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Examination and Chemical Analysis by GC-MS of Syzygium Cumini Extract (Bark)

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Abstract:

The investigation focused on the analysis of the hexane extract obtained from the bark of Syzygium cumini originating from India. Through chromatographic techniques, both traditional column chromatography and gas chromatography mass spectrometry (GC-MS), the oily fraction of the bark was examined, leading to the identification of numerous ester and hydrocarbon compounds. While the initial hexane eluate yielded waxy substances, which were challenging to separate through column chromatography due to their limited quantity, GC-MS analysis facilitated the identification of 39 compounds. These compounds were matched with existing literature through comparisons of retention times, Kovats indexes, and interpretation of mass spectra. Many of these identified compounds find applications across industries such as perfumery, flavoring, deodorants, antiseptics, and pharmaceuticals. This study aimed to elucidate the chemical composition of the hexane extract from S. cumini bark, particularly focusing on the identification of hydrocarbons and esters.

Key words: *Syzygium cumini* (bark), esters, hydrocarbons, signals, peak gas chromatography -mass spectrometry

Introduction

Gas chromatography-mass spectrometry (GC-MS) is a hyphenated analytical technique that combines two distinct methods to analyze chemical mixtures. Gas chromatography separates the components of the mixture, while mass spectrometry characterizes each individual component. This combined approach allows analytical chemists to assess solutions containing multiple chemicals both qualitatively and quantitatively.

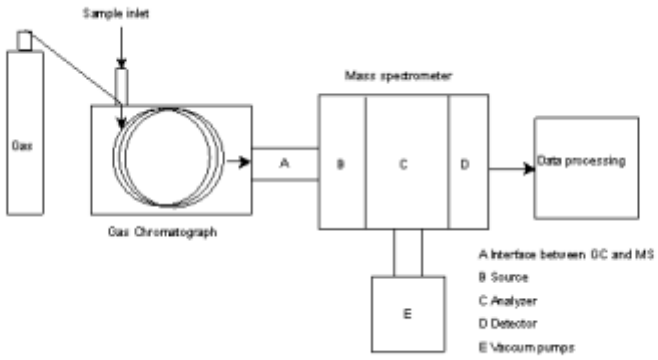

Fig. No. 1: Block diagram of Gas chromatography-mass spectroscopy (GC-MS) technique.

3.1: Gas Chromatography

Chromatography is a technique which uses to separate the chemical mixtures in their individual components for analysis. This process involves a mobile phase, which carries the sample mixture through a stationary phase. In liquid chromatography (LC), the mobile phase is a solvent, while in gas chromatography (GC), it is an inert gas like helium. A stationary phase selectively interacts with the components of a mixture as they pass through the column, causing them to separate based on their different interaction rates.

Note that, the total gas flow flows:

1. through the column (green thing at bottom),
2. out the septum purge, and
3. out the split vent and always add up to the incoming flow, 49 ml/min in this example (1 + 2 + 46 = 49 ml/min)

As depicted in the diagram, the gas flow traverses through the septum purge and split vent, with a portion of a injected sample undergoing vaporization and exiting through out the split vent. In this instance, the split ratio is 1:49, indicating that for every 49 parts injected, only 1 part enters the column for analysis.

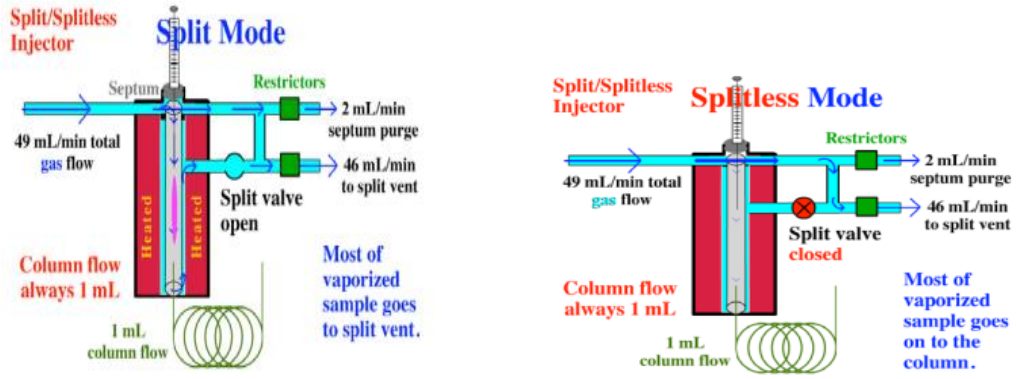

Fig.No. 2 Representing sampling mode in GC instrument.

Mass Spectroscopy

As the compounds exit the GC column, they enter the electron ionization detector where they encounter a stream of electrons. This electron bombardment causes the compounds to fragment into smaller pieces. The gas molecules leaving the GC column are subjected to a high-energy electron beam with an energy of 70 electron volts (eV). When an electron collides with a molecule, it may transfer sufficient energy to eject another electron from that molecule. For instance, methanol undergoes the following reaction in the ionization region:

$CH_3OH + 1\ e^- \rightarrow CH_3OH^{+\cdot} + 2\ e^-$

(The symbols +. indicate that a radical cation was formed)

Electron impact ionization (EI) typically generates singly charged ions characterized by possessing one unpaired electron. When a charged molecule retains its original structure, it is referred to as the molecular ion. However, the

molecular ion may become unstable due to the energy transferred during electron impact, leading it to split into smaller fragments. For instance, methanol ions can undergo various fragmentation pathways, resulting in one fragment retaining the charge while another remains uncharged.

For example:

CH3OH+. (molecular ion) CH2OH+ (fragment ion) + H.

(or)

CH3OH+. (molecular ion) CH3 + (fragment ion) + . OH

3.3 Present work:

For the present work we, have taken the reddish brown coloured oily fraction of *S.cumini* plant as isolated from the separation of hexane extract.

MATERIALS AND METHODS

Bark

Plant material from S. cumini was gathered in the vicinity of jaunpur, Uttar Pradesh, and a voucher specimen was archived at Achary Narendra Dev Agricultural University Ayodhya . The essential oil was extracted from the fresh bark through a four-hour steam distillation process. This species yielded a yellowish oil with a pleasant aroma, constituting 0.30% of the fresh weight.

Extraction

The air-dried bark of S. cumini underwent Soxhlet extraction using n-hexane, followed by fractionation on a silica gel column. Various solvents were employed for elution in a sequence of increasing polarity.(Table.1)

GC-MS Technique

A Hewlett-Packard 5890 Series II Chromatograph, equipped with a flame ionization detector (FID) and HP-2 fused silica columns (25 m × 0.32 mm, with a film thickness of 0.25 μm), was utilized. Samples, dissolved in hexane, were injected in splitless mode into a helium carrier gas. Injector and detector temperatures were maintained at 250°C. The column temperature was programmed to increase from 60°C (after 2 minutes) to 220°C at a rate of 4°C per minute, and the final temperature was sustained for 20 minutes. Peak areas and retention times were electronically integrated by a computer. Relative amounts of individual components were determined based on the obtained peak areas, without correction for FID response factors.

GC-MS analyses were conducted using a Hewlett-Packard 5970A mass selective detector (MSD), directly linked to an HP 5790A gas chromatograph. A column measuring 26 m in length and 0.22 mm in diameter, coated with 0.13 μm of CP-Sil 5CB, was utilized with helium as the carrier gas. The oven temperature program began at 60°C for 3 minutes, followed by a ramp of 5°C per minute until reaching 250°C, which was then held for 30 minutes. All other parameters remained consistent with those described for GC. Electron ionization (EI) mass spectra were recorded across a mass range of 10 to 400 Da at a rate of 2 scans per second.

Processing of Hexane extract of Bark of Syzygium*cumini*

The n-hexane eluate of n-hexane extract of *S.cumini* yielded a yellowish, waxy fraction, which was rechromatographed on alumina column. Its DCM (pure) eluate, designated as Sample No-1 yielded a waxy liquid which was small in amount and was not separated by column chromatography. Hence it was separated by GC-MS analysis which revealed presence of 11 compounds.

The sample was separated and analyzed by the GC-MS technique from Saurashtra University, Rajkot (Mehta B K *et al.* 2012).

The compounds were identified by comparing their retention time and covate indices that of literature and by interpretation of mass spectra. The quantitative estimation of each peak was made by estimating area of peak by computer, attached by GC-MS instrument. The result of GC-MS analysis is given in table No.1.

Results and discussion

Total 11 compounds were identified from the above fractions and are given below:

Sample No 1: Cyclopentanol, 5, 7, 9 trimethyl tridecanoic acid, Stigmast-5-en-3-ol, cyclopropanamine, urs-12-ene,Pregnane,5,7 dimethyl tetratricontane, 2,2-dimethyl heptanes; 7,10,13 trimethylhexatriacontane,2Methyl-3,13-octadecadienol (Table No: 1; Fig No).

Table No.1: Identification of Chloroform fraction of Hexane extract of *S.cumini* (Bark): (Sample No. 1)

Peak No.	Retention Time	Area %	Molecular mass(M^+)	Molecular formula	Compound Name
1	3.877	12.93	86	$C_5H_{10}O$	Cyclopentanol
2	9.918	4.94	256	$C_{16}H_{32}O_2$	5,7,9 trimethyl tridecanoic acid
3	10.853	6.35	280	$C_{19}H_{36}O$	2Methyl-3,13-octadecadienol
4	11.197	37.33	147	$C_{10}H_{13}N$	Cyclopropanamine
5	11.999	7.20	410	$C_{10}H_{50}$	Urs-12-ene
6	14.044	11.92	288	$C_{21}H_{36}$	Pregnane
7	14.630	3.05	506	$C_{36}H_{74}$	5,7 dimethyl tetratricontane
8	15.311	3.26	128	C_9H_{20}	2,2-dimethyl Heptane
9	16.130	4.67	506	$C_{36}H_{74}$	7,10,13 trimethyl tritriacontane
10	18.810	6.07	414	$C_{29}H_{50}O$	Stigmast-5-en-3-ol
11	19.615	2.28	220	$C_{15}H_{24}O$	Cedroxyde or 2,6-di-tert-butyl-4-methyl phenol

Identification of compounds

The identification of the compounds present in the VLC fractions of hexane extract was based on direct comparison of retention times and mass spectral data for standard compounds, and by computer matching with Wiley 229, Nist 107, 21 Library, also by comparison of the fragmentation patterns of mass spectra with reported in the literature (Abok and Manulu, 2017; Muthumperumal *et al.* 2016; Ullah, *et al.*2017; Kataria S *et al.* 2011; McLafferty and Turecek, 1993; Siddiqui and Patil *et al*. 2015;Chauhan *et. al*. 2014. Devi and Singh, 2013; Timotius K H, *et. al.* 2015. Intan S. Ismail *et al.* 2010; Supabphol1 and Tangjitjareonkun, 2014; Hussain and Maqbool, 2014; Joseph *et al*. 2015).

Experimental values

Identification of chloroform fraction of n-hexane extracts (Sample No 1)

Peak: 1 (RT: 3.877, M^+86, Molecular Formula: $C_5H_{10}O$, Cyclopentanol, Fig.No.4.1)

The mass spectrum of peak-1 of SSC-03 sample showed the molecular ion peak (M^+) at m/z 86 which was suggested the molecular formula $C_5H_{10}O$. The base peak was obtained at m/z 57. The peak was obtained at 70 due to the moiety formed by loss of –OH unit from the parent molecule other abundant fragments were obtained at m/z, 57 and 50. Thus the compound was identified as Cyclopentanol.

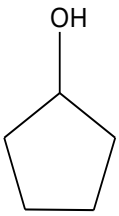

Fig.No.4.1 ($C_5H_{10}O$, Cyclopentanol,)

Peak: 2 (RT: 9.918, M^+256, Molecular Formula: $C_{16}H_{32}O_2$, 5, 7, 9 trimethyl tridecanoic acid, Fig. No.4.2)

The mass spectrum of peak-2 of SSC-03 showed the molecular ion peak (M^+) at m/z 256 which was suggested the molecular formula $C_{16}H_{32}O_2$.The base peak was obtained at m/z 60 formed by the Mc lafferty rearrangement in the molecule. Other abundant fragments were 213, 171, and 129 confirmed the presence of aliphatic chain in the molecule. This type of fragmentation pattern confirmed and identified the compound as 5,7,9 trimethyl tridecanoic acid.

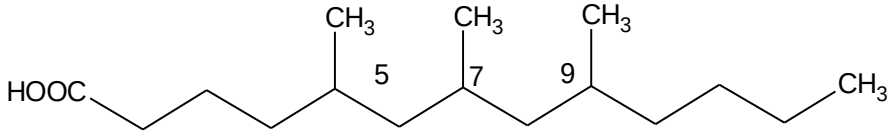

Fig. No.4.2 ($C_{16}H_{32}O_2$, 5, 7, 9 trimethyl tridecanoic acid)

Peak: 3 (RT: 10.853, M^+280, Molecular Formula: $C_{19}H_{36}O$, 2Methyl-3, 13-octadecadienol, Fig.No.4.3)

The mass spectrum of peak-3 of SSC-03 showed the molecular ion peak (M^+) at m/z 280 which was suggested the molecular formula $C_{19}H_{36}O$.The most abundant peak (base peak) was obtained at m/z 55.Other abundant fragments were obtained at 259,244,225,209,193,183,165,148,123,98,81,67,57,55. Thus the compound was identified as 2Methyl-3, 13-octadecadienol.

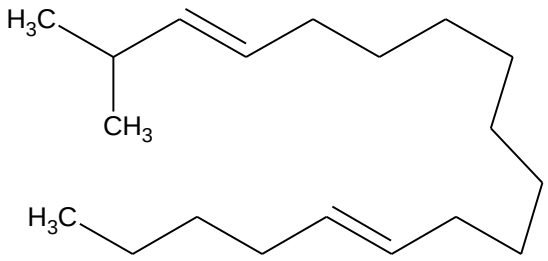

Fig.No.4.3 ($C_{19}H_{36}O$, 2Methyl-3, 13-octadecadienol)

Peak: 4 (RT: 11.197, M^+147, Molecular Formula: $C_{10}H_{13}N$, Cyclopropanamine, Fig. No. 4.4)

The mass spectrum of peak-4of SSC-03 showed the molecular ion peak (M^+) at m/z 147 which was suggested the molecular formula$C_{10}H_{13}N$ .The most abundant peak (base peak) was obtained at m/z 147. Thus the compound was identified as Cyclopropanamine.

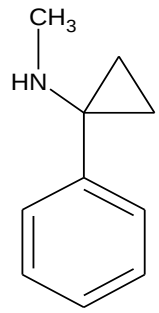

Fig. No. 4.4 ($C_{10}H_{13}N$, Cyclopropanamine,)

Peak: 5 (RT: 11.999, M^+410, Molecular Formula: $C_{10}H_{50}$, Urs-12-ene, Fig. No.4.5)

The mass spectrum of peak-5 of SSC-03 showed the molecular ion peak (M^+) at m/z 410 which was suggested the molecular formula $C_{10}H_{50}$. The base peak was obtained at m/z 218.Other successive fragments were 389, 372, 355, 340, 334, 299, 282, 227, 190, 175, 147, 134, 122, 107, 95, 81. Thus the compound was identified as Urs-12-ene.

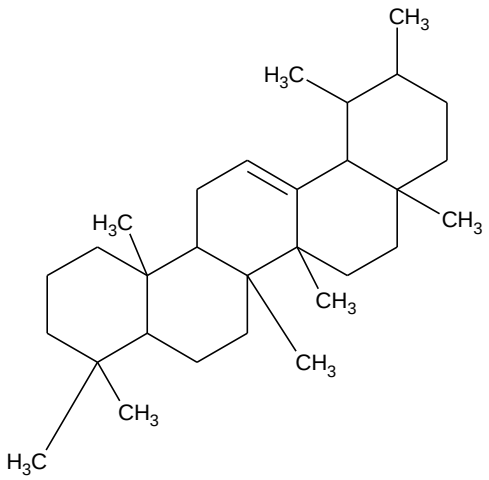

Fig. No.4.5 ($C_{10}H_{50}$, Urs-12-ene)

Peak: 6 (RT: 14.044, M^+288, Molecular Formula: $C_{21}H_{36}$, Pregnane, Fig. No. 4.6)

The mass spectrum of peak-6 of SSC-03 showed the molecular ion peak (M^+) at m/z 288 which was suggested the molecular formula$C_{21}H_{36}$. The base peak was obtained at m/z 57. Other successive fragments were 273, 246, 220, 205, 179,161, 137, 125, 109, 95, 71, 57, and 55. Thus the compound was identified as Pregnane.

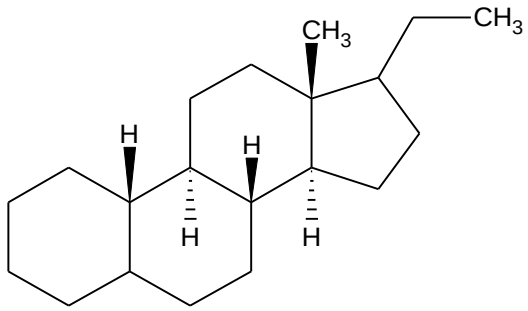

Fig. No. 4.6 ($C_{21}H_{36}$, Pregnane,)

Peak: 7 (RT: 14.630, M^+506, Molecular Formula: $C_{36}H_{74}$, 5, 7 dimethyl tetratricontane, Fig. No.4.7)

The mass spectrum peak-7 of SSC-03 of showed the molecular ion peak (M^+) at m/z 478 which was suggested the molecular formula $C_{34}H_{70}$. The base peak was obtained at m/z 57. Other abundant fragments were obtained at447,417,403,364,349,333,301,268,251,224,211,194,168,154,141,127,113,99,85,71,and 57. Thus the compound was identified as 5, 7 dimethyl tetratricontane.

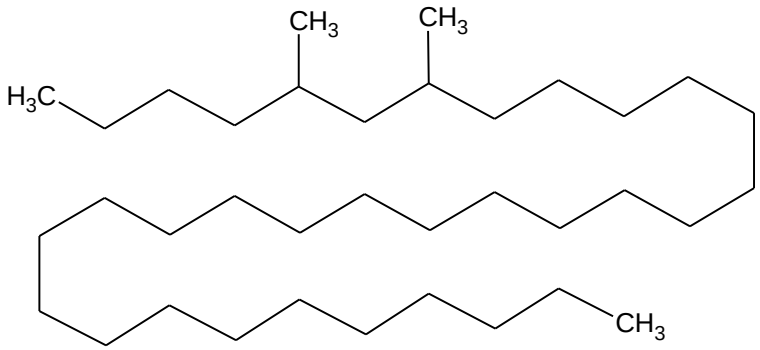

Fig. No.4.7 ($C_{36}H_{74}$, 5, 7 dimethyl tetratricontane,)

Peak: 8 (RT: 15.311, M^+128,Molecular Formula: C_9H_{20}, 2,2-dimethyl Heptane, Fig.No.4.8)

The mass spectrum peak-8 of SSC-03 of showed the molecular ion peak (M^+) at m/z 128 which was suggested the molecular formula. The base peak was obtained at m/z 57 due to presence of $C(CH_3)_3$. Thus the compound was identified 2, 2-dimethyl Heptane.

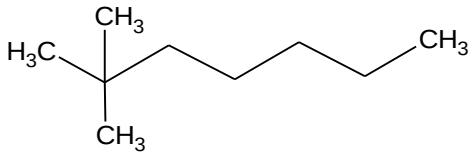

Fig.No.4.8 (C_9H_{20}, 2,2-dimethyl Heptane)

Peak:9 (RT: 16.130, M^+506,Molecular Formula: $C_{36}H_{74}$, 7, 10, 13trimethyl tritriacontane, Fig.No.4.9)

Mass spectrum shows that compound is a long chain saturated aliphatic hydrocarbon,i.e. 7,10,13 trimethyl tritriacontane (M^+506).Predominance of C_nH_{2n+1} peaks with separation of 14 u with less abundant molecular ion peak at 506 proves long chain saturated aliphatic nature of the compound. Most abundant peaks at 127, 113, 99, 85, and 57.

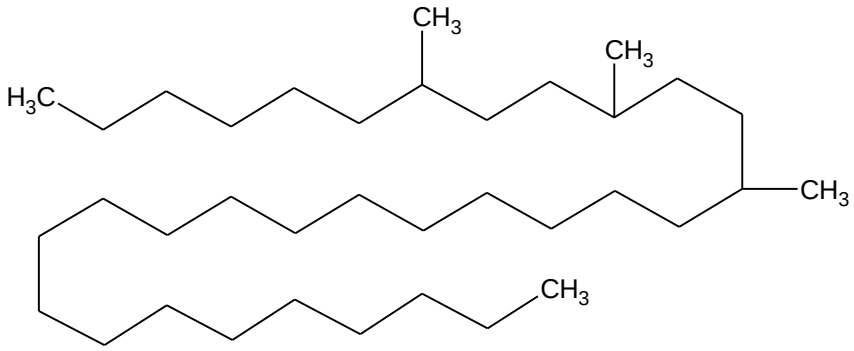

Fig.No.4.9 ($C_{36}H_{74}$, 7, 10, 13trimethyl tritriacontane)

Peak: 10 (RT: 18.810,M^+414,Molecular Formula: $C_{29}H_{50}O$, Stigmast-5-en-3-ol, Fig.No.4.10)

Mass spectrum showed that the compound is Stigmast-5-en-3-ol.Most abundant peak is present at 57.The molecular ion peak is present at m/z,M^+414.Other successive abundant fragments are present at 396,381,367,357,338,329,321,303,285,271,255,250,230,213, 203, 191,175, 160,133,120,105,91,81 and 57.

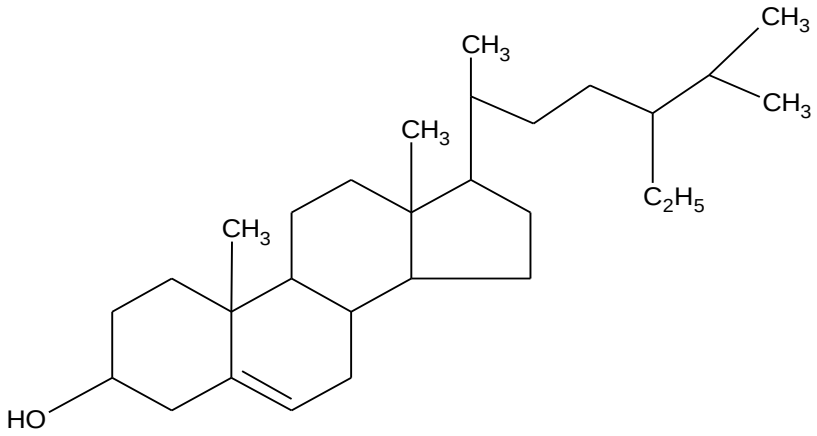

Fig.No.4.10 ($C_{29}H_{50}O$, Stigmast-5-en-3-ol,)

Peak: 11(RT:19.615, M^+ 220,Molecular Formula:$C_{15}H_{24}O$,Cedroxyde or 2,6-di-tert-butyl-4-methyl phenol, Fig.No.4.11)

The mass spectrum of peak-11 of SSC-03 sample showed the molecular ion peak (M^+) at m/z 220 which was suggested the molecular formula C_{15} H_{24} O. The base peak was obtained at m/z 69,which suggested the aromatic

nature of molecule. Other abundant fragments were obtained at m/z, 211, 197, 189, 163, 145, 128, 107, 81, 69 and 55. Thus the compound was identified as Cedroxyde or 2, 6-di-tert-butyl-4-methyl phenol.

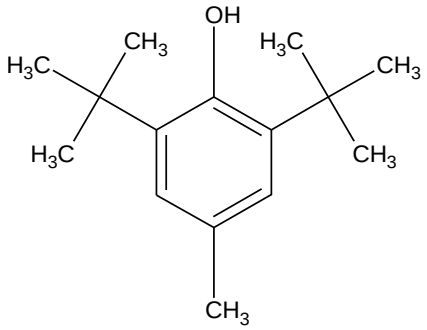

Fig.No.4.11 ($C_{15}H_{24}O$,Cedroxyde or 2,6-di-tert-butyl-4-methyl phenol,)

Conclusion:

This technique can be used to detect identify and quantify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitrogen compounds present in plant part extracts (Shree Devi *et al.*2015). Thus the GC-MS is an advanced technique that cannot be compared with other modern analytical tools and it has broad range of applications that cater to academic research, quality control as well as industrial applications. Its concise, efficient, automated system gives fast, reproducible and effective results that serve a key role in advancement of "Science and technology". This multipurpose and investigate technique could be explored for better prospects in future.

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