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Comparative Analysis on GCMS, Physicochemical and Anti-Microbial Properties of Aerial Parts of Plant *Artemisia vulgaris* Obtained from Two Different Altitudes

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Abstract: ***The oil obtained by hydro distillation of Artemisia vulgaris using Clevenger apparatus revealed presence of alkanes, oxygenated and non-oxygenated monoterpenes, and sesquiterpenes. The oxygenated monoterpenes were either alcohols or esters, and the oxygenated sesquiterpenes were in the form of acetates and oxides. AVO1 contained 70 different constituents whereas AVO2 contained about 42 different chemical constituents. The physiochemical characters showed that the yield percentage varied from 1.43 to 1.28, acid value varied from 0.397 to 0.45and specific gravity varied from 0.878 to 0.902 in AVO1 and AVO2 respectively. Likewise the oil was found to be insoluble in water and fixed oil and soluble in most organic compounds. Volatile oil from both the altitude showed a significant antibacterial and antifungal effect. A large inhibition was seen in S. aureus, E.coli and enterococci species. Both the sample showed antifungal activity against Candida species.***

Keywords: Artemisia vulgaris, hydrodistilation, essential oil, GCMS, oil yield value, sesquiterpene, organoleptic characters

1. Introduction

Since immemorial times medicinal plants have been virtually used in all cultures as a source of medicine. Plants are rich source of effective and safe medicines and from ancient times as a main source of primary health care herbal medicines have been used in many nations. About 80% of world populations are still dependent on traditional medicines [1]. More than 900 types of precious medicinal plants are said to be found in Nepal among 7000 species of medicinal plants recognized all over the world but only few of them are used for their medicinal value[2]. In developing countries a major part of the total population still uses traditional folk medicine obtained from plant resources for primary health care. According to WHO estimation 80% of world's population in rural areas rely on herbal traditional medicines as their primary health care [3].This leads in getting growing interest for the study on properties and uses of medicinal plants .It is estimated that only 15-20% of the population of Nepal - living in and around urban areas have access to modern medicinal facilities, whereas the rest depend on traditional medicines [4].

Nepal is a country with wide range of geographical altitude and climate variations. From various independent researches, it has been found that number and percentage of chemicals vary to a great extent when using plants from different location, altitude, storage conditions and growth period [5]. Thus modern research should focus not only on what chemical the plant possess but in what proportion and how best to cultivate it so as to get maximum yield. Artemisia vulgaris is a magical plant which has been used since ancient times in Nepal mainly as ethno medicine and folk medicine. Volatile oil of Artemisia have been investigated as antimicrobial, antioxidant, cytotoxic and anticonvulsant agents [6].Variation in the volatile components of these plants may occur during plant ontogeny or growth at different altitudes. This study observe chemical variation of Artemisia vulgaris collected from two different altitude and variation in its antimicrobial property.

The active components in the extract of Artemisia vulgaris are flavonoids, coumarins, sesquiterpene, lactones, volatile oils, inulin, and traces of alkaloids. Volatile oil contain about 70 different phytochemicals. The chief compounds of volatile oils include camphor, camphene, α-thujone, germacrene D, 1, 8-cineole and β-caryophyllene [7].

Artemisia vulgaris are rhizomatous perennial herbs exhibiting extreme variation in morphology. It has dark green 1–10 cm long and 3–7.5 cm wide leaves with the upper surface slightly hairy and the lower surface covered with silvery-white wooly hairs (< 1 mm long)[8] .The leaves on the lower portion of the stem are coarsely segmented, with each segment further dissected. The middle to upper leaves are smaller, but are more coarsely toothed than primary leaves [9].

Figure 1: Leaf of Artemisia vulgaris

2. Experimental section

2.1 Sample collection and extraction

The plant leaves were collected from two different location in September from Kirtipur region of Kathmandu district at an altitude of 1400m above sea level, labelled as AV01 and from Itahari region of Sunsari district at an altitude of 200m above sea level, labelled as AV02.

Fresh leaves of both Artemisia vulgaris samples were subjected to essential oil analysis. A 100 g sample of fresh leaves were mixed with 700 ml distilled water and subjected to hydro distillation in a Clevenger-type distilling apparatus for 2h. The obtained oil was dried over anhydrous sodium sulphate at 4°C and subjected to GC-MS analysis .The test Method was ISO 7609:1985 performed in Department of Plant resources, Nepal Government, Thapathali, and Kathmandu, Nepal.

2.2 Physiochemical Character Study

The following physiochemical properties were studied.

2.2.1 Percentage Yield [10]

The percentage oil yield was calculated by using following relation

$$\text{Percentage yield} = \frac{\text{weight of oil}}{\text{Total weight of material used for extraction}} \times 100$$

2.2.2 Organoleptic characters

The organoleptic characters were identified by means of sense of sight, smell and touch.

2.2.3 Solubility

A known amount of the solvent was put in a test tube. Then the substance whose solubility is to be determined (oil) was added, a clear layer observed indicates insolubility.

2.2.4 Specific gravity [10, 11]

Specific gravity was determined using specific gravity bottle using the formula

$$D = \frac{W2 - W0}{W1 - W0}$$

Here,
W0 is the mass of empty specific gravity bottle.
W1 is the mass of specific gravity bottle filled with water and
W2 is the mass of specific gravity bottle filled with oil

2.2.5 Acid Value determination [10]

5ml of extracted oil and 25mL of neutral alcohol were mixed and heated on a steam bath for 10-15 minutes so as to dissolve the oil. The content was titrated using 0.1N KOH using phenolphthalein as indicator. The acid value was determined by the following formulae

$$\%I = V \quad X \quad C \quad X \quad \frac{56.11}{m}$$

Here,
V is volume of the KOH solution used for the titration,
C is molarity of KOH and
m is mass in g of the test sample.

2.3 Antimicrobial and Antifungal activity[12]

The antibacterial and antifungal activity were evaluated through agar cup diffusion method. The media was sterilized in the autoclave at 121 ^{o}C for 21 minutes. Then the sterilized media was cooled and the poured to sterilized petri dish with size 90mm diameter and left to solidify. Bacterial suspension was prepared by inoculating loop full of desired microorganisms. The suspension was then swabbed in the media with sterile cotton swab. 20 µl of oil was then poured in the bore with help of a micropipette. Three plates were used for each microorganism. This was then refrigerated and kept in incubator for 24 to 48 hours. The Mean zone of Inhibition for both the samples AV01 and AV02 were taken and recorded.

2.4 Microorganisms used

Bacterial strains consisted of *E.coli, Staphylococcus aureus, Enterococci sp, Psudomonas Sp* and *Salmonella typhii*. Similarly fungal stain used was *Candida.*

3. Results

3.1 Results of physiochemical characteristics

The results of different physiochemical characters have been reported in Table1, Table 2, Table 3 and report of GCMS analysis have been reported in Table 4. The antimicrobial test results have been shown in Figure 2 and Figure 3.

Table 1: Organoleptic Characteristics

Characteristics	*Sample AV01*	*Sample AV02*
Colour	Dark Green	Dark Green
Odour	Pungent Camphorous	Pungent Camphorous
Appearance at room temperature	Transparent	Transparent

Table 2: Solubility in different solvents

Characteristics	*Sample of AV01 and AV02*
Hexane	Soluble at any volume
90% alcohol	clearly soluble at any volume
Chloroform	Soluble at any volume
Distilled water	Insoluble
Acetone	Slightly soluble
Coconut Oil	Insoluble

Table 3: Acid Value, Yield percentage and Specific gravity

Parameters	*Sample AV01*	*Sample AV02*
Acid Value	0.397	0.45
Oil Yield Percentage	1.43	1.28
Specific Gravity	0.8786	0.9023

3.2 GC-MS analysis

GC-MS result of both the samples oil showed that AVO1 contained 64 different chemical constituents whereas AVO2 contained only 42 constituents. The oil consisted of alkanes, oxygenated and non-oxygenated monoterpenes, and sesquiterpenes. The oxygenated monoterpenes were either alcohols or esters, and the oxygenated sesquiterpenes were in the form of acetates and oxides. Major constituents were identified as sabinene (2.68 and 5.85%), Eucalyptol (3.59 and 6.02 %) thujone (5.51 and 3.55 %) camphor (7.46 and 4.52%) borneol (3.36 and 3.12%) Carophyleene (4.97 and 7.90%). It was seen that amount of major chemical constituents were more in AVO1 as compared to AVO2. Some of the chemical constituents are as follows

Table 4: List of most common chemical constituents

S.N.	*Chemical constituents*	*AV01*		*AV02*	
		Area %	*Retention time*	*Area %*	*Retention time*
1	Sabinene	2.68	13.617	5.85	13.617
2	Limonene	1.05	16.253	0.58	16.253
3	Eucalyptol	3.59	16.389	6.02	16.261
4	Chrysanthenone	-	-	1.71	19.971
5	Thujone(β)	5.51	20.066	3.55	20.059
6	S.N.	3.93	20.582	6.98	20.601
7	Camphor	7.46	21.979	4.52	21.958
8	Borneol	3.36	22.998	3.12	22.993
9	Caryophyllene(E)	4.97	34.507	7.90	34.515
10	Viridiflorol	0.60	41.958	3.14	40.836
11	Santolinatriene	2.95	10.631	0.41	10.634
12	Piene(α)	1.37	11.769	0.52	11.776
13	Camphene	1.53	12.438	0.74	12.445
14	Pieneβ	1.11	13.754	-	-
15	Myrene	0.45	14.455	-	-
16	Bergamal	0.99	17.777	-	-
17	Terpinolene	0.34	19.183	0.80	19.691
18	Isoborneal	0.49	22.545	-	-
19	Myrtenol	0.35	24.464	-	-
20	Cupaeneα	0.73	32.585	0.80	32.591
21	Cubenene	0.56	33.397	0.52	33.206
22	Maalieneβ	1.05	34.066	2.74	34.076
23	Fencheneα	-	-	0.62	12.377
24	Intermedeol	1.28	43.896	0.96	43.841
25	Cadineneγ	0.51	38.589	-	-

3.3 Antimicrobial results

Antimicrobial result showed that both samples have a significant zone of inhibition against most of the bacteria and fungi. Out of the 5 strains of antimicrobial agent tested only Pseudomonas was resistant to it while all other showed significant sensitivity. Similarly, both the sample showed antifungal activity against Candida species.

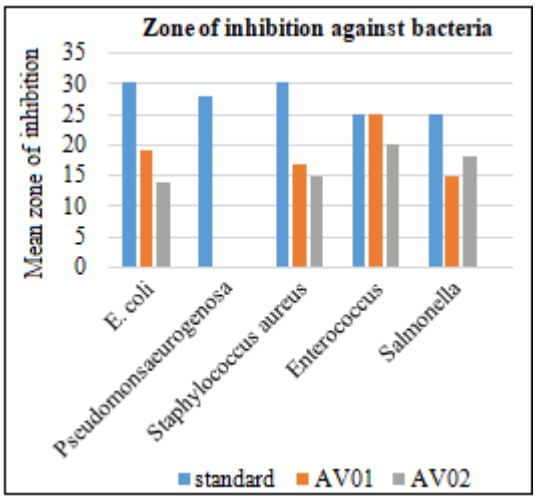

Figure 2: Zone of inhibition against bacteria

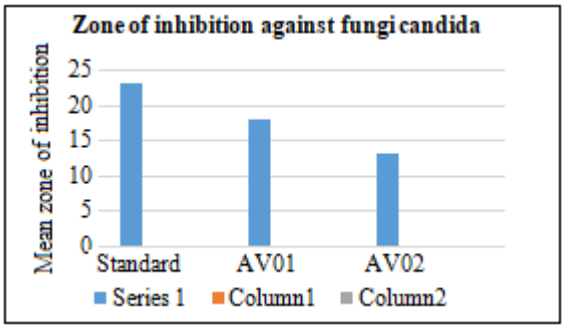

Figure 3: Zone of inhibition against fungi

4. Discussion and Conclusion

This work comprised of comparative study of plant description, microscopy, chemical constitution, physiochemical constitution, and anti-bacterial activities of volatile oil obtained from aerial parts of Artemisia vulgaris collected from two different altitudes. The volatile oil of Artemisia vulgaris contains a number of chemical constituents which can be evidenced from GC-MS analysis and shows anti-bacterial and antifungal activities.

This study reveals that aqueous extracts of aerial part consists of a wide variety of photochemical which differs in altitudes. In this study we found that oil yield percentage by hydro distillation were dependent on a number of factor. The first one being the temperature. It was observed that maximum yield was when temperature was maintained at 60 to 70 degree Celsius. Temperature greater than that showed less yield as well as bumping of the Artemisia-water paste. Another crucial

factor was size of the plant. Smaller plant were found to be devoid of volatile oil. As we compare the physiochemical studies of these oil the yield percentage of AVO1 was greater with less acid value as compared to AVO2 with less yield percentage and more acid value. Organoleptic characters and solubility were similar whereas specific gravity showed a very minimum difference.

As we focus on the anti-bacterial activity, it reveals that maximum activity is shown by oil obtained from AVO1 as compared to AVO2. The study by Asghari et. al. [12], has also demonstrated that the oil was active against S. aureus, E. coli, Enterococci and L. monocytogenes [13]. The most susceptible microorganisms to both the oil were E. coli, Staphylococcus aureus and Enterococci respectively. Also, Pseudomonas showed least sensitivity and was resistant to both the oil respectively. This demonstrates that the actual anti-bacterial constituent from the oil can be used as a narrow spectrum antibiotic for Staphylococcus aureus, E coli and Enterococcus Species. The study by Gyawali et al[13] , has demonstrated that Artemisia vulgaris was sensitive against S. aureus, E. coli and K. pneumoniae, respectively with MIC value of 125/20, 3.9/20 and 800 µg/20 µl, respectively[14]

This plant has many other essential features which needs to be explored. As very less study on this plant has been done, it has a virgin area of research .We recommend further studies on different parts of this plant and the need for isolation of the active chemical constituents along with molding it into formulations for use.

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