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Phytochemical profiling and GC-MS analysis of *Kyllinga nemoralis* (J. R. forst. & G. forst.) dandy ex hutch. & dalziel (*Cyperaceae*)

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Abstract
*Kyllinga nemoralis (*J. R. Forst. & G. Forst.) Dandy ex Hutch & Dalziel is a perennial herb from the sedge family, Cyperaceae. The plant is commonly known as White Water Sedge. The present study were carried out to detect the presence of phytochemicals in the methanol extracts, its quantification and essential oil profiling using GC-MS. Preliminary phytochemical analysis of *K. nemoralis* revealed the presence of Carbohydrates, Phenols, Flavonoids, Flavones, Tannins, Saponins, Alkaloids, Steroids, Terpenoids, Coumarins, Quinones, Lignins and Fats and Oilsin all the plant parts. Quantitative phytochemical analysis resulted in the detection of highest percentage of phytocompounds in the plant extract. Phenols were found to be high in rhizome (37.08µg/mg). Polyphenol content (22.97µg/mg) in the rhizome (19.97µg/mg) and whole plant was relatively higher than in the aerial part (13.13µg/mg). Total tannin (223.75µg/mg) and flavonoid (156µg/mg) content was high in whole plant. Terpenoid content was observed to be high in aerial portion (80.61µg/mg). GC-MS analysis of the essential oil of rhizome of *K. nemoralis* revealed the presence of Terpenoid compounds in high amount [a'-elemene (63.15%)].Thus it is evident that the phytocompounds from this plant could be possibly isolated and utilized effectively as sources of phytochemicals.

Keywords: *Kyllinga nemoralis*, phytocompounds, phytochemical profiling, a'-elemene

Introduction
Phytochemicals protect plants from disease and damage, and also contribute to the plant's colour, aroma and flavour (Gibson *et al*., 1998) [7] (Mathai, 2000) [17] Recently, it has been clearly shown that they also have roles in the protection of human health, when their dietary intake is insignificant (Samrot *et al*., 2009) [24] (Dipak *et al*., 2010) [4]. Aside from the non-volatile organic compounds, the volatile essential oils are also considered as biologically active. Essential oil is used in aromatherapy and phytotherapy. They also have antibacterial, antifungal potential and anti-cancerous properties. Essential oils from different parts of the same plant may have completely different scents and properties. The quantity of essential oil extracted from the plant is determined by many interrelated factors, such as climatic, seasonal and geographical conditions, harvest period and extraction techniques (Pannizi *et al*., 1993) [22].
The plant material selected for the present study was *Kyllinga nemoralis* (J. R. Forst. & G. Forst.) Dandy ex Hutch. & Dalziel. It is a perennial herb from the family Cyperaceae, commonly known as the white water sedge or white head spike sedge. These grow chiefly in marshy and wet places, shaded meadows, rock crevices and road sides. The plant is grass-like in habit and grows up to 50cm in height. Rhizomes serve as the organs of perennation. The shoot is grass- like solid and triangular. Leaves are simple, tristichous, grass- like, generally crowded near the base of the stem having head inflorescence. The seed is a nut and is brown in colour.
The plants have a characteristic rich aroma and are considered medicinal by traditional practitioners. Rhizomes of the plant are fragrant, sweet and the paste of rhizomes mixed with milk is used internally against worm infections (Silja *et al*., 2008) [26]. Plant leaves have been used traditionally for relief from malarial chills, pruritus of the skin; thirst due to fever and diabetes (Panghal *et al*., 2010) [21] and as anti-venom (Oudhia, 1999) [20]. The rhizomes of the plant contain many biologically active chemicals, and the extracts have been used in traditional folk medicine to treat many diseaseconditions. It is considered as an anti-diarrhoeal, diuretic, stomachic and expectorant (Sivarajan & Balachandran, 1994) [27]. It is also used in fever, hepatopathy, splenopathy, diabetes and tumours (Warier *et al*., 2008) [32] (Somasundaram *et al*., 2010) [29].The rhizomes and roots contain allelopathic oils that contribute to its weediness (Komai & Tang 1989) [16]. The present study focussed on the qualitative and quantitative analysis of phytochemicals from the methanolic extracts of plant parts of *Kyllinga nemoralis* and the GC-MS analysis of its essential oil.

Materials and methods
1. Plant material
Kyllinga nemoralis seedlings were raised in the field of Department of Botany, University of Kerala, Kariavattom. The plant was authenticated by Dr. G. Valsala Devi, Curator, Department of Botany. Fresh plants were collected from the experimental field for the studies.

2. Preparation of the plant extract
The collected fresh plants were cleaned, segregated into three categories (whole plants, rhizomatous and aerial portions) shade dried and stored separately. The shade dried samples were ground to fine powder and subjected to Soxhlet extraction using methanol. About 20g of powdered plant samples was extracted with 250ml of methanol for about six hours. The residual plant extract was stored in a refrigerator and later used for phytochemical analysis.

Preliminary Phytochemical Analysis

1. Qualitative analysis

Preliminary phytochemical analyses of plant powder from different parts of the plant such as whole plant, aerial portions and rhizomes were carried out using the standard procedures of Harborne (1973) [11] (Harborne and Williams,2000) [10] (Trease and Evans, 2002) [30]

Table 1: List of phytochemicals qualitatively analysed

S. No.	Name of the phytochemical	Tests
1	Carbohydrates	Molisch's test, Fehling's test, Benedicts test,
2	Glycosides	Borntragers test,Keller killiani test,
3	Proteins	Biuret test, Xanthoproteic test
4	Aminoacids	Millions test, Ninhydrin test
5	Phenols	Ferric chloride test
6	Flavanoids	Shinodas test, Alkalaine reagent test, Lead acetate test
7	Flavanols	Mineral acid test
8	Flavones	Lead acetate test
9	Tannins	Ferric chloride test
10	Phlobatannins	Lead acetate test, Hydrochloric acid test
11	Saponins	Foam and froth test
12	Alkaloids	Mayers test, Dragendorff's test, Wagners test
13	Steroids	Libermann-Buchard's test, Salkwoski reaction
14	Terpenoid	Salkowski's test, LibermannBuchard's test
15	Dextrose sugar,	Keller killiani test
16	Coumarins,	Test with alcoholic NaOH and Conc:HCL
17	Quinones,	Test using Conc:H_2SO_4
18	Anthraquinones,	Test using Chloroform and Ammonia
19	Lignin	Saffranin test
20	Fixed oils&fats	Hydrodistilation
21	Gums and	Test using absolute alcohol
22	Mucilage,	Test using absolute alcohol
23	Resins	HCL test
24	Acids	Sodium bicarbonate test

Courtesy: (J B Harborne 1973, 2000) [11, 10] (Trease and Evans. 2002) [30]

2. Quantitative study of phytochemicals

Table 2: List of phytochemicals quantitatively analysed

Sl no:	Phytochemicals quantified	Reference	Standards used
1	Total phenol content	Ghasemzadeh *et al.*, 2010 [6]	Gallic acid
2	Total polyphenol content	Hakiman and Maziah, 2002 [8]	Gallic acid
3	Total flavonoid content	Har and Intan, 2012 [9]	Morin
4	Total tannin content	Saad *et al*., 2014 [23]	Tannic acid
5	Total alkaloid content	Shamsa, *et al*., 2008 [25]	Atropine
6	Total terpenoid content	Indumathi *et al*.,2014 [14]	Linalool
7	Total saponin content	Hiai *etal.*, 1976 [12]	Quillaja saponin

Quantitative analyses of phytochemicals (7 phytocompounds) were carried out using the standard procedures. All the standards were purchased from Sigma Aldrich. The optical density of all the samples and standards were calculated and compared using the UV-Visible spectrophotometer (Shimadzu, Japan).

OIL Extraction for GC- MS analysis

The fresh plant parts of *Kyllinga nemoralis* was separated into aerial and rhizome portions. About 500g of each of the plant parts were subjected to hydro-distillation in a Clevenger apparatus for six to eight hours. The oil was collected, and stored in a refrigerator for further analysis.

GC-MS analysis

The analysis of the oil was performed using GC-MS (Model: GC-MS-QP 2010, Shimadzu, Tokyo, Japan) equipped with a VF 5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. The carrier gas was Helium (99.99%) used at a constant flow rate of 1.51 ml/min. injector and mass transfer line temperature were set at 200°C and 240°C respectively. The oven temperature was set from 70 to 220°C at 10°C/min, held isothermal for three minutes and finally raised to 300°C at 10°C/min. Two micro liters of the sample was injected in a split mode with a scan range of 40 – 1000 m/z. The total running time of GC-MS was 30 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

1. Identification of components

The chemical identity of the components in the oil samples was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with the NIST08

LIB library sources and a literature source (Adams R. P., 1989) [1].

Results

In the present study, the methanol extract was prepared after Soxhlet extraction and the extract was subjected to qualitative and quantitative study. The results are provided in the following table.

Table 3: Yield percentage of methanol extracts of *K. nemoralis*

Kyllinga nemoralis	Yield percentage/ gram
Aerial	1.5
Rhizome	2.5
Whole plant	4.0

Note: Highest amount of extract (4gm) was obtained from the whole plant.

Table 4: Qualitative phytochemical analysis of (Methanol extract) plant parts of *K.nemoralis*

Si no	Phytocompounds	Type of analysis	KNA*	KNR*	KNW*
1	Carbohydrates	Molisch's test	+	+	+
		Fehling's test	-	-	-
		Benedicts test	-	+	+
2	Glycosides	Borntragers test	-	-	-
		Keller killiani test	-	-	-
3	Proteins	Biuret test	-	-	-
		Xanthoproteic test	-	-	-
4	Amino acids	Millions test	-	-	-
		Ninhydrin test	-	-	-
6	Phenols	Ferric chloride test	+	+	+
7	Flavonoids	Shinodas test	-	-	-
		Alkaline reagent test	+	+	+
		Lead acetate test	+	+	+
8	Flavonols	Mineral acid test	-	-	-
9	Flavones	Lead acetate test	+	+	+
10	Tannins	Ferric chloride test	+	+	+
11	Phlobatannins	Lead acetate test	-	-	-
		Hydrochloric acid test	-	-	-
12	Saponins	Froth and foam test	+	+	+
13	Alkaloids	Mayers test	-	-	-
		Dragendorff's test	+	+	+
		Wagners test	-	-	-
14	Steroids	Libbermanbuchards test	+	+	+
		Salkowskis test	+	+	+
15	Terpenoids	Salkowskis test	+	+	+
16	Dextrose sugar	Keller killiani test	-	-	-
17	Coumarins	Test with alcoholic NaOH and Conc:HCL	+	+	+
18	Quinones	Test using Conc:H_2SO_4	+	+	+
19	Anthraquinones	Test using Chloroform and Ammonia	-	-	-
20	Lignin	Saffranin test	+	+	+
21	Fixed oils & fats	Hydrodistilation	+	+	+
22	Gums	Test using absolute alcohol	-	-	-
	Mucilage	Test using absolute alcohol	-	-	-
23	Resins	HCL Test	-	-	-
24	Acids	Sodium bicarbonate test	-	-	-

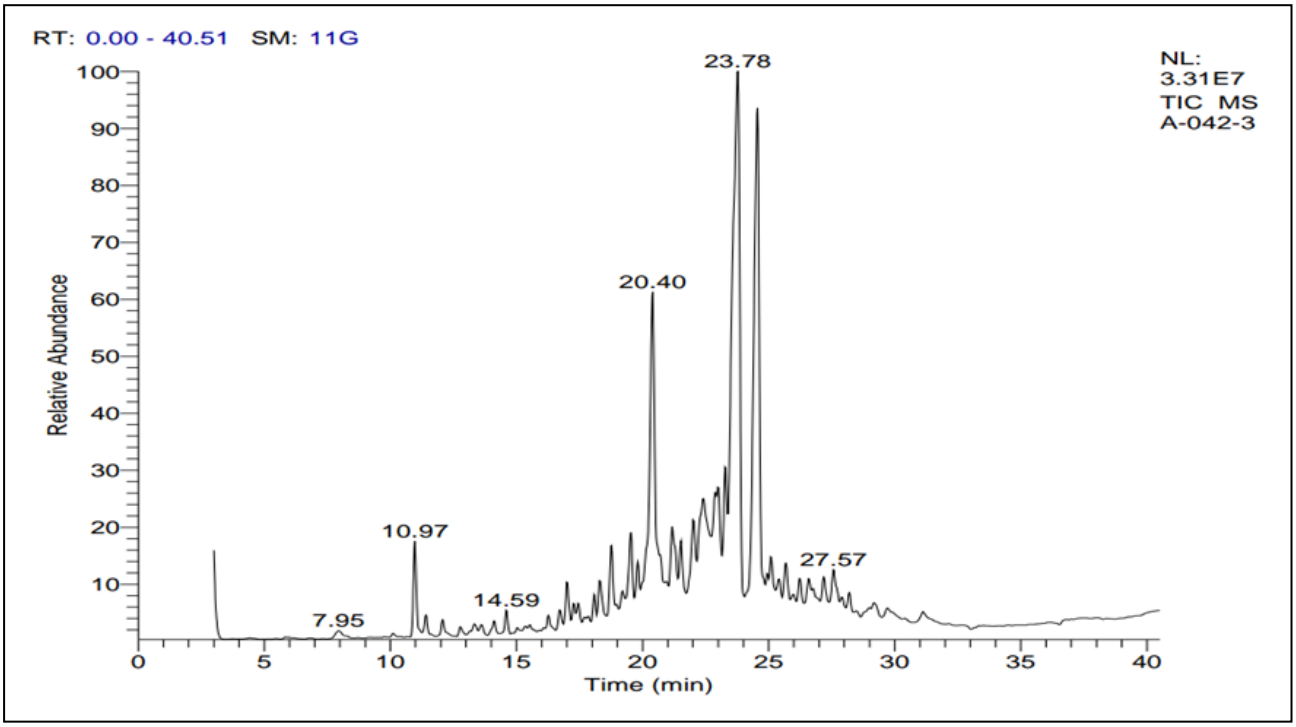
*KNW: *Kyllinga nemoralis* whole plant,
*KNA: *Kyllinga nemoralis* aerial part,
*KNR: *Kyllinga nemoralis* rhizome

Out of the 24 phytochemicals analysed, positive results were obtained for13 phytochemicals. Among the three parts analysed, intense colour reactions were observed in the rhizome extract probably indicating a higher content of these phytochemicals.

Table 5: Quantitative phytochemical analysis of Methanol extract of plant parts of *K.nemoralis*

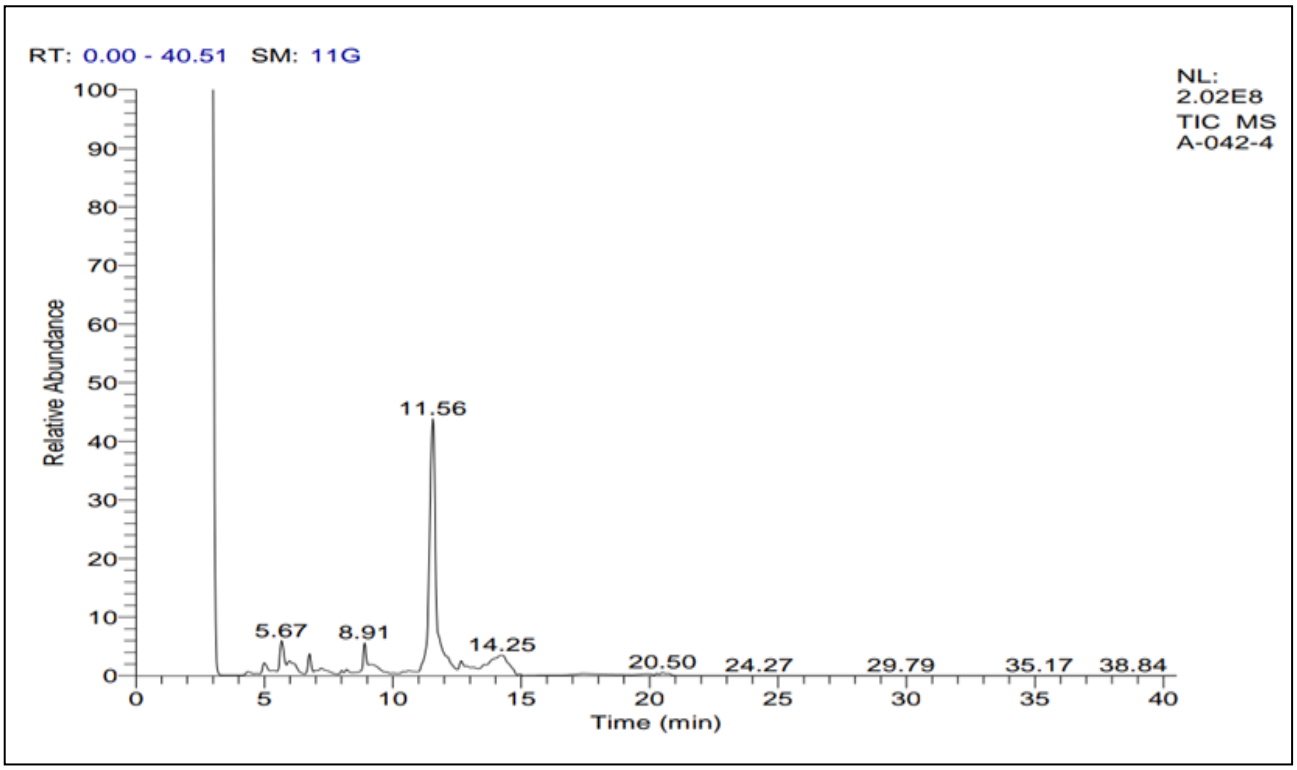
Sl no:	Phytochemicals	Concentration of phytochemicals in each plant part (µg/mg of extract)		
		KNA	KNR	KNW
1.	Phenol	12.36	37.08	33.98
2.	Polyphenol	13.13	22.97	19.97
5.	Tannins	67.00	181.00	223.75
3.	Flavonoids	62.00	110.00	156.00
4.	Alkaloid	15.33	15.00	12.76
6.	Terpenoid	30.61	80.61	40.05
7.	Saponin	0.18	0.11	0.14

Among the seven phytochemicals analyzed, the tannin content was the highest. Quantitative phytochemical analysis showed that the tannins and flavonoids were highest in the whole plant (223.75 and 156 µg/mg) extract followed by rhizome (181 and 110µg/mg) respectively. Phenol content was higher in the rhizome (37.08µg/mg) and whole plant (33.98µg/mg) compared to the aerial part (12.36µg/mg). Likewise the polyphenol content (rhizome-22.97 and whole plant 19.97 µg/mg) was almost double the amount in the aerial part (13.13µg/mg). The alkaloid content was comparable in all the plant parts (whole plant, rhizome and aerial portions). Terpenoid content was highest in the rhizome extract followed by whole plant extract. Saponin content was comparable in all the samples.



Note: The peak with highest area percentage is 2-naphthalenol, 2, 3, 4, 4a, 5, 6, 7-octahydro-1,4a-dimethyl-7-(2-hydroxy-1-methylethyl). Total compounds identified in the analysis was 30

Fig 1: GC- MS Chromatogram of *Kyllinga nemoralis* essential oil (aerial part)



Note: The total compounds identified in the *Kyllinga nemoralis* essential oil (rhizome was 29. The highest peak was represented by "a'-elemene".

Fig 2: GC- MS Chromatogram of *Kyllinga nemoralis* essential oil (rhizome)

Discussion

The extract yield was observed to be highest for the rhizomes of *K. nemoralis* compared to that from aerial part.

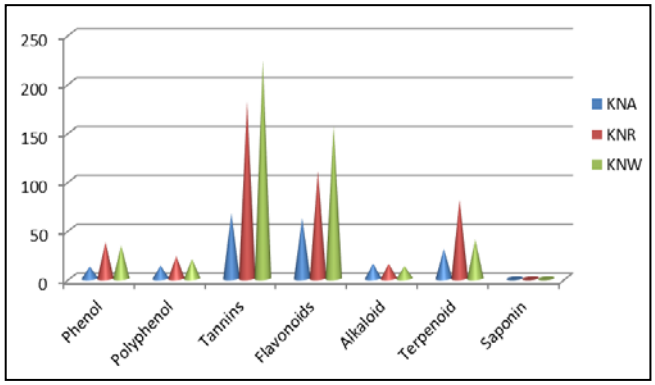


Fig 3: Result of quantification of phytochemicals

Quantitative phytochemical analysis revealed the presence of tannins and flavonoids in higher quantities in the rhizome and whole plant methanol extract compared to aerial part. The whole plant extract as well as the rhizome extract consisted of almost comparable amounts of phenols, polyphenols, flavonoids, alkaloids, terpenoids and saponins, while the aerial parts had comparatively lesser amounts of phytocompounds.

The presence of multiple functional groups in the chemical structure of tannins, such as hydroxyls, provides them with the ability to create bonds to reach a stable cross-linked association within different molecules, such as proteins or carbohydrates. This unique characteristic lets them be differentiated from the common group of polyphenols (Smeriglio, 2017) [28]. The antioxidant, antimicrobial, anthelmintic, antiviral, and anti-inflammatory activities of diverse tannins may be differently administrated and even included as food additives. Tannins are also used in dyeing, photography, refining beer and wine as well as astringent in medicines. Significantly tannins form a vital element of tea (Nonaka *et al.*, 1981) [19].

Flavonoids are important groups of polyphenols widely distributed among the plant flora. Numerous reports supports their use as antioxidants or free radical scavengers (Kar, 2007) [15] (Nishizuka, 1998) [18] (Hunter, 1995) [13]. Flavonoids have miscellaneous favorable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), atherosclerosis, etc. (Burak M & Imen Y., 1999) [2]. Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of neutraceutical, pharmaceutical, medicinal and cosmetic applications (Castaneda *et al*., 2009) [3]. Phenols or polyphenols have an important role in plant defense against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections (Kar, 2007) [15].

Terpenes are among the most widespread and chemically diverse groups of natural products. Plant steroids (or steroid glycosides) also referred to as 'cardiac glycosides' are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs (Firn, 2010) [5].

Table 6: Predominant compounds in *K.nemoralis* detected by GC-MS analysis in aerial part (KNA)

Peak no	Name of compound	Retention time	Molecular formula	Molecular weight	Area%
1	Eugenol	10.7	$C_{10}H_{12}O_2$	164	2.90
2	Longifolene Aldehyde	18.75	$C_{15}H_{24}O$	220	2.22
3	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	19.55	$C_{13}H_{18}O$	190	2.31
4	DIEPI-a'-CEDRENEEPOXIDE	20.40	$C_{15}H_{24}O$	220	9.52
5	2,7-OctanedIone,4,4-dimethyl3-[2-(1-hydroxy-1-methylethyl)-3-methy1-3-butenylidene	21.18	$C_{18}H_{30}O3$	294	2.45
6	6-sopropenyl-4,8a'-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol	22.40	$C_{15}H_{24}O_2$	236	2.80
7	Spiro -10-(tricycloo[5.5.0.0(5,9)]decane-7,8-diol-2'(oxirane),1-methyl-4-isoprppyl-(8S-)	23.01	$C_{15}H_{24}O_3$	252	5.15
8	2-naphthalenol,2,3,4,4a,5,6,7-octahydro-1,4a-dimethyl-7-(2-hydroxy-1-methylethyl)	23.78	$C_{15}H_{26}O_2$	238	32.87

Table 7: Predominant compounds in rhizome of *K.nemoralis* (KNR)

Peak no:	Name of compound	Retention time	Molecular formula	Molecular weight	Area percentage
1	o-cymene	5.67	$C_{10}H_{14}$	134	7.23
2	o-cymene	5.98	$C_{10}H_{14}$	134	3.10
3	LINALYLCETATE	6.75	$C_{10}H_{18}O$	154	3.97
4	Thymol methyl ether	8.91	$C_{11}H_{16}O$	164	4.96
5	a'ELEMENE	11.56	$C_{15}H_{24}$	204	63.15
6	a'-muurolene	14.29	$C_{15}H_{24}$	204	2.18

GC-MS analysis of essential oils from the aerial and rhizome portion of *Kyllinga nemoralis* revealed the presence of about 30 phytocompounds in the aerial part and 29 compounds in rhizome portion. The highest amount of compounds identified from the rhizome portion was "a'elemene"[alpha elemene]. Elemenes are sesquiterpenes, they contributes to the floral aromas of some plants and are used as pheromones by some insects. a'- Elemenes are a group of closely related natural chemical compounds founds in variety of plants.

Elemenes includes α-, β-, γ-. The δ-elemenes are the structural isomers of each other and are classified as sesquiterpenes. Experiments in invitro showed that β-, elemene acts as a rho kinase inhibitor and has anti proliferative effects toward some cancer cell types indicating its probability of use in the field of

chemotherapy. (Wang *et al*., 2005) [31] (Yao *et al*., 2008) [33]. Likewise**,** o-cymene is an organic compound classified as an aromatic hydrocarbon. Its structure consists of a benzene ring ortho substituted with a methyl group and an iso propyl group. It is a flammable colorless liquid which is insoluble in water but soluble in inorganic solvents.

The present study highlights the importance of the plant *K. nemoralis*. Although it is considered as a weed, many biologically active compounds such as tannins, flavonoids, terpenes and the essential oil component, a'- Elemene can be extracted out from the plant.

Conclusion

Phytochemicals from, *Kyllingia nemoralis* including the essential oil from the plant might serve as potential molecules for the formulation of novel drugs.

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