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GC-MS of Carissa *carandas* and Formation of Ointment from the Fruit

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ABSTRACT

The karonda is widely cultivated in subcontinents. It has been reported that plant is extensively used in local remedies. The fruit of plant contain vitamin c 8.8mg/100ml and calcium 32mg/g whereas seed contains the calcium 44.3mg/g of seed sample. The ointment prepared from the fresh fruit and dry fruit of Carissa carandas has potential to heal the wound. For this purpose 26 rabbits were divided into 4 groups. One group was treated with standard ointment, other two groups were treated with dry fruit ointment and fresh fruits ointment. The fourth group was kept untreated. The contraction of wound was calculated in all the groups. Moreover the phytochemical analysis of fruit, leaves and flowers of the plant was also performed with the help of GC-MS, titration and other laboratory reagents.

KEYWORDS: GC-MS, Phytochemistry, Ointment, Percentage of Vitamin C, Percentage of Calcium

Introduction:-

The Carissa carandas Linn is a tropical shrub or a small tree of the family of the Apocynaceae. The common name of plant is changed regionally e.g in India and Malaysia it is known as Karanda, in Thailand nahm-daeng, in Philippines caramba and in China ci huangguo. The cultivated regions of this plant are India, Taiwan, Indonesia, Malaysia, Burma and Sri Lanka. The plant had been widely used by natives in aurvedic medicines for the treatments of ailments. The unripe fruit is used for the treatments of liver dysfunction; counteract purification of blood. The fruit has also been used as analgesic, anti-inflammatory and anti-diabetic [1]. The ripe fruit of Carissa carndas was used as antiscorbutic and a remedy for biliousness. The chemical constituents of the roots of Carissa carandas are salicylic acid cardiac glycosides that help in the depression of blood pressure [2]. Roots also contain methyl ester and carissone which have anti-anthelmintic and anti-diarrheal properties. The leave extracts have cytotoxic effect on HeLa cells, PC3 cells and 3T3 cells [1]. The leaves contain betulinic acid [3], Oleanoic acid, carandiol [1], ursolic acid, β-sitosterol-3-0-β-D-

glucopyranoside and 4-hydroxybenzoic acid. The fruit appear rich in Vitamin C [4].

Medicinal Importance:- Various species of Carissa are grown as ornamental flowers. Besides this it is extensively used in medicines.

The bark of Allamanda cathartica L (Golden trumpet) is astringent, tonic, anthelmintic, purgative and antiperiodic. It is an important cure in perpetual the runs and the propelled phases of looseness of the bowels. Its smooth juice is connected to ulcers and blended with oil in ear infection. Sap, gum and roots are utilized as a part of growth [5].

The roots of Ichnocarpus frutescens (L.) are utilized as a part of fever, dyspepsia, skin inconveniences, diabetes and stone in the bladder. A decoction of the stems and leaves is utilized as a part of fevers. Leaves are connected to cerebral pains, wounds and sore between fingers [6].

The bark of Holarrhena antidysenterica (L.) and Nerium oleander Linn have anticancer properties, among them vincristine and vinblastine are effectively utilized against leukemia and Hodgkin's malady.

The root of Tabernaemontana divaricata (L.) is recommended for biliousness, epilepsy and loss of motion. Its latex is applied to wounds to present irritation.

Experiment:-

Extraction of Essential Oils:-

Some flowers and leaves of the C.*carrandas*.L were kept under the shad for 5-7 days of their drying. The clean dried sample was grounded with the help of mortar and pistil to make a fine powder that could pass through 0.45mm mesh. The 80 grams of this fine powder was subjected to hydro-distillation process for 45 minutes in Clevenger's type apparatus, where dichloromethane was used as the collecting solvent. To remove the water from the extract Magnesium Sulphate was used as a drying agent. The sample was lypholized at -4°C in a refrigerator before subjecting to GC-MS analysis.

GC-MS Analysis:-

GC separation of volatile constituents of essential oil was performed by using, a Clarus500 PerkinElmer gas chromatograph was used fitted with an HP-INNO Wax fused silica capillary column (30 m × 0.25 mm, film thickness 0.50 μm). Oven temperature ranged 70–250°C, with the temperature ramp being programmed as 45°C for 10 min then increased 5.0°C/min to 290°C, then 10.0 °C per min to 290°C, with a final hold time of 5 min. An injection volume of 2μL was employed in split mode with split ratio of 1:30. Helium gas was utilized as carrier gas with a flow rate of 1μL/min. The mass detector conditions used were as follows; transfer line was 250 °C and source temperature was 150 °C and a solvent delay of 3.5 min were selected, the mass range was set between (m/z = 35-550) and a multi-channel plate voltage of 2200V was used. Temperature detector was 270°C. The ion source temperature of 240°C and ionizing voltage of 75eV was utilized.

Methanolic & Aqueous Fractions:

The ripped and some unripe fruits were collected, washed properly and were kept under the shade for 3-5 days for drying.

The seeds were separated from the fruits and these were also kept under the shade for drying purpose. The dried sample of both fruits and seeds were crushed into a fine powder with the help of a mortar and pistal. This dried fine powder of fruit sample was divided into two fractions, one fraction was soaked into 100ml methanol for 5-7 days. The second fraction of dried sample of fruit was soaked in 250ml distilled water in 500ml beaker. The mixture was heated at 50°C for 2.5 hours on a hot plate till the 150 ml water evaporated. In the same way the two fractions of dried seeds were treated, one with 100 ml methanol and other with distilled water. After 7 days, both (methanolic fractions of seeds and fruits) were filtered. On the other hand the aqueous fractions of fruits and seeds were filtered when 150 ml water was evaporated at 50°C, so, the remaining 100 ml aqueous fractions were filtered on approaching the temperature of fraction at room temperature. All four fractions that are methanolic and aqueous fractions of the seeds and fruits were stored in four sampling tubes of 100 ml. The sample was lypholized at -4° until the further analysis was performed. After that the sample was sent to investigate its phytochemical composition by various laboratory methods.

Phytochemical Analysis of the Fruit:-

Both (methanolic and aqueous) fractions of seeds and fruits were subjected to react with different laboratory reagents in order to investigate their phyto chemistry. The presence of different phytochemical constituents, for example alkaloids, carbohydrates, phenolic compounds, gums and mucilage, saponins, proteins and fats was confirmed by Mayer's test, Wagner's test, Molish test, Ferric chloride test, Lead acetate test etc. [7]

Determination of Vitamin C & Ca

The amount of vitamin c and minerals was found by the titration method. The amount of vitamin c in the fruits of *Carissa carandas* was found by the redox titration method. The fruit extract was titrated against iodine solution whereas starch was used as an indicator. On the other hand the amount of calcium was found by the complexometric titration. In this titration the fruit extract was titrated against EDTA solution using EBT as an indicator [8].

Wound Healing Activity of the Ointment:

Animals:-

The male Blanc de Termonde rabbits weighing 1500-1600 g were chosen to investigate the wound healing activity in them. For experiment 24 animals were divided into four groups that were one reference group, one control group and two experimental groups with six rabbits in each group. The animals of a group were kept separately in cages with free supply of food and water. The cages of animals were kept under the standard conditions. The animals were kept hungry for 12 hours. After starving, by the open mask method, the animals were given anesthetic ether before injuring. It is as per animal moral technique that before doing any animal examination such as surgery or incision, the animal should be famished to get an ideal impact of sedation, so that the

effect is for long duration and with least suffering to animals [9].

Making Abrasion on Skin:-

The hairs were removed from the dorsal side of rabbit. The shaved area was antisepticised by iodine tincture under sterile condition. A small abrasion of 1cm was made on the dorsal side of rabbit with the help of a peeler.

Preparation of Ointment:-

The ointment base was prepared by mixing the following constituents in given quantity,

Sr.no	Names of constituents	Quantity
1	E-Wax	12g
2	Beeswax	600mg
3	Parafin oil	30ml

The fine powder 5.3g of dry fruits was taken and moisture was removed by keeping in dehumidification apparatus for 5 minutes. The fine powder was added into base and mixture was homogenized by homogenizer. The prepared ointment was marked as D.F ointment and was kept below 25°C [9].

The fresh fruits were taken and it was washed with distilled water. The fresh fruits were grinded into a paste by grinder. The 30 ml distilled water was added in grinder to remove the paste from the walls of grinder. The paste was filtered by cotton cloth and filtrate was placed in water bath to remove excess water. After removing excess water from the past, the paste was incorporated with ointment base and mixture was homogenized. The ointment was marked as F.F ointment and it was kept below 25°C [10].

Experimentation:-

The abrasion day was considered as day zero (0). From day zero the experiment was started on control, reference and experimental groups. The rabbits of both experimental groups were treated with D.F ointment and F.F ointment on abrasion site respectively. The rabbits of reference group were treated with standard Bepanthen ointment and the rabbits of control group were untreated [11]. In order to check the wound healing activity of F.F ointment and D.F ointment, the time taken for wound healing in all the groups was compared. The result was noted in the forms of table. The percentage contraction of wound healing was calculated as,

$$\text{Percentage contraction} = \frac{\text{wound length on day 1} - \text{wound length on day } x \times 100}{\textit{wound length on day 1}}$$

Evaluation of the Ointment:-

The physical properties of ointment, such as viscosity, pH, color, texture and homogeneity were evaluated [12].

Organoleptic Evaluation:-

In order to evaluate the organoleptic properties of ointment, such as texture, color, odor and homogeneity, the small quantity of ointment was spread between slides and analyzed visually. The outcome of the homogeneity and texture were given as (+++) = excellent, (++) = very good, (+) = good, (-) = poor.

Determination of pH:-

The pH of ointment was evaluated by mixing 1g of ointment in 100ml of distilled water followed by warming and rapid shaking to cool. The cooled mixture was kept for two hours at room temperature. After that three concordant readings of pH were taken by dipping the electrode of pH meter (Jenway, UK) in the solution of ointment.

Viscosity:-

The viscosity of ointment about 100% of torque was noticed at 10 rpm by Brookfield Viscometer (Fungilab, Spain). At room temperature the spindle of viscometer was inserted in ointment and readings were noted.

Results:-

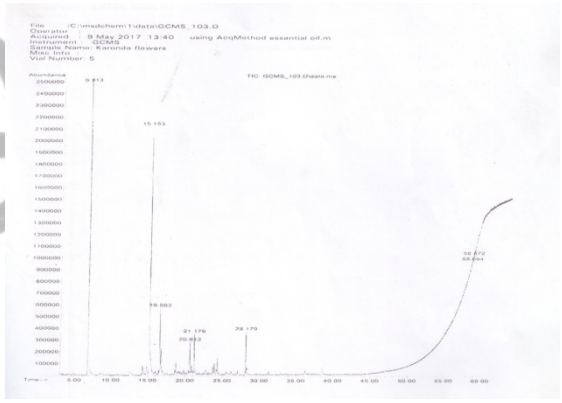
GC-MS of Essential Oil:-

Volatile constituents of the essential oil of the fruits and leaves were identified on the basis of matching their mass spectral data with the standard data given in NIST-05 mass spectral library and previous literature found for C.carandas.L.

Sr.no	Ret t	Names of Compounds
1	6.8	Furfural
2	14.3	Cyclohexanone-4-diene
3	14.8	3-aminopyrazine-1-oxide
4	15.1	2-furancarboxaldehyde-5-methyle
5	16.5	Phenol (carbolic acid)
6	16.75	1,2-cyclohexanediole
7	18.8	L-serine
8	19.00	Ortho-hydroxyanisol
9	19.14	2,5-furandione-3,4-dimethyle
10	19.23	Nona-3,5-diene
11	20.6	Ortho-guaiacol
12	21.17	3-ethyle-3-methyle cyclopentanone
13	28.17	2-methoxy-4-vinylphenol
14	23.8	1-octane-2-one-4,4-diethyle-3-methylene
15	24.00	Benzene acetic acid
16	24.2	trans-2-Caren-4-ol
17	24.4	Methyl salicylate
18	24.6	(z)-7-hexadecanal
19	38.5	Dodecane-1-cyclopentyle

Major Constituents of the Leaves of C.carandas.L

Sr.no	Ret t	Names of Compounds
1	1.3	Neo-hexane
2	1.42	Iso-hexane
3	1.45	Pentane-3-methyl
4	1.6	n-hexane
5	1.8	Methyl cyclohexane
6	2.1	Cyclohexane
7	20.6	Guaiacol
8	28.18	4-vinyl guaiacol
9	36.0	Diethyl phthalate
10	38.0	Hexadecane thiol
11	58.9	Hexasiloxane



Phytochemical Constituents of Fruit:-

The presence of alkaloids in aqueous and methanolic extracts was confirmed by Mayer's test and Wagner's test. The confirmatory test for carbohydrates showed negative results both for aqueous and methanolic extracts. The lead acetate test and ferric chloride test give positive results for phenolic compounds. Both test Biurete test and Millon test, give negative results for proteins. The sponin and fats were also identified in extract. Moreover

the percentage of vitamin c in fruit extract was calculated as 8.8mg per 100ml. The calculations showed that 1g of fruit sample contains 32mg of calcium and 1g of seed sample contains 44.8mg of calcium.

Percentage Contraction of Wound

Groups	% Wound Contraction					Healing
	Day 4	Day 8	Day 10	Day 12	Day 14	
Control	20.03 ± 0.2	48.0 ± 0.05	66.02 ± 0.3	71.93 ± 0.1	85.0 ± 0.43	25.04 ± 0.6
Exp F.F	22.01 ± 0.4	51.06 ± 0.7	70.02 ± 0.3	87.01 ± 0.7	98.03 ± 0.5	17.07 ± 0.3
Exp D.F	24.06 ± 0.2	53.07 ± 0.8	72.01 ± 0.4	89.05 ± 0.1	98.02 ± 0.9	17.02 ± 0.8
Ref	26.07 ± 0.3	57.02 ± 0.8	77.04 ± 0.6	91.01 ± 0.9	99.07 ± 0.3	15.09 ± 0.1

The above table is showing the results of wound healing in the four groups of rabbits. The group which was marked as a control group and was kept treated took 25 days for complete healing. The groups which were treated with fresh fruits ointment and dry fruit ointment took almost 17 days for complete healing. The reference group which was treated with standard ointment took 16 days for complete healing. So it was seen that the ointment prepared from the fruits of C.carandas.L has potential to heal the wound. It is maybe due to the presence of Vitmin c, calcium and some anti-inflamatory agents present in fruit of C.carandas.L.

Organoleptic properties of ointment:-

Sr no.	Parameter	Fresh Fruit Ointment	Dry Fruit Ointment
1	**Color**	Pink	Brown
2	**Texture**	+++	+
3	**Homogeneity**	+++	++
4	**Smell**	Fruity	Nil
5	**pH**	5.0	6.1
6	**Viscosity (torque) at 10 rpm & 37°C**	40.2%	32.5%

The organoleptic properties of ointments are given in the above table. The color of fresh fruit ointment is pink and the color of dry fruit ointment is brown. The texture of fresh fruit ointment is smooth while the texture of dry fruit ointment is slightly rough due to the presence of fine powder of dry fruit. The components of fresh fruits are equally homogenized as compared to dry fruit ointment. Fresh fruit ointment has fruity smell while no typical smell was observed from dry fruit ointment. The pH values of fresh fruit and dry fruit ointment is 5.0 and 6.1 respectively. The viscosity of fresh fruit and dry fruit ointment was recorded in viscometer 40.2% and 32.5% respectively at 37°C with revolution of 10rpm.

Conclusion:-

The research showed that the fruit of Carissa carandas has potential in wound healing. The phytochemical analysis of the fruit of Carissa carandas showed the presence of vitamin c, calcium and some anti-inflamatory agents. The presence of these constituents helps in the wound healing. So the ointment obtain from the fruit extract of Carissa carandas has potential in wound contraction.

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