps, 2022.

2.2. Preliminary phytochemical screening tests

Qualitative phytochemical screening for alkaloids, flavonoids, glycoside, phenol, saponins, tannins, terpenoids were done in the methanol extract following standard methods as described in the literature, the result is presented in Table 1.

2.1.1. Test for alkaloids

Wagner's Test: The extract (5 mL) was mixed with 2 mL of HCl. To this acidic medium, 1 mL of Wagner's reagent was added to the mixture. A reddish-brown precipitate was observed for positive results [13].

2.1.2. Test for flavonoids

Shinoda Test (NaOH test): A few drops of ammonia were added to 3 mL of extract and then a few drops of concentrated sulfuric acid were added. The formation of a yellow colour was observed [14] in the presence of flavonoids.

2.1.3. Test for glycoside

Killer-Killiani Test: Few drops of $FeCl_3$ and conc. H_2SO_4 was added to an extract in glacial acetic acid, formation of a reddish-brown colour at the junction of two layers and changing of the upper layer into bluish-green indicated the presence of glycoside [15].

2.1.4. Test for phenols

Ferric chloride test: To 3 mL of extract two drops of 1% ferric chloride were added. The formation of blue green colour indicated the presence of a phenolic compound [16].

2.1.5. Test for saponins

Froth Test: The extract (0.2 g) was dissolved in 3 mL of distilled water. The mixture was shaken vigorously. Stable and persistent froth was formed [17].

2.1.6. Test for tannins

Ferric chloride test: The extract (0.1 g) was dissolved in 2 mL of distilled water, and then a few drops of 1% ferric chloride solution were added to give a blue-black colouration [18]

2.1.7. Test for terpenoids

Salkowski test: The crude extract (0.1 g) was shaken with chloroform (2 mL) in the test tube followed by the addition of a few drops of concentrated sulfuric acid along the side of the test tube using the dropper, and a reddish-brown colouration of the interface indicated the presence of terpenoids [19]

2.3. Extraction of plant material

The powder of plant material weighing 400g was soaked in 1.6L of DCM and shaken for 72hrs. Then the solution was filtered with Whatman no.1 (150mm) filter paper. The filtered solution was concentrated with a rotary evaporator at 40 °C and was collected in two Petri dishes. The crude weighing 3.2g was collected after two days by scratching with a spatula. This yield was found to be much less than the minimum amount needed for column packing and therefore kept separately. Similarly, the marc (i.e. an insoluble solid residue left over after the extraction by DCM) weighing 380 g was soaked in 3 L of methanol and left on a thermostatic bath shaker for three days. After 3 days the solution was filtered using Whatman no.1 (150 mm) filter paper. The filtered solution was concentrated using a rotary evaporator at a temperature of 40 °C and collected in five Petri dishes. Then the concentrate was air dried, and finally, the crude weighing 46.6 g was collected by scratching the Petri dishes with a spatula. This yield was appreciable and accounted for 12.26% of 380 g marc soaked with methanol

2.4. Compound isolation

The methanol extract weighing 30g was meshed into a fine powder and adsorbed with 10g of silica gel. Then, it was subjected to 170g of silica gel column chromatography packed with n-hexane and eluted with n-hexane and ethyl acetate whose elution ratio was set to 1:0, 0.99:0.01 and 0.98:0.02 (n-hex: ethyl acetate). The result is presented in Table 1.

Table 1. The solvent system and fractions collected from methanol crude extracts of Maesa lanceolata.

Fractions	Eluent	Ratio	Total volume (mL)	spot
1-6	n-hex : EtOAc	1:0	300	none
7-41	>>	0.99:0.01	1050	none
42-46	>>	0.99:0.01	180	Single spot
47-59	>>	0.99:0.01	390	none
60-87	>>	0.99:0.01	840	Single spot
88-139	>>	0.98:0.02	2500	Single spot

2.5. Antimicrobial Activity

The disc diffusion method was used for antibacterial activity evaluation. The antibacterial activity of the methanol extract, as well as the two isolated compounds, were tested against two Gram-positive bacterial strains such as S. pyogen (ATCC19615) and S. aureus (ATCC25923) and two Gram-negative bacterial strains such as P. aeruginosa (ATCC27853) and E. coli (ATCC25922) using Muller-Hinton agar medium. All of the bacterial strains were cultured in the Biology Laboratory of Adama Science and Technology University, Adama, Ethiopia. Standard antibiotic ampicillin was used as a positive control and DMSO was used as a negative control. The methanol crude extract and the isolated compounds were prepared for investigation by dissolving 3.5mg and 4mg of each in 10 mL of DMSO to afford two separate concentrations 350µg/mL and 400 µg/mL, respectively. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the discs.

3. Results and discussion

3.1. Extract Yield

The percentage yield of crude extracts is given in Tale 2.

Table 2. The solvent used, amount of sample extracted and weight of crude afforded

No.	Solvents used in serial extraction	Weight of sample extracted	Weight of crude afforded
1.	DCM	400g	3.2g
2.	Methanol	380g	46.6g

$$\text{Yield (\%)} = \frac{\text{weight of dried crude extrac}}{\text{weight of sample}} \text{x } 100\% \quad (1)$$

$$DCM\ extract = \frac{3.2g}{400g} \text{ x } 100\ \% = 0.8\% \text{ of DCM extract} \quad (2)$$

$$\text{Methanol extract} \quad = \frac{46.6g}{380g} \text{x}100\% \quad (3)$$

$$= 12.26\% \text{ of memethanoltract}$$

Therefore, the ground 400g stem bark powder of Maesa lanceolata afforded 3.2g (0.8%) crude when extracted with DCM, and 380g of the marc afforded 46.6g (12.26%) in the successive extraction with methanol.

3.2. Phytochemical analysis

The phytochemical analysis result of the secondary metabolites in the methanol crude extract of Maesa lanceolata stem bark is given in Table 3.

Table 3. Secondary metabolites and tests were conducted.

No.	Metabolites	Solvent of extraction	Test employed	Status
1	Alkaloids	Methanol	Draggendorff's	++
2	Flavonoids	>>	Shinoda test	++
3	Terpenoids	>>	Salkowski test:	++
4	Saponins	>>	Froth	++
5	Phenols	>>	Lead acetate	++
6	Glycosides	>>	Kellar-Kiliani	++
7	Tannins	>>	KOH	++

Remark: ++ symbol was used to represent the "Presence" status.

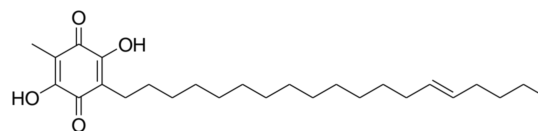
3.3. Structural elucidation of isolated compounds

A compound which was latter known as maesaquinone was isolated from a combined fraction of 60-87 and 88-139 in an elution ratio of 0.99:0.01 and 0.98:0.02 (n-hex: ethyl acetate), respectively. Both of the separate combinations showed similar Rf values of 0.57. Besides, they were orange-yellow coloured powders weighing 27.8mg that were subjected to NMR and IR analysis at Addis Ababa University Chemistry Laboratory. Its IR spectrum showed characteristic absorption bands at 3326 cm-1 to hydroxyl, 1612 cm^{-1} to quinoid carbonyl and 2850-2920 cm^{-1} attributed to –C-H vibrations of the side chain.

The 1H-NMR spectrum showed some typical signals at δ 5.37 (m, 2H, -CH=CH-), δ 2.42 (t, 2H.-CH_2-), δ 2.19 (m, 4H, C=C-CH-), δ 1.30 (m. 18H, CH_2) and 0.92 ppm (t, 3H. CH_3). The 13C-NMR spectrum with the aid of DEPT-135 showed the presence of the peak at δ 129.6 and δ 129.7 accounted for the presence of an olefinic group along the side chain. Besides, the signals ranging from δ13.5- 31.8 are assigned for 16 C (15CH_2 +1CH_3) across the olefinic side chain and one terminal carbon. This was further confirmed with signals observed in DEPT-135. The peaks at δ 111.01 and 115.51 brought clues about two carbons in the ring. The remaining oxygen-bearing four quaternary carbons didn't show any peaks and therefore it matches spectral information obtained from literature. [20, 21, 22,] and a comparison of spectra is shown below (Table 4)

Table 4: 1H-NMR ($CDCl_3$, 400 MHz), 13C-NMR and DEPT-135 (100MHz) spectral data of compound 32 and reported NMR (20,21).

Position	^{13}C-NMR	^{13}C-NMR Literature [20,21]	^{1}H-NMR	1H-NMR Literature [20,21]	DEPT-135
1, 2,4 & 5	-	-	-	-	-
6	111.01	111.0	-	-	-
3	115.51	116	-	-	-
3"	6.62	7.7	2.42	2.43	CH_3
1'	22.45	22.7	2.42	2.45	CH_2
2'	26.87	27.2	1.26	1.29	CH_2
3'-12'	29.1	29.7	1.30	1.30	CH_2
13'	28.01	27.7	2.19	2.18	CH_2
14'	129.6	129.9	5.37	5.45	CH
15'	129.67	130.0	5.37	5.45	CH
16'	28.01	27.4	2.19	2.18	CH_2
17'	31.8	32.0	1.26	1.29	CH_2
18'	22.5	22.8	1.32	1.31	CH_2
19'	13.5	13.9	0.92	0.92	CH_3



2, 5-dihydroxy-3-methyl-6-(nonadec-14-enyl)-1, 4-benzoquinone

Fig 2. The proposed structure of compound- maesaquinone.

A compound which was latter known as isopropyl oleate was isolated from combined fractions of 42-46 in elution ratio of 0.99:0.01 (n-hex: ethyl acetate). Of the combined fractions a sample weighing 24.3mg was subjected to NMR and IR analysis at Addis Ababa University Chemistry Laboratory. The sample was oily in nature and yellow in colour.

Its IR spectrum showed characteristic absorption bands at 2850-2920cm-1 and 1738 cm-1 attributed to –C-H vibrations and the ester functional group, respectively. Also, the absorption band at 1094 cm^{-1} further confirms the existence of –C-O bond vibration.

The ^{1}H-spectrum showed a peak at δ 0.88 (3H) due to the presence of protons of methyl (-CH_3) groups; the peaks at ~δ 1.30 (20H) indicate protons of aliphatic methylene (CH_2) group while the peak at δ 2.02 ppm indicates the presence of protons of methylene groups that is bonded to C=C bond; the peak at δ 2.29 ppm indicates protons of methylene that is bonded to a carbonyl group; the peak at 5.13 ppm indicates the presence of proton in methine group that is bonded with oxygen of ester functional group; the peak at δ 1.27 ppm indicates protons of methyl carbons in isopropyl group and the peak at δ 5.36 ppm indicates presence of olefinic protons.

The ^{13}C-NMR spectrum peaks at δ 129.6 and 129.9 ppm indicated the presence of a C=C bond in the compound; a single peak at δ 172.7 ppm indicated a quaternary carbon signal of carbonyl carbon of oleate. The peak at δ 62.8 ppm indicated the presence of carbon

bonded with oxygen in the ester functional group. On the other hand, the chemical shift values in the range of 14.0 to 34.0 ppm indicate the presence of methyl (-CH3) and methylene (-CH2) carbons. The DEPT-135 spectrum showed the single peak for the presence of methyl (-CH3) carbon at δ 13.82 ppm and assured the presence of another methyl carbon at δ 22.3 ppm for the terminal isopropyl group. Besides, it also gave a clue to assure that it is methine carbon (δ 62.8ppm) that is bonded with oxygen in the ester group. The carbons at δ 22.7 to 34.0 are said methylene carbons by assessing the overall spectrum thoroughly and comparing with literature the isolated compound was found to be isopropyl oleate [23,24] and a comparison of spectra is shown below (Table 5)

Table 5 : 1H-NMR (CDCl3, 400 MHz), 13C-NMR and DEPT-135 (100MHz) spectral data of compound 32 and reported NMR (23,24).

Position	^{13}C-NMR	^{13}C-NMR Literature [23,24]	^{1}H-NMR	^{1}H-NMR Literature [23,24]	DEPT-135
1	172.77	173.43	-	-	Quaternary
2	34.2	34.71	2.29	2.27	CH_2
3	24.25	24.6	1.61	1.62	CH_2
4	29.01	29.1	1.30	1.30	CH_2
5	29.01	29.2	1.30	1.30	CH_2
6	29.01	29.4	1.30	1.30	CH_2
7	29.01	29.6	1.30	1.30	CH_2
8	27.79	27.1	2.05	2.02	CH_2
9	129.69	129.74	5.35	5.36	CH
10	129.95	129.97	5.35	5.36	CH
11	27.79	27.2	2.05	2.02	CH_2
12	29.01	29.5	1.30	1.30	CH_2
13	29.01	29.3	1.30	1.30	CH_2
14	29.01	29.1	1.30	1.30	CH_2
15	29.01	29.2	1.30	1.30	CH_2
16	31.7	31.9	1.30	1.30	CH_2
17	22.4	22.7	130	1.30	CH_2
18	13.5	14.1	0.88	0.89	CH_3
1’	62.82	64.0	5.13	5.02	CH
1’’	22.3	21.2	1.27	1.25	CH_3
2’	22.3	21.2	1.27	1.25	CH_3

Figure 3 bellow describes the proposed structure of the isolated isopropyl oleate based on comparison of obtained spectral data and literature

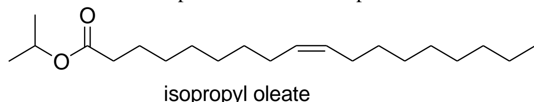


Fig 3. The proposed structure of the compound isopropyl oleate.

3.4. Antibacterial activity

The antibacterial activities of the methanol extract and isolated compounds are given in Table 6 below.

Table 6. Antibacterial activity testing of samples and bacterial strains used.

Samples with conc.		Bacteria Species and Zone of Inhibitions in mm: *E. coli* ATCC25922	*S. aureus* ATCC25923	*P. aeruginosa* ATCC27853	*S. pyogen* ATCC19615
Methanol crude extract	A_1=350 µg/ml	6.5	7	6.5	7.5
	A_2= 400 µg/ml	7.5	8.5	7	8
Compound-2	B_1= 350 µg/ml	7	8	7	8
	B_2= 400 ug/ml	8.5	9	8	9.5
Compound-1	C_1= 350 µg/ml	8	8.5	8	9
	C_2= 400 µg/ml	9.5	10	9	11
Ampicillin (+ve control)		12	12.5	12	13.5

The results further indicated the tendency of an increment in the inhibition zone with concentration. Figure 4 further depicts antibacterial activity test results in a clustered column graph.

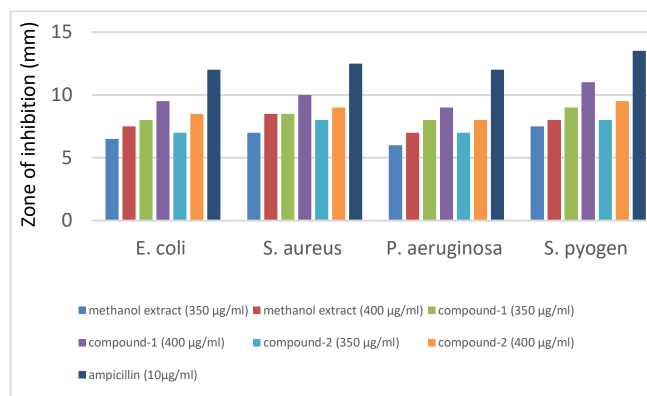


Fig 4. Inhibition of *E. coli, S. aureus, P. aeruginosa, S. pyogen* by the methanol crude extract, isolated compounds and positive control (ampicillin) at different concentrations.

4. Conclusions

For decades, medicinal plants have been in use and continue to be an alternative approach for the treatment of several ailments caused by diseases causing micro-organisms. Maesa lanceolata is one of the typical medicinal plants which are widely used among Hadiya community. The phytochemical screening results on methanol crude extract revealed the presence of alkaloids, terpenoids, phenols, flavonoids, saponins, tannins, and glycosides. Silica gel column chromatographic separation in its turn afforded two compounds such as maesaquinone and isopropyl oleate. Their respective structures were thoroughly interpreted from spectral information obtained from IR, 1H-NMR, 13C-NMR and DEPT-135 in comparison with data from the literature.

The methanol crude extract and both isolated compounds were evaluated for their antibacterial activity by disc diffusion method against four bacterial strains; two-gram positive bacteria S. aureus and S. pyogen, and two-gram negative bacteria E. coli and P. aeruginosa. Three of the samples showed higher zones of inhibition ranging 7-11mm against tested bacterial strains at a concentration of 400µg/ml and 350 µg/ml. The results showed an increase in activity with concentration. The results further validate the use of the Maesa lanceolata in traditional medicine.

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