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Identification of Novel Bio-Active Compounds from *Kyllinga nemoralis* (Cyperaceae)

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***Abstract*:** *Kyllinga nemoralis* (Hutch and Dalz), a member of the Cyperaceae family, known for its diverse medicinal properties. The aim of the study is to identify and characterize the bioactive compounds present in *K.nemoralis.* Phytochemical screening revealed the presence of phenols, flavonoids, tannins, saponins, steroids, and terpenoids, with high amounts of phenols (109 mg/g) and tannins (59.4mg/g). GC-MS analysis detected 19 compounds, including cyclo octa siloxane hexadecamethyl, 7 methyl bicyclo (3.2.0) hept-3-ene-2-one etc., exhibiting antibacterial, antifungal, and anticancer activities. This study highlights the potential of *K. nemoralis* as a valuable source of natural products for healthcare solutions, contributing to the advancement of medicinal plant-based research.

***Keywords*:** *Kyllinga nemoralis*, Phytochemical compounds, GC-MS analysis

In India, most of the people also depend on traditional herbal medicine systems and still explored number of medicinal plants for their therapeutic activities (Savithramma et al., 2012). Phytochemicals are naturally occurring constituents in medicinal plants, present in all parts which utilizes for defense mechanisms to protect themselves. This specificity was precisely utilized to cure human diseases. Identification of plant phytochemicals through screening is considered to be an effective discovering method. phytochemical constituents of medicinal plants responsible for important physiological functions in living beings (Ballesta et al., 2010). The phytochemicals are mainly two groups i.e., primary and secondary metabolites. Fatty acids, common sugars and proteins are included under primary metabolites. Terpenoids, alkaloids, phenols under secondary metabolites. They show anti-oxidant, free radical scavenging activities, anti-inflammatory, anti-spasmodic, anti-defense, anti-diuretic as anti-diabetic, anti-cancerous, anti-viral and helps plant to fight against pathogenic fungi (Lingarao et al., 2011), also relieve cardio-vascular diseases (Yugandhar and Savithramma 2017).

Considerable research on metabolites has been conducted on dicotyledonous plants, while monocotyledonous plants have received relatively less attention. Cyperaceae family, which comprises a significant group of monocots, remains largely underexplored, with only a handful of studies reported. The densely tangled rhizomes of Cyperaceae species contribute significantly to erosion control and water purification. *K. nemoralis* a perennial herb, also called as water clover or sedge, traditionally used to treat fever, digestive issues and respiratory problems, was investigated for its phytometabolites. As a rich traditional folk medicine, leaves are used to treat snakebites, malarial chills, and diabetes exhibiting analgesic, anti-oxidant, anti-microbial, anti-diabetic and anti-cancer properties. This study aims to investigate the phytochemical properties of *K.nemoralis* focusing on the qualitative and quantitative analysis of secondary metabolites using different polar and no-polar solvents. Additionally, GC-MS analysis was employed to identify the bioactive compounds responsible for its medicinal properties.

MATERIAL AND METHODS

Collection of plant: The plants were collected from the Botanical Garden of Sri Venkateswara University, Tirupati (13.628927°N; 79.419307°E) and identified using dictionary of flowering plants of Chittoor District, Andhra Pradesh and authenticated by Dr.N.Savithramma, Department of Botany, SVU, Tirupati. Leaves of plant are three-angled, 2-15cm long, and 0.2-0.4 cm wide. Stem is triangular, solid, and glabrous. Flower grouped together in terminal head, sessile, white or brown. Petals are absent and fruit is a nut.

Preparation of plant extracts: The plant material along with its roots was washed 2-3 times under tap water followed by distilled water to remove the soil and dirt particles, shade dried and powdered. 5 gms of dried powder was taken and subjected to extraction under with 100 ml of different solvents i.e., distilled water, methanol, chloroform, ethyl acetate and isopropanol. The plant extracts were filtered, stored in the refrigerator for further studies.

Phytochemical screening: Various tests were performed with plant extracts to unveil the metabolites like alkaloids, phenols, flavonoids, saponins, terpenoids (Harbone 1998, Savithramma et al., 2011).

Quantification of secondary metabolites: Phenols,

flavonoids, tannins and steroids of the plant were estimated (Okeke and Ekekwa 2003, De silva et al., 2017)

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: During a GC-MS analysis, the sample is vaporized and carried by an inert gas (like helium) through a column coated with a stationary phase. As the sample travels through the column, its components separate based on their interactions with the stationary phase and their boiling points. The separated components enter the mass spectrometer, where they are ionized typically by electron impact. The ions are then sorted and detected based on their mass to charge ration (m/z). The resulting spectrum provides a unique finger print for identifying and quantifying the compounds. GC-MS analysis of crude methanolic extract was carried out using GC-MS QP2010 Shimadzu (Japan) system comprising a gas chromatograph interfaced to a mass spectrometer. The details of column used, carrier gas and maintenance of column temperature were followed as per the method Konappa et al. (2020).

The plant had shown the presence of many secondary metabolites. More number of compounds were identified in aqueous extract (11) followed by methanol and chloroform. phenols, flavonoids and tannins were recorded in all the solvents. Steroids were not observed in aqueous extract but

Table 1. Preliminary screening of secondary metabolites from *K. nemoralis*

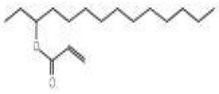
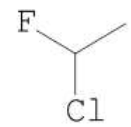
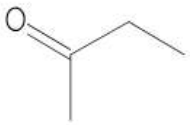
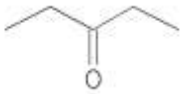
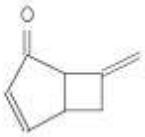
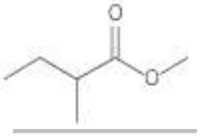
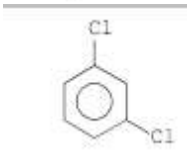
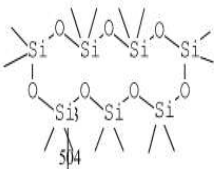
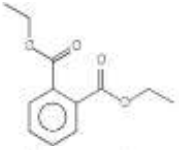
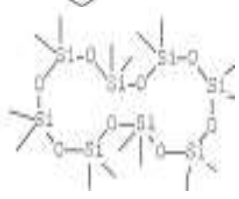
Phytochemical constituent	Aqueous	Methanol	Chloroform	Ethyl Acetate	Isopropanol
Alkaloids	-	-	-	-	-
Steroids	-	+	+	+	+
Phenols	+	+	+	+	+
Flavonoids	+	+	+	+	+
Terpenoids	+	+	+	-	-
Saponins	+	+	+	-	+
Tannins	+	+	+	+	+
Anthroquniones	+	+	-	-	+
Reducing Sugars	+	-	-	-	-
Phlobatannins	+	-	-	-	-
Leucoanthocyanins	+	+	-	+	+
Triterpenoids	+	-	-	+	+
Anthocyanins	+	-	-	-	-
Emodins	-	-	+	-	-

+: present; -: not present

Table 2. Quantitative Estimation of secondary metabolites from aqueous extracts of *K. nemoralis*

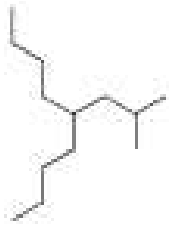
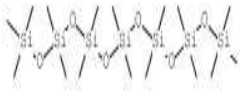
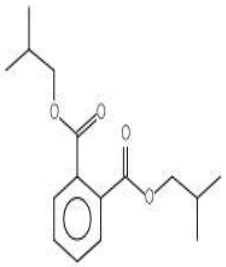
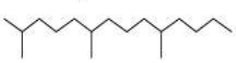
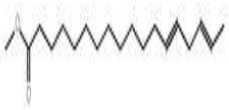
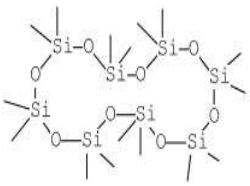
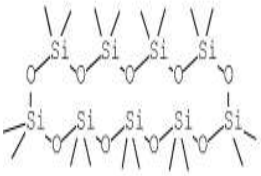
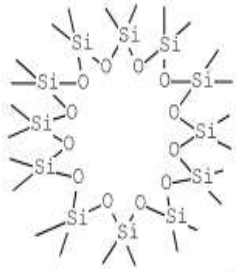
Secondary metabolites	Amount present (mg/g)	Uses
Phenols	109±0.75	Used as fungicide, pesticide, antiseptic and disinfectant, in manufacture of resins; anti-inflammatory, antitoxic, antiviral and anti-microbial, anti-tumor agent. (Shaheen and Savithramma 2022)
Flavonoids	20.8± 0.43	regulation of plant growth, development, disease resistance; anti-inflammatory, analgesic, anti-oxidant, anti-fungal and immune stimulant (Abdallah 2015)
Tannins	59.4± 0.37	Astringent i.e., fasten the wound healing; anti-oxidant, anti-microbial and anti-inflammatory; treats intestinal disorders such as diarrhea and dysentery (Koleckar et al 2008)
Steroids	19.71± 0.29	Has relationship with endocrinal hormones of human beings in their chemical composition (Yaswanthi et al 2024).

Table 3. GC-MS analysis of bioactive compounds found in methanolic extract of *K. nemoralis*

RT value	Name of the compound	Molecular formula	Molecular weight	Peak area	Structure of the compound	Uses
0.122	3Prop-2enoyloxy tetradecane	$C_{17}H_{32}O_2$	248	38		Imparts olfactory properties making it potential ingredient in perfumes or flavouring, its properties make it useful as insecticide or repellant.
1.088	Ethane 1-chloro 1-fluoro	C_2H_4ClF	82	39.6		Used as corrosion inhibitor for steel i.e., binds to water and prevent formation of H bonds with other substances like fatty acids. It is also used as sold catalyst for reaction of chloride with fluorine.
1.629	2-Butanone	C_4H_8O	72	0.8		It is colourless liquid with sweet odour. Used in glues and as cleansing agent. Its long term exposure was reported to have slight neurological,liver, kidney and respiratory effects
1.846	3-Pentanone	$C_5H_{10}O$	86	0.5		It is precursor to Vitamin - E. possess odour like that of acetone, used as solvent in paint.
2.385	7-methylene bicycle (3.2.0) hept-3-ene-2-one	C_8H_8O	120	0.06		Possess biological activities like bacteriostatic, fungistatic and anti-parasitic.
2.515	Butanoic acid 2-methyl-methyl ester	$C_6H_{12}O_2$	116	0.4		It is used as chiral stationary phase in Gas chromatography to allow separation of enantiomers.
5.735	Benzene 1,3-dichloro	$C_6H_4C_{l2}$	146	0.56		It is combustible and toxic to aquatic life.
12.592	Cycloheptasiloxane, tetradcamethyl	$C_{14}H_{42}O_7Si_7$	518	2.6		These are among the wider class of organosilicon. Commonly used in cosmetic industries, to produce deodrats, hairsprays and skin care. It also used in cookware industry and Kitchen utensils, effective industrial cleansing agents in dry cleaning industries. They are good source of electric insulation, low chemical conductivity, low toxicity, hgh gas permeability.
14.461	Diethyl Pthalate	$C_{12}H_{14}O_4$	222	3.6		It is colourless, odourless oily substance used to improve the performance and durability of many products. It is added to plastic polymers to maintain flexibility.
15.542	Cyclo octa siloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	3.26		It exhibits anti-microbial activity especially against 5 pathogensi.e., *P. florescence, P. aeruginosa, S. aureus, Vibreo damsela, Aeromonas hydrophila*

Cont...

Table 3. GC-MS analysis of bioactive compounds found in methanolic extract of *K. nemoralis*

RT value	Name of the compound	Molecular formula	Molecular weight	Peak area	Structure of the compound	Uses
16.077	Nonane 5- (2-methyl propyl)	$C_{13}H_{28}$	184	0.23		Not noticed with any application.
17.815	Heptasiloxane, hexadecamethyl	$C_{16}H_{48}O_6Si_7$	532	2.4		It is used in cosmetics as a film forming polymer. It is also used as antifungal agent by treating many fungal infections. It has alos been shown to have anti-inflammatory properties. This effect may be due to its ability to inhibit prostaglandin synthesis by reversibly binding to enzyme cyclooxygenase.
18.300	1,2 Benzene dicarboxylic acid, bis (2-methyl propyl)	$C_{16}H_{22}O_4$	278	2.9		It is used in adhesives, sealants, paints, coatings, and plastic and rubber products. Some studies have shown that it has potential as a chemoprotective or chemo therapeutic against osteosarcoma.
18.845	Tetradecane 2,6,10-trimethyl	$C_{17}H_{36}$	240	0.7		Anti-bacterial, anti-fungal and nematicidal activity.
20.794	13,16 Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.76		Possess anti-microbial, anti-oxidant and anti-inflammatory activity
21.165	Cyclo 3octa siloxane hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	0.7		It exhibits anti-microbial activity.
28.746	Cyclononasiloxane octadecamethyl	$C_{18}H_{54}O_9Si_9$	666	5.5		It is used as cleansing agent in cosmetics, textiles. Also possess antifungal property, biologically resistant to termites.
30.671	Tetracosamethyl cyclododeca siloxane	$C_{24}H_{72}O_{12}Si_{12}$	888	2.09		Its unique structure gives it excellent lubricating and emollient properties. It has low surface tension which makes it an excellent surfactant reducing interfacial tension between two substances. It is shown to have low toxicity.

detected its presence in all the other extracts of polar and non-polar solvents. Saponins were present in all solvents except ethyl acetate. Leucoanthocyanins, were absent in chloroform extract and emodins were observed only in chloroform extract whereas, reducing sugars and phobia tannins only in aqueous extract. Anthocyanins are seen only in aqueous extract, Triterpenoids are absent in methanol and chloroform extracts and anthraquinones in aqueous, methanol and isopropanol extracts.

Alkaloids are one of the largest group of phytochemicals in plants having amazing effects based on their toxicity against cells of foreign organisms. But alkaloids are absent in *K.nemoralis* whereas present in other members of Cyperaceae which include *Cyperus rotendus, Frimbristylis eragrostis, Fimbristylis monostachya, Paspalidium flavidum*. Phenols, flavonoids, steroids, tannins were present in *K.nemoralis* and found in other members of Cyperaceae i.e.,*Fimbristylis cymosa, Fimbristylis eragostis, Kyllinga triceps, Paspallidum flavidum* but found to be absent in *Cyperus difformis, Scleria lithosperma* (Haribabu and Savithramma 2014).

Among the metabolites identified, phenols (109) were in higher amounts followed by tannins (59.4) from aqueous extracts. Saponins are also present in the plant. Traditionally saponins have been extensively used as detergent as pesticides and molluscides. Saponins have a relation with a hormone oxytocin which is involved in controlling the onset of labour pains in female and the subsequent release of milk. Saponins enhance nutrient absorption and aid in animal digestion. They are bitter in taste and can resume plant palatability. Saponins possess hypocholesterolemic property for the control of high blood lipids in addition to their industrial applications as foaming and surface active agents it also have beneficial health effects (Komuraiah 2014).

GC-MS analysis: Gas chromatography analyses in combination with mass spectra of methanolic extracts were analyzed to identify different phytochemical compounds along with their molecular weight and molecular formula. Nearly 18 compounds are identified with their potential uses (Table 3). Among them cyclo octa siloxane hexadecamethyl, tetradecane 2,6,10-trimethyl, 13, 16 octa decadienoic acid, methyl ester; exhibits anti-bacterial activity. Cyclonona siloxane octa deca methyl, hepta siloxane hexa decamethyl, 7 methyl bicyclo (3.2.0) hept-3-ene-2-one especially possess fungistatic activity. The GC-MS revealed the plant may be used to treat cancer, act against bacteria, fungi and nematodes; as a cleansing agent, also in industrial applications such as chiral stationary phase in paper chromatography.

CONCLUSION

The *Kyllinga nemoralis* is rich in phenols, flavonoids, tannins and steroids. The GC-MS analysis further confirmed the presence of these compounds and provided an information on their chemical structure. The *K. nemoralis* is a rich source of bioactive compounds with pharmacological applications and highlights its potential as a source of novel bioactive compounds.

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