Cyperus scariosus rhizome essential oil - physio-chemical characteristics and anxiolytic potential

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Abstract- Essential oils are important plant products that often have a complicated composition made up of the volatile plant components. Numerous biological actions are reportedly connected to essential oils, and a new class of aromaceuticals is being developed. The market for essential oils has increased since they are used to cure these types of ailments as a result of the emergence of various mental issues due to changing living standards, such as depression, anxiety, sleeplessness, and stress. Studies on animal models have shown that the mode of action of essential oils, which cause physiological changes in the brain, involves a number of neurotransmitter systems. Despite mounting proof that essential oils have quantifiable effects on animal behavior.

Therefore the current study is proposed to investigate the physical and chemical characteristics of the essential oil obtained from the rhizome of *Cyperus scariosus* and assess its anxiolytic effects on the central nervous system in rodent models.

Hydro distillation was used to extract the essential oil from the rhizomes of the plant. Total yield, physical properties like viscosity, specific gravity, refractive index, acidity, saponification, iodine no, etc were examined .Further to determine its impact on the central nervous system in a rodent model studies were performed at a dose level of 250 mg/kg body weight and compared to the benchmark medication caffeine @100 mg/kg body weight**.**

The essential oil has a sustainably stimulating impact on the central nervous system in a proportion of 88% compared to caffeine's 68%, according to CNS locomotors count and physicochemical features that were in good accordance with literature value.

Key words: *Cyperus scariosus*, anxiolytic, essential oil CNS.

Introduction

Essential oils are volatile complex compounds which are characterized by a strong odour and are formed naturally by aromatic plants as secondary metabolites. They are rich sources of biologically active compounds[1]. Essential oils are secreted in different parts of the plant. Essential oils from a broad spectrum of plant species have shown antinociceptive, anti-inflammatory,

antimicrobial, antiviral, antitumoral and antioxidant activities [2]. Some oil constituents can act as pro-oxidants (such as free fatty acids and hydroperoxides) or as antioxidants, including tocopherols, phenols, and possibly phospholipids together with other components. Moreover, recently there has been a profound interest in the antimicrobial characteristics of extracts from aromatic medicinal plants, specifically essential oils.

Essential oils are gaining remarkable interest for their multipurpose use as antioxidant, antifungal, and antiseptic agent; Study of various physicochemical characteristics explores the practical importance of herbal oils in daily life[3]. The quality of both fixed and essential oils is indirectly influenced by physical-chemical properties of the oil, such as colour, specific gravity, specific viscosity, refractive index, acid value, saponification value, ester value, carbonyl percentage, phenol and solubility tests, etc. The majority of an oil's commercial significance is determined by its physicochemical features, which provide a basis for assessing its suitability for consumption. The current study is proposed in order to investigate the physical chemical makeup of the essential oil extracted from *Cyperus scariosus*rhizome a very well known medicinal plant and assess its anxiolytic impact on the central nervous system in rodent models.Cypreus *scariosus* known by the common name "Nagarmotha" and Nagar Mustakais a small grass-like herb with angular softstem and underground rhizomatous tubers.Stolons are thin, measuring between 0.8 and 5.0 cm by 0.25 cm, and are covered in elliptic, acute, laxly striate, concolorous scales stem 40-90cm long, thin, and triquetrous at the apex, 1/8inch long1/24-1/16inch in diameter, variable, usually short (less than 1/3 inch. The rhizome of this plant contains anamber or light brown viscous essential oil .This premium essential oil-bearing plant is well-known around the world for its superior digestive and carminative properties. It is used to treate a variety of diseases including diarrhea, epilepsy, fever, gonorrhea, liver damage, syphilis and act as an important ingredient of several prescriptions used in indegenoius system of medicine.Oil reportedly kills intestinal worms effectively and also acts as a diuretic, astringent, anti-inflammatory, antibacterial, hypotensive, central nervous system stimulant, and antipyretic[4-10].

Material and Methods

Preparation of substrate-The rhizome of *Cyperus scariosus* was collected from a local mark of Dehradun authenticated by Systematic Botany Division, Forest Research Institute, Dehradun, Uttarakhand, India. The rhizomes were cut into small slice followed by oven drying at 60 °C - 80 °C for 48 hrs .The dried parts were then stored at room temperature and then used for the experiment.

Extraction of oil from leaves and rhizomes of *Cyperus scariosus*- In a Clevenger's apparatus, hydro-distillation (6-8 hrs) was applied to 300 g of Cyperus scariosus rhizomes. The resultant volatile fraction showed two separate layers: an upper aromatic oily layer and a lower colourless watery layer. These layers are transferred into separating funnel with addition of diethyl-ether in it. The upper layer was collected and dried over anhydrous sodium sulphate.

Poured the solution into a small, dry, empty beaker, weighed it out, and then placed it

over a water heater until all the ether has evaporated. The oil-filled beaker was again weighed to calculate the yield of oil from rhizome.

The oil was stored at low temperature (4 - 6°C) for further use[11].

Studies of characteristics of extracted oil[12]

Physicochemical characteristics provide a base line for aptness of oil. These characteristics of oil NM -1 studied were colour , Specific gravity, Specific Viscosity, Refractive index, optical rotation **,** acid number & saponification number[13-14]

(a) **Specific gravity**

1. A cleaned and dried empty pycnometer was taken and weighed.
2.The pycnometer was filled upto the mark with double distilled water and weighed again.
3.The weight of water was recorded. After removing the water the pycnometer was dried in the oven and filled upto the mark with the essential oil under experimentation.
4. The weight of pycnometer with oil was calculated using the following formula.

$$\textbf{Specific gravity} = \frac{wt.\ of\ the\ oil}{wt.\ of\ an\ equal\ volume\ of\ water}$$

(b) **Specific Viscosity**

1. An Ostwald viscometer was cleaned and dried. Ten ml of 1% solution (in acetone) of the oil under experimentation was filled in the bulb of the viscometer.
2. The solution was sucked upto upper mark and the viscometer left as much.
3. The time taken by the solution to percolate down from the upper mark of the viscometer to the lower mark was recorded.
4. The process was repeated by filling the viscometer with pure acetone. The relative viscosity of the oil was calculated as per following formula:

$$\eta\ \textbf{Rel} = \frac{\textbf{Flow time of 1\%solution of essential oil in solvent}}{\textbf{Flow time of pure solvent}}$$

The value of relative viscosity thus obtained, was converted to specific viscosity by the formula as under:

$\eta\ sp = \eta\ rel^{-1}$

where,

η sp = Specific viscosity

$\eta\ rel^{-1}$ = relative viscosity

(c) Refractive index

1. A type of refractometer was used to determine the refractive indices of the oils.

2.The double prisms of the apparatus were cleaned with alcohol and one drop of the oil was placed between them.
3. The prisms were closed by tightening the screw heads and the refractometer was allowed to stand for few minutes to equate the temperature of the oils and the apparatus.
4.The alidade of the refractometer was moved backward of forward to get a broader line which was a band of colour.
5.A sharp colourless line was obtained by rotating the screw heads of the compensator. Finally, the line was adjusted in such a way that it fell on the point of intersection of the cross-hairs.
6. The refractive index was head directly on the scales of the sector.

(d) Acid number- 1. One ml of the essential oil was dissolved in 15 ml of 95 % ethanol in a conical flask.

2.Three drops of 1% phenolpthaline were added to the contents of the flask and it

was titrated against 0.1 N sodium hydroxide solution.
3. The first appearance of pink colouration that did not fade with in 10 second was considered as the end point.
4. Another set without oil was also run parallel to treatment and the difference in the amount of alkali used while titrating the treatment and the set without oil gave the amount of alkali consumed for determination of the acid number of the oil.
5. The acid number was calculated by the following formula :

$$\textbf{Acid number} = \frac{\textbf{Volume of 0.1 N Alkali consumed}}{\textbf{Weight of 1ml essential oil}} \textbf{ X 5.61}$$

(e) Saponification number- 1. One ml of the essential oil was taken into a 100 ml saponification flask. Ten ml of 0.5 N alcoholic sodium hydroxide solution was added to the flask and an air cooled glass condenser (1 meter in length and 1 cm in diameter) was attached to it.

2. The mixture was refluxed for an hour on a water bath and then allowed to cool down to room temperature.
3. The contents were the titrated against 0.5 N aquous hydrochloric acid using 3 drops of 1% phenolpathalin solution as the indicator.
4. Another set, without oil was also run parallel to the treatment set and the difference in the amount of acid consumed for the determination of saponification number of the oil, which was calculated by the following formula:

$$\textbf{Sap. No.} = \frac{\textbf{Volume of 0.5 N Acid consumed}}{\textbf{Weight of 1ml essential oil}} \textbf{ X 28.05}$$

(f) **Optical Rotation-** 1. Place the 100mm polarimeter tube containing the oil or liquid under the examination in the trough of the instrument between the polarizer and analyser.

2. Slowly turn the analyser until both halves of the field, viewed through the telescope, show equal intensities of illumination.
3. At the proper setting, a small rotation to the right or to the left will immediately caused a pronounced inequality in the intensities of illumination of the two halves of the field.
4. Determine the direction of rotation. If the analyser was turned counter-clockwise from the zero position to obtain the final reading, the rotation is laevo and if clockwise, it is dextro.
5. After the direction of rotation has been established, carefully readjust the analyser until equal illumination of two halves of the field is obtained. Adjust the eyepiece of the telescope to give clear, sharp lines between the two halves of the field.
6. Determine the rotation by means of protactor; read the degrees directly , and the minutes with the aid of either of two fixed verniers, the movable magnifying glasses will aid in obtaining greater accuracy.
7. A second reading should be taken; it should not differ by more than 5' from the previous reading.

Toxicity Study

Acute Toxicity Study[15]; For the evaluation of acute toxicity, five groups (n = 5) of male

albino mice were employed. Animals from all groups were given a single dose of NH-1 (50, 100, 150, 250, 500, 1000, 1500, and 2000 Mg/Kg body weight) after fasting for the previous night. Animal behaviour was tracked during 72 hours following oil administration in a group of animals that had received an equivalent volume of P.B.S. Animals were watched for 14 days for any signs of toxicity and mortality was observed .

Antianxolytic Activity

The antianxolytic activity or locomotor activity (horizontal activity) can be easily measured using actophotometer which operates on photoelectric cells which are connected in a circuit with a counter. When a beam of light falling on the photocell is at on the animal a count is recorded. An actophotometer could have either a circular or square area in which the animal moves. Both rats and mice may be used for testing in this equipment.

Wistar rats of both sex (male and female) weighing 150-250 gms with a variation of ± 2.0 gm were taken for study. Test rats were kept in individual elastic ages with wire tops, prior to the use for screening. All the animals were fasted for at least 12 hours before use, allowing only access to water. The rats were divided into groups of five animals each. Each rat was weighed individually and was marked to distinguish one from another. The equipment was checked and was sure that all the photocell counter was recorded for each group for 10 mins. At the end of counting each group of rats were removed from the counting chamber. Drugs were given orally as per the following schedule and after 60 minutes they were retested for activity scores for 10 minutes. The dose for drug is 250 mg/kg body weight of rat while the dose for standard reference drug caffeine is 100mg/kg body weight. Animals were divided into 5 groups and received different drugs as Control group receiving Tween 80, Standard groups receiving caffiene and Test groups receivingNM-1 @ 250 mg/Kg body wt. Table 4

CNS motor activity was calculated as per the following formula: -

$$\% \text{ CNS activity} = \frac{\text{Initial no. of counts} - \text{Final no. of counts}}{\text{Initial no. of counts}} \times 100$$

Statistically Data

All data were expressed as mean SEM ± wherever applicable, the data were analysed statistically by student's t- test, using graph pad instant version 2.05a and one way ANOVA. The level of significance was $p < 0.05$ and n represents five per group.

Results

Various physicochemical properties viz. yield (%), specific gravity, specific viscosity, refractive index,optical rotation **,** acid number&saponification number are summarized in **Table-1**. Acute toxicity studies of Essential oil NM-1 was also carried out and further screened for their effect on Central Nervous System on rat model using actophotometer. Results are summarized in Table-2 and 3.

Table-1 Characteristics of NM-1

Parameter studied	Value NM-1
Yied	0.5%
Colour	Light brown
Specific gravity	0.9450
Specific viscosity	0.195
Refractive index	1.5106
Acid number	10.756
Saponification number	34.346

Table-2 Determination of LD50 of NM-1

Treatment	Dose (mg/kg PO)	No. of Animal	No. of Animal Dead	No. of Animal Survive	% dead animals
NM-1	50	5	0	5	0
	100	5	0	5	0
	150	5	0	5	0
	250	5	0	5	0
	500	5	0	5	0
	1000	5	0	5	0
	1500	5	0	5	0
	2000	5	0	5	0

Table-3 Antiaxyolytic (CNS) potential of NM-1

S.No.	Drugs	Dose	CNS Activity		% CNS Activity
			Before	After	
1.	Caffiene	100 mg/kg	37.6± 0.28	63.2±0.25	**68.08**
2	NM-1	250 mg/kg Body weight	40.6± 0.22	70.6±0.19	**88**

Discussion

Physicochemical properties analysis which indirectly influence the quality of essential oil revealed that, oils has comparable properties**.** Using an actophotometer, essential oil was further examined for acute toxicity tests and their impact on the Central Nervous System in a rat model. According to investigations on acute toxicity, sample NM-1 did not cause any toxic symptoms or mortality at a dose of 50, 100, 150, 200, 500, 1000, 1500, or 2000 mg/kg body weight when given orally to mice. The aim of biological activity was to study effect of essential oil on the central nervous system, which was done in terms of locomotor activity of rats using actophotometer (activity cage).Most of the central nervous system acting drug influence the locomotor activities in human beings and animal the CNS depressant drug such as barbiturates and alcohol reduce motor activity while the stimulants such as caffeine and amphetamines increase the activity .in other words the locomotor activity on be in debt alertness of mental activity. Essential oils exhibit stimulation properties which lie in their structure closely in resemblance with actual hormones .[16-17]One of the important characteristics of these oils used in aromatherapy is their ability to penetrate the skin and reach the subcutaneous tissues. When breathed, essential oils are integrated into a biological signal of the receptor cells in the nose, which is how they work. Through the olfactory bulb, the signal is delivered to the limbic and hypothalamic regions of the brain. These signals trigger the production of neurotransmitters in the brain, including serotonin, endorphin, and others, that connect our nerve and other bodily systems to ensure the desired change and to give us a sense of relaxation. To have the desired effect on the body and mind, relaxing oil, euphoric oil, and stimulating oil, respectively, release serotonin, endorphin, and noradrenaline in adequate quantity thus gives stimulation signals. From activity data summarized in table3 . It is evident that in total NM-1 has exhibited CNS stimulant potential (88% @ 250 mg/ Kg body wtlocomotor count as compared to caffeine (68 % @ 100mg/ Kg body wt**)** locomotor count .

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