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# SEED VOLATILE OIL ANALYSIS AND *INVITRO* ANTIMICROBIAL ACTIVITY OF POTENTIAL MEDICINAL HERB: *ACHYRANTHUS ASPERA* (WILLD)

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**ABSTRACT**

The present paper deals with hydro distillation of Achyranthus aspera seeds and GC-MS analysis. The total composition of the oil was 76.7%. The Achyranthus aspera seed volatile oil was tested against pathogenic bacteria and Fungi.

**Keywords:** Achyranthus aspera, GC-MS Analysis Volatile oil, Antimicrobial activity, Antifungal activity.

## INTRODUCTION

*Achyranthus aspera* (Amaranthaceae) a perennial stiff erect herb, 0.2, 2.0 m high is growing up to 1000 m height. Stems are square, leaves elliptic ovate or broadly rhombate, 5.22 cm long, 2.5 cm broad, and pubescent. The inflorescences are 8 - 30 cm long, with many single, white or red flowers, 3 - 7 mm wide. Flowering time is in summer. The plant is widespread in the world as a weed. In the northern part of India it is known as a medicinal plant in different systems of folk medicine. Plant volatile oils are predominant in many industries, particularly in pharmacy, clinical and food preservative industries. The oil and its constituents are well documented as antimicrobial agents [1,2]. The volatile oils are complex mixtures of compounds which mainly having monoterpines, sesquiterpines hydrocarbons with general formula $(C_5H_8)_n$. [3,4]. Compounds in the seeds of *A. aspera* are the saponins A and B. They are glycosides of oleanolic acid. The carbohydrate components are the sugars D-glucose, L-rhamnose, Dglucuronicacid (= Saponin A). Saponin B is the ß-D-galactopyranosyl ester of Saponin A. The

content of free oleanolic acid in *A. aspera* roots is 0.54 % (4, 34).From the roots ecdysterone and oleanolic acid have been isolated. In the unripe seeds saponines, oleanolic acid, amino acids and hentriacontane, a long chained carbohydrate, have been found. In the shoots an aliphatic dihydroxyketone 36,37-dihydroxyhenpentacontan-4-on and triacontanol could be found. Two long chain compounds, isolated from the shoots, have been characterized as 27-cyclohexylheptacosan-7-ol and 16-hydroxy.26-methylheptacosan-2-on by chemical and spectral investigations .The petrol extract of the shoots produced a yellow semi-solid mass. From this a pink coloured essential oil with a pleasant odour and an aliphatic alcohol (17-pentatriacontanol) were found[5,6.]

**EXPERIMENTAL**

The seeds of *Achyranthus aspera* were collected from Nallamalai foresti, Andhra Pradesh., India. The seeds (1Kg.) were fine powdered and hydro-distilled at $100^0$C in a Clevenger apparatus[7]. The obtained volatile oil was collected, dried over anhydrous Sodium Sulphate, stored in brown bottles and finally kept in refrigerator for further GC-MS analysis.

**GC-MS Analysis:-**

A Shimadzu 17A GC coupled with Shimadzu QP5050 A (quadruple) Mass Spectrometer (Shimadzu, Japan), equipped with EI and a fused Silica Column DB-5(30mx0.25mm i.d.) of 0.25μm film thickness The oven temperature at $50^0$C for 5 minutes and then programmed from 50-280 $^0$C for 40 minutes. Helium flow rate of 2ml/min, with a split ratio of 1:30 mode was used for sample injection of 1μl and ionization voltage of MS-analysis was run by EI technique at 70ev. The volatile oil constituents were identified by matching their MS and retention index data with those of standards ethnic spectra and by matching their fragmentation pattern in Mass Spectra with those of WILEY 139.LIB & NIST 12.LIB.(3) The retention indices were calculated by Kovat's procedure[8,9].

**Antimicrobial susceptibility test:-**

Gram-positive and gram-negative bacterial species, yeast and dermatophyte species were used to test the antimicrobial activity of the oil extracted from *Achyranthus aspera*. Antimicrobial activity by the disc diffusion

method was determined using the Kirby-Bauer method.[10, 11.] The discs of 6mm diameter were prepared with Whatmann no 1 filter paper. The plant volatile oil of concentration 20µg for the test was applied to the discs for the test. Inoculum was prepared with fresh cultures of bacterial strains, which were cultured in Tryptic-soy agar for 18hrs at $37\pm1^0$C with physiological saline, $3x10^6$ cells $ml^{-1}$. Inoculum density was compared with Mac-Farlands standard solution of $BaSO_4$ (0.1ml of 1% $BaCl_2$+9.9ml of 1% $H_2SO_4$). fungi, yeasts and dermatophytes were cultivated on Sabouraud dextrose agar with addition of 50mg/l Chloramphenicol (sigma, Germany) for 5 days for yeasts and 10 days for fungi and dermatophytes at $25\pm1^0$C. 1ml of inoculum was mixed with 22±5ml of Muller Hington agar for bacterial strains, and the same amount of inoculum was cultured in Sabouraud agar for fungi. Then the agar was inoculated with the culture and incubated at room temperature for 25minutes. 20µg oil concentrated discs were arranged on the surface of the inoculated agar plates and pressed the discs gently to adhere perfectly to the surface of the agar. Under aerobic condition the plates were incubated for 24-48hrs at $35\text{-}37^0$C. After incubation the diameter of the zone of inhibition was measured and recorded the results.[12,13]

**RESULTS AND DISCUSSION**

The preliminary GC-MS analysis of the *A.aspera* volatile oils shows 29compounds as shown in Table.1. Which includes two new chemical compounds viz. *2(1H)-Naphthalenone*($C_{11}H_{18}O$) and *Uvdin* ($C_{15}H_{24}O_3$).

The results of antimicrobial activity of the *A.aspera* volatile oil by the disc diffusion method are presented in table-2. The comparative study with the standard, the zone of inhibition in case of oil against *staph.,* is 18mm even though the quantity of oil used was 100 times less than the quantity of standard which clearly reveals that the oil is very effective as antimicrobial agent.

CONCLUSIONS

*A.aspera* was already considered as medicinal plant. The antimicrobial activity of plant oil has been further highlighted in this report that can pave the way for the emergence of new herbal drug, which acts against gram positive, gram negative and Fungi. Hence the oil can be used as good herbal antibiotic drug.[14-18]

Table-1: Premier Chemical composition of Seeds of *Achyranthus aspera* volatile oil constituents.

| Compound | Percentage |
|---|---|
| 3-Methyl Nonane | 0.6 |
| α-Pinene | 1.2 |
| Sabinene | 1.4 |
| β-Pinene | 1.3 |
| Myrcene | 0.6 |
| n-Decane | 0.4 |
| α-Phellandrene | 0.6 |
| *p*-Cymene | 9.0 |
| 1-Methyl-3-propyl benzene | 0.7 |
| γ-Terpinene | 0.5 |
| 1-Ethyl-2,3-dimethyl benzene | 0.2 |
| *2(1H)-Naphthalenone* | 2.6 |
| Fenchone | 1.1 |
| Terpinen-4-ol | 0.7 |
| *p*-Cymene-8-ol | 0.4 |
| Nerol | 1.3 |
| Dihydrocarvone | 0.3 |
| Carvone | 2.0 |
| Thymoquinone | 11.8 |
| Trans-Anethole | 27.1 |
| Carvacrol | 3.7 |
| α-Longipinene | 0.3 |
| *n*-Tetradane | 0.2 |
| Uvidine | 1.3 |
| *n*-Hexadecane | 0.2 |
| Apiole | 1.0 |

**Table-2: Antimicrobial activity of the *A.aspera* volatile oil by Disc diffusion method.**

| | Microbes | Inhibition Zone in mm Oil disc (20µg) | Standard |
|---|---|---|---|
| Gram-Positive Bacterial species | *Staphylococcus aureus* MTCC 737 | 17 | 25 |
| | *Streptococcus pneumoniae* MFBF | 14 | nt |
| | *Bacillus subtilus* MTCC 121 | 12 | nt |
| | *Micrococcus luteus* MTCC 1541 | 14 | 20 |
| Gram-Negative Bacterial species | *Pseudomonas aeruginosa* MTCC 1688 | 12 | 11 |
| | *E.Coli* MTCC 1687 | 16 | 17 |
| | *Salmonella typhi* MFBF | 10 | nt |
| | *Proteus vulgaris* MTCC 1771 | 12 | 12 |
| Yeast like fungi | *Candida albicans* MTCC 184 | 10 | 12 |
| Fungi | *Aspergillus niger* MTCC 1344 | 10 | 13 |
| | *Aspergillus flavus* | 06 | 14 |
| | *Tricoderma vibriae* | 0 | 16 |
| | *Penicillium rubrum* | 0 | 16 |
| | *Chaetomium globosum* | 07 | 12 |
| Dermatophyte | *Trichophyton mentagrophytes* | 06 | 12 |

nt=not tested., Clindamycin 2mg/ml for S.aureus, Gentamicin 2mg/ml for *Ps. aeruginosa, Proteus vulgaris*; Tetracycline 3mg/mlE.Coli and *B.subtilus* Clotrimazole 5mg/ml for *C. albicans*; Nystain 10mg/ml for *A niger* and *T.mentagrophytes*.
MFBF: number of strains from the collection of microorganisms of the Dept. of Microbiology and biotechnology, Anantapur.
MTCC: Microbial type culture collection centre.

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