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Phytochemical investigation on leaf extract of *Adhatoda schimperiana*, Ethiopia

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Interest in obtaining biologically active compounds from natural sources has recently spiked due to their low toxicity, complete biodegradability, availability from renewable sources, and in most cases, low cost. This research project is aimed at isolating, phytochemical screening and partial characterizing the chemical constituents of *Adhatoda schimperiana* of Ethiopia. The leaves of *Adhatoda schimperiana* was extracted with 1.5 L of n-Hexane for 72 h. The extract was evaporated under reduced pressure at temperature of 40 ^{0}C using rotary evaporator. Partitioning was done using n-hexane, chloroform, ethyl acetate, ethanol and methanol. The organic and aqueous layer was concentrated to dryness under reduced pressure to afforded 3.10 g and 2.50 g crude extract respectively. Phytochemical screening revealed the presence of alkaloids, polyphenols, flavonoids, glycosides, phytosterols, saponins, triterpenes, and quinines as a major class of compounds. However, less presence of tannins was observed. The selective extraction of triterpenes allowed me to obtain 0.71% yields. Separation on column chromatography conducted to a major fraction of triterpenes and a major compound from defatted pricarp leaves of chloroform extract. Partial characterization of the pure compounds was done using a combination of spectroscopic techniques including UV-Vis, IR, 1D NMR (^{1}H-NMR,^{13}C-NMR and DEPT).

Keyword: *Adhatoda schimperiana*, Leaves, Phytochemical screening, and characterization.

1. Introduction

Medicinal plants have a long history of use in most communities throughout the world. It has been confirmed by WHO that herbal medicines serve the health needs about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries [1]. In Africa, the use of traditional medicine has persisted over the years and the last few decades have witnessed an upsurge of interest in traditional medicine and other alternative forms of healthcare in the developing and developed countries [2].

The family Acanthaceae (or Acanthus family) which consists of 250 genera and 2500 species. This genus consists of 600 species distributed in tropical and temperate regions of which most are tropical herbs or twining vines; some are epiphytes [3]. *Adhatoda schimperiana* is one of the plants that belong to the family Acanthaceae that has a synonym *Justicia schimperiana* or *Gendarussa schimperiana*. *Adhatoda schimperiana* is a very common erect shrub, usually much branched from the base. It is relatively fast growing and prefers altitude of 8,000 ft. or above. The shrub is abundant in Ethiopia, Kenya and Tanzania. The plant is used as a live fence. It is an erect shrub up to 4 m high; stem woody and with internodes; leaves decussate, estipulate, simple, ovate-oblong in outline; inflorescence thyroid, with densely

flowered spikes; corolla bilabiate white to creamy white; fruit capsule [4, 5].

Traditionally the decoction of the leaf of the plant was taken mixed with local beer as a remedy against bronchial asthma. The leaves are used by local people for protection against contagious diseases [5]. Also different Traditional uses of the plant have been recorded, in Eastern Ethiopia, the plant is used as a laxative [5, 6]. In Northern Ethiopia the plant alone or in combination with other plants is used for various diseases such as epilepsy, mental illness, eye diseases, jaundice, malaria, leprosy, syphilis, gonorrhea, rabies, measles, relapsing fever, vitiligo, gout and acute febrile illness [7]. In Southwest Ethiopia, it is used for malaria, scabies, where the fresh leaves are crushed and macerated in water and then the affected area is washed with the macerate [8].

The determination of the phytochemical constituents of plant extracts are essential in order to ensure the reliability and repeatability of pharmacological and clinical research, to understand their bioactivities and possible side effects of active compounds and to enhance product quality control. Thus, with regard there is much to be explored. Therefore, the aim of this study was to validate the ethno medicinal use and subsequently the isolation and characterization of the chemical constituents of *Adhatoda schimperiana* leaf which will be added to the potential lists of drugs.

2. Materials and Methods

2.1. Plant Material

The leaves pericarp of the *Adhatoda schimperiana* plant were collected from mountains located at South Western Ethiopia (Hadiya area). The plant material used in this study was collected in June, 2009 from SNNPR of East Badawacho Woreda Center, Shone area, which is 110 km South East of Hadiya Zone Center, Hosanna, 475 km from Addis Ababa and 150 km from Hawassa. The plant sample was identified by the authors. The plant specimen was given a voucher number of ASC-007 and deposited in the Biological Science Laboratory of the ecology, Department of Biology, University Addis Ababa.

The plant organ were cut into small pieces and shade dried at room temperature (20 ^{0}C) for three weeks, finely powdered plant materials were stored in airtight polythene bags protected from sunlight until use.

2.2. Extraction and Isolation

2.2.1. Extraction

215 g powder of air dried leaf of *Adhatoda schimperiana* was first soaked with 1L n-Hexane for 3 days. After filtration the extract was evaporated under reduced pressure and temperature of 40 ^{0}C using rotary evaporator. The dried marc was extracted with ethanol after soaking for 3 days at room temperature. The extract was concentrated in rotary vapor and afforded 7 g dark green residue which was collected with distilled water and added to a separator funnel with 215 ml diethyl ether to separate organic part and the aqueous parts. The filtrates were evaporated under reduced pressure using rotary evaporator and afforded 3.1gm green solid and 2.5 gm yellow solid for organic part and aqueous part respectively. When TLC was developed for the crude extract for the organic part on solvent chloroform: ethyl acetate (7:3) had shown four colored spots and for aqueous part ethyl acetate: ethanol (7:3) had showed 6 colored spots.

2.2.2. Isolation

110 gm of silica gel was measured and mixed with 200 mL of n-Hexane. Then the mixture was packed into a column, 2 ml of n-Hexane was added to the 3.1 gm dried sample of the crude extract. This concentrated sample was then applied on to the top of packed silica gel using a dropper. Elution was done with 100 mL pure n-Hexane, followed by 100 ml n-Hexane: CF (1:1), 100 mL pure CF 100 mL CF: EtOAc (1:1), 100 ml pure EtOAc and then 100 ml EtOAc: MeOH the elution stopped when the darkest part at top reached the bottom. A total of 30 fractions were collected (Table 3). Similarly 2.5 g crude extract of aqueous part was subjected to column chromatography elution with solvents systems; Chloroform, Chloroform: Ethyl acetate, Ethyl

acetate: Ethanol and Ethanol and a total of 20 fractions (Table 4) were collected. As follows:

2.3. Fractionation

2.3.1. Organic Part Fractionation

The fractions collected other than 14 were discarded because their TLC results did not show spots. Fractions 14 showed pure spots. The solvent system used for the TLC examination of 14 was CF: EtOAc (70:30), the fraction 14 was left in a hood for 12hrs. While pale yellow solid were observed containing fraction 14. Fraction 14

as labeled compound ASH-14, its amount was 30 mg. The IR, NMR (1H, 13C, DEPT) and UV-VIS spectra for compound ASH-14 were recorded and were characterized

Table 1: Solvent systems and fractions collected from crude extract organic part

Fraction	Solve system	Ratio	Volume	Remark
1	n-He	Purely n-Hexane	100 ml	ASH_1
2	n-He			ASH_2
3	n-He			ASH_3
4	n-He			ASH_4
5	n-He			ASH_5
6	n-He: CF	1:1	100 ml	ASH_6
7	n-He: CF			ASH_7
8	n-He: CF			ASH_8
9	n-He: CF			ASH_9
10	n-He: CF			ASH_10
11	CF	Purely chloroform	100 ml	ASH_11
12	CF			ASH_12
13	CF			ASH_13
14	CF			ASH-14
15	CF			ASH_15
16	CF: EtOAc		100 ml	ASH_16
17	CF: :EtOAc			ASH_17
18	CF: EtOAc			ASH_18
19	CF: EtOAc			ASH_19
20	CF: EtOAc			ASH_20
21	EtOAc	Purely ethyl acetate	100 ml	ASH_21
22	EtOAc			ASH_22
23	EtOAc			ASH_23
24	EtOAc			ASH_24
25	EtOAc			ASH_25
26	EtOAc: MeOH	1:1	100 ml	ASH_26
27	EtOAc: MeOH			ASH_27
28	EtOAc: MeOH			ASH_28
29	EtOAc: MeOH			ASH_29
30	EtOAc: MeOH			ASH_30

2.3.2. Isolation of Compound ASH-14

2.3.2.1. Physical Data

Nature: Compound ASH-14 is pale yellow solid with, Rf 0.58 (CF: EtOAc, 3.5:1.5), mp 167-170 ^{0}C.

2.3.2.2. Spectroscopic Data

IR Vmax (4000 cm^{-1} (KBr) to an olefinic system (1458 cm^{-1}), the methyl C-H stretching in this compound is indicated by a sharp peak at 2920 cm^{-1}.). Three peaks near to 1625 cm^{-1}, 1625 cm^{-1} and 1462 cm^{-1} are indicative of aromatic ring.

1H NMR δ (400MHz, CDCl3), (Appendix 3): 5.40(t, 1H, H-3), 2.30(t, 1H, H-21), 2.8(d, 1H, H-9), 2.20-1.15(m, 22H), 1.03(s, 6H, C-23 Me & C-26 Me), 0.87(s, 6H, C-24 Me, C -25Me),**13C NMR** (100Hz, CDCl3) δ ppm 14.08 (C-30), 22.65 (C-24 & C-25), 25.52(C-16), 27.17(C-23 and C-26), 27.13 (C-15), 29.10 (C-29), 29.21 (C-27), 29.49(C-20), 29.67 (C-21), 29.73 (C-7), 31.87 (C-22) 33.44 (C-6), 35.82 (C-19), 49.40 (C-14, quaternary), 49.62 (C-13, quaternary), 49.83 (C-8, quaternary), 48.98 (C-17, quaternary), 48.98 (C-18, quaternary), 63.75 (C-9), 116.17 (C-11), 118.44 (C-12), 128.83 (C-5 and C-10, quaternary), 129.19 (C-2 and C-4) 129.71 (C-3) and 130.01 (C-30)

2.4. Phytochemical Screening

Each extract of *Adhatoda schimperiana* was screened for the presence of various secondary metabolites (phytochemicals) such as alkaloids, Polyphenols, Flavonoids, tannins, glycosides, phytosterols/withanoids, saponins, terpenoids, Quinones. The methods of analysis employed were those described by [9, 10] for the presence of various active components.

2.4.1. Test for alkaloids: Extract 300 mg was digested with 2 M HCl, and the acidic filtrate was mixed with:

a) Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.
b) Hager's reagent, presence of alkaloids confirmed by the yellow colored precipitate.

2.4.2. Test for polyphenol: 100 mg of extract in the test tube was treated with 3% ferric chloride. The deep blue color of solution shows the presence of phenol.

2.4.3. Test for flavonoids

a) Alkaline reagent test: Extract was treated with 10% NaOH solution, formation of intense yellow colour indicates presence of Flavonoid.
b) Mg turning test: Extract were treated with Mg turning and add conc. HCl to this solution add 5 ml of 95% ethanol, formation of crimson red colour indicates Flavonoid.

2.4.4. Test for tannins: To an aliquot of extract (dissolved in water) 2 ml of sodium chloride was added, filtered and mixed with 5 ml 1% gelatin solution. The absence of precipitation indicates the absence of tannins.

2.4.5. Glycosides: Keller-Killani Test: extract 100 mg treated with 2 ml glacial acetic acid containing a drop of FeCl3. A brown colour ring indicates the presence of positive test.

2.4.6. Test for phytosterol: Salkowski's test: Extract (150 mg) was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated H_2SO_4 and shakes, allow standing, appearance of golden red indicates the positive test.

2.4.7. Test for saponins: Extract (300 mg) was boiled with 5 ml water for two minutes; the mixture was cooled and mixed vigorously and left for three minutes. The formation of frothing indicates the presence of saponins.

2.4.8. Test for triterpens: Extract (300 mg) was mixed with 5 ml chloroform and warmed for 30 minutes. Few drops of concentrated sulphuric

acid was added and mixed well. The appearance of red color indicates the presence of triterpens.

2.4.9. Test for quinone: Addition of 200 mg extract with 5 ml hydrochloric acid result in yellow colored precipitate denoting the presence of quinone.

3. Results and Discussion

Table 2: The phytochemical screening of the *Adhatoda schimperiana* was tabulated below

S. No	Phytochemical	Reagents	Chloroform Extract [+ means Present; - means Absent]
1.	Alkaloids	Wagner Test	+
		Hager Test	+
2.	Polyphenols	Salkowski Test	+
3.	Flavonoids	Alkaline reagent test	+
		Mg turning test	+
4.	Tannin	2 ml Ferric chloride 2% and 5 ml gelatin solution 1%	-
5.	Glycosides (Oligosaccharides)	2 ml glacial acetic acid and a drop of $FeCl_3$	+
6.	Phytosterols/withanoids	$CHCl_3$/conc. H_2SO_4 Libermann-Bruchard`s	+
7.	Saponins	Honey comb froth formation	+
8.	Triterpens	Anisaldehyde	+
9.	Quinone	5ml hydrochloric acid	+

3.1. Partial Characterization of Compound ASH-14

Ground leave parts (215 g) of *Adhatoda schimperiana* were subjected to exhaustive extraction successively with ethanol. The solvent from each extract was recovered under reduced pressure using rotavapor to obtain ethanol extract (ET, 7 g). Chromatographic purification of the ethanol extract gave a compound coded; ASH-14 and H_12 the structures of this compound have been elucidated on the basis of spectroscopic evidences.

Compound ASH-14 is a peal yellow solid obtained from fraction 14 of Ethanol extract of organic part. Its TLC plate gave deep yellow color when sprayed with Sulphuric acid, which is a characteristic of terpenes.

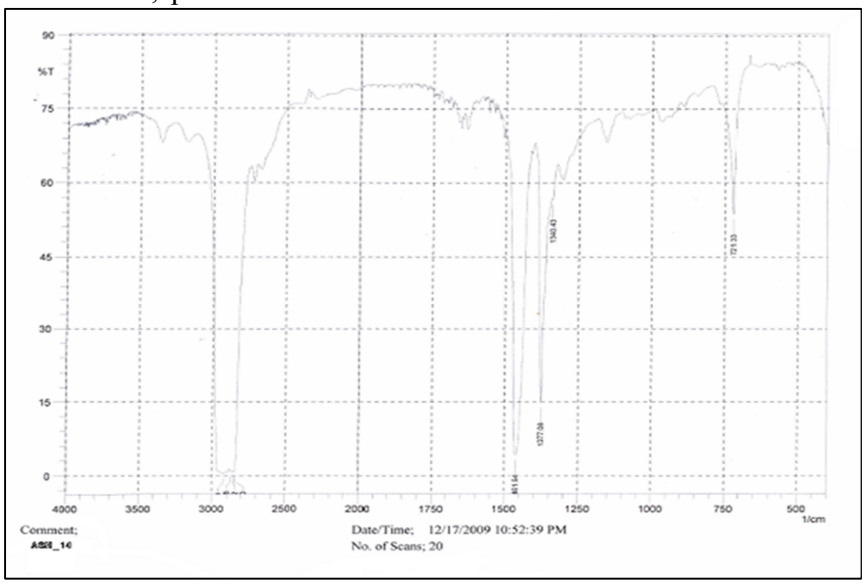
The UV spectrum of compound ASH-14 (Appendix 1) shows absorbance peaks at 280 nm and 350 nm which may support that the compound is both aromatic and olefinic carbons. This region of absorbance may tell us the possibility of conjugation. The presence of an aromatic group and olefinic group in compound ASH-14 is also supported by its 1H NMR spectrum (Appendix3)

In the IR spectrum (Appendix 2) showed absorption bands assigned to an olefinic system (1458 cm^{-1}), the methyl C-H stretching in this compound is indicated by a sharp peak at 2920 cm^{-1} cm-1.). Three sharp peaks near to 1625 cm^{-1}, 1625 cm^{-1} and 1462 cm^{-1} are indicative of aromatic ring. It also revealed the presence of a week alkenes C-H stretching at 3010 cm^{-1} another

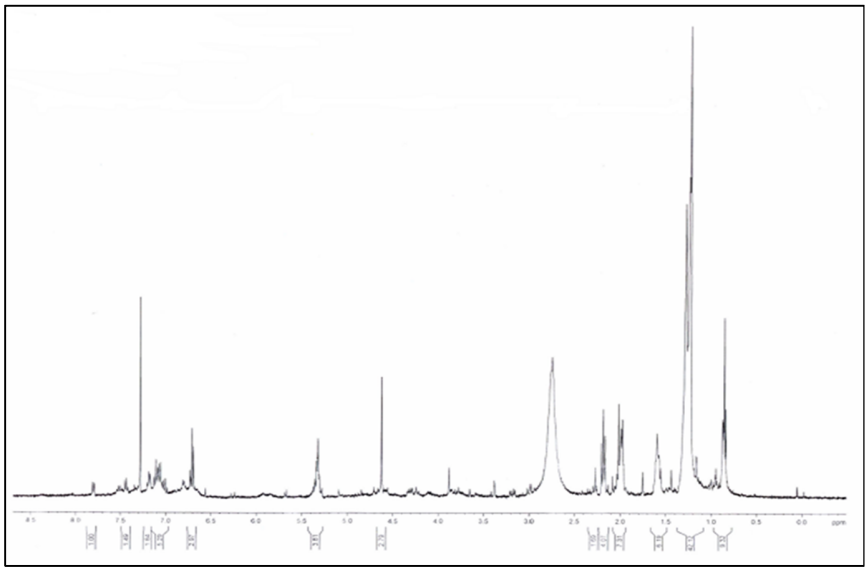
peaks around 900 cm^{-1} is for a methyl group attached to a quaternary carbon. Therefore the IR spectrum depicts the presence of aromatic group, olefinic group, methyl group and methyl group attached to a quaternary carbon in the compound.

1H NMR δ (400MHz, CDCl3): δ 4.70 and δ 5.40 multiplet and integrated for one proton indicate olefinic proton, δ 7.25 (sharp singlet the solvent $CDCl_3$), δ 3.12 with multiplet multiplicity indicate a proton attached with a chiral carbon, δ 2.80 with doublet multiplicity and integrated for one proton indicate methine, protons in δ 2.19-1.15 multiplet and integrated for 22H showed methylene protons, a δ 1.03 singlet and integrated for 6H, showed methyl protons, δ 1.12 singlet and integrated to 3H, 0.87 doublet & integrated to 6H

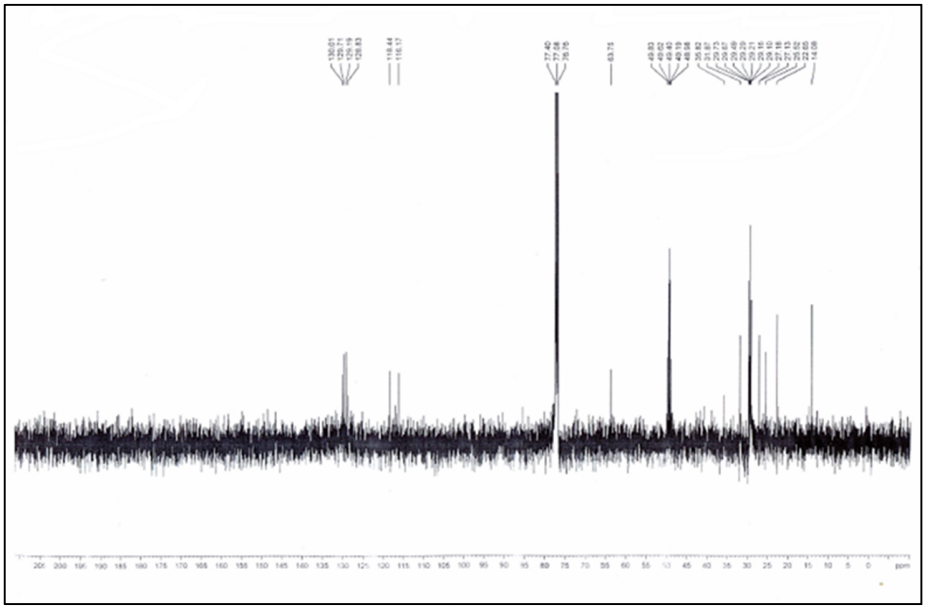
showed methyl protons, also the aromatic protons are observed at δ 6.70 multiplet and integrated to 1H of C-1 and C-2 ,7.50 multiplet and integrated to 1H of C-2 and δ 7.10 multiplet and integrated to 1H of C-3.



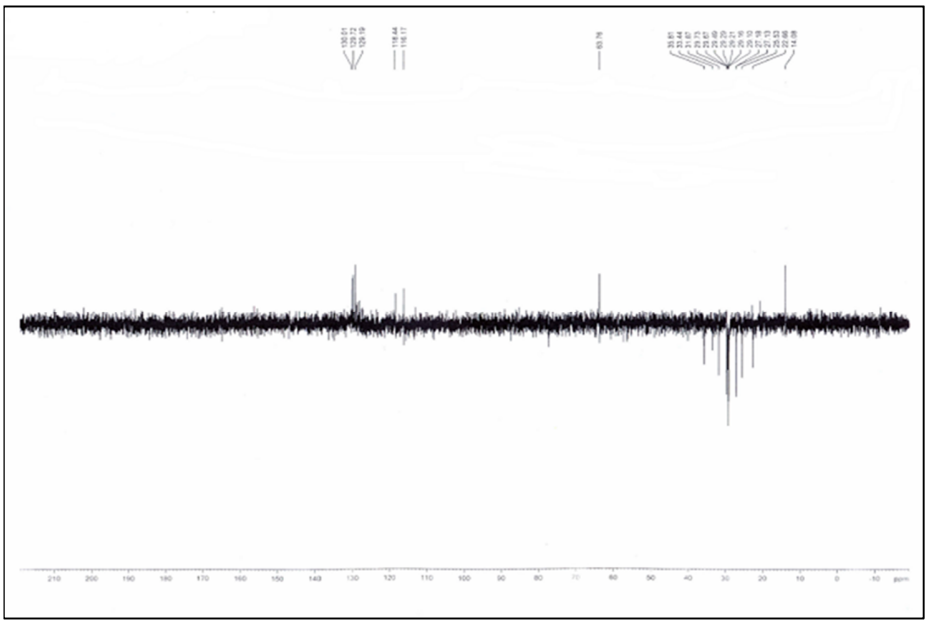
Appendix 1: IR Spectera of compound ASH_1



Appendix 2: 1HNMR spectera of compound ASH-14



Appendix 3: 13 NMR Spectera of compound ASH-14



Appendix 4: Dept Spectera of compound ASH-14

The 13C spectrum of compound ASH-14 (Appendix 4, Table 4) also strengthens the fact that there are two different chemical shift regions, one from δ 110- 131 which is for the aromatic and olefinic carbons and the other from δ 14-50 which is for the aliphatic group and there is peak at 63.75 which is to tertiary carbons. The 13C spectrum of compound ASH-14 is therefore in line with the 1H NMR spectrum. The region from δ 14 to δ 64 has twenty one peaks for twenty three carbon atoms. The peaks at δ 22.65 and 27.13 are each for two carbon atoms that

overlapped. There is a peak at δ 76.76- δ 77.4 which is for solvent, ($CDCl_3$). The region from δ120 to δ130 is that of the aromatic and olefinic carbons of compound ASH-14. In the 13C spectrum, there are six peaks for eight carbon atoms in the aromatic and olefinic region. Therefore, compound ASH-14 has a total of twenty-seven peaks that for thirty carbon atoms.

The DEPT (Appendix D) showed twenty -one peaks corresponding to twenty- four carbon atoms. Compound ASH-14 have five methyl groups at δ 14.08, δ 22.65 and δ 27.13, The peaks at δ 22.65 and δ 27.13 tells us there is an overlapping of two methyl carbons which are the same in chemical shift. There are eleven methylene groups at δ 25.53, δ 27.20, δ 29.10, 29.21, δ 29.29, δ 29.49, δ 29.67, δ 29.73, δ 31.20, δ 33.44, and δ 35.81.Also there are seven methine groups, out of which one is in the aliphatic region at δ 63.76, two are in the olefinic region at δ 116.19, δ 118.44, and four are in the aromatic region at δ 129.19, δ 129.71, which are for two carbon atoms overlapped, and δ 130.01. The difference in the number of carbon atoms of the 13C spectrum and the DEPT spectrum is six. Therefore, compound ASH-14 has six quaternary carbon atoms at δ 48.98, δ 49.19, δ 49.40, δ 49.62, δ 49.83 and 128.83, which is for two carbon atoms attached.

From all the above data, the following structure suggested for compound ASH-14.

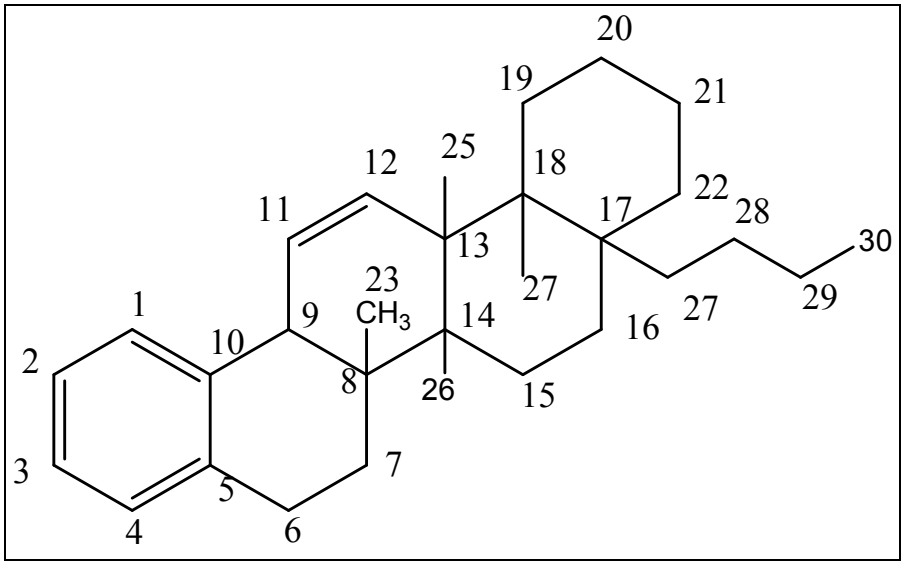


Fig 3: Suggested structure of compound ASH-14

4. Conclusion

Present study deals with phytochemical investigation of leaves extract of *Adhatoda schimperiana*. Table 2 shows the results of Phytochemical Screening of leaves of *Adhatoda schimperiana*. Chloroform extract of leaves of *Adhatoda schimperiana* shows the presence of alkaloids, polyphenols, flavonoids, glycosides, phytosterols, saponins, triterpens, and quinines as a major class of compounds. However, less presence of tannins was observed.

The presence of oily components, the difficulty to remove green color from laboratory equipment and the absence previous work on this plant made separation difficult. However, with repeated use of column chromatography one compound was isolated and partially characterized ASH-14. ASH-14 was partially characterized from *Adhatoda schimperiana* for the first time. To the best of our knowledge compound is reported for the first time.

The secondary metabolities of this plant are seasonal; therefore who wants to perform phytochemical investigation on this plant must collect the plant materials on seasons June-August. The difficulty to remove the green color

from laboratory equipments suggests that *Adhatoda schimperiana* as *Adhatoda zeylanica* may be also used to manufacture dyes.

5. References

1. Akerele O. WHO's traditional medicine program: progress and perspectives. WHO Chron 1984; 38(2):76-81.
2. Duru S, Grierson DS, Afolayan AJ. Antimicrobial activity of Solanum aculeastrum. Pharm Biol 2006; 44:283–286.
3. Ayehu A, Abebe D. Medicinal plants and enigmatic health practices of Northern Ethiopia Addis Ababa, Ethiopia, 1993.
4. Abebe D, Ayehu A. Medicinal plants and enigmatic health practices of Northern Ethiopia. Berhanina Selam Printing Enterprise, Addis Ababa, Ethiopia, 1993; 341.
5. Murthy PN, Moges G, Hymete A, Gebremariam T. Antimicrobial and phytochemical screening of Justicia Schimperiana. Eth Pharm 1993; 11:47-53.
6. Getahun A. Some common medicinal and poisonous plants used in Ethiopian folk medicine. Addis Ababa University, Addis Ababa, 1976, 3-5.
7. Abebe D. The role of Herbal Remedies and the Approaches Towards their Development, In Proceedings of the Workshop on Development and Utilization of Herbal Remedies in Ethiopia, Nazareth, Ethiopia, 1996, 111:29.
8. Teferi G. The use of medicinal plants in self-care in rural Ethiopia. J Ethnopharmacol 2003; 87:155-161.
9. Kokate CK. Practical Pharmacognosy. Edn 4, Vallabh Prakashan, New Delhi, India, 2000, 107-111.
10. Harbone JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Edn 3, Chapman & Hall, London, UK, 1998, 60-66.