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Identification of Major Volatile (Essential Oil) Constituents of "Carrom Seeds" and "Clove Buds"

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ABSTRACT:

The major volatile constituents of essential oil obtained from carrom seeds and clove buds have been analyzed by GC and FT-IR. Physio-chemical and phyto-chemical properties of carrom oil and clove bud oil were checked and confirmed with standard oil samples. Among carrom oil, thymol and p-cymene were the major constituents constituting 61% and 14%. In clove oil, eugenol was main compound constituting 78%. FT-IR results confirmed the presence of two major phenolic group i.e. thymol and eugenol in carrom oil and clove oil respectively.

KEYWORDS: Essential oil, GC, FT- IR, phyto-chemical, physio-chemical, eugenol, iso-eugenol, thymol, p-cymene, carrom seeds, clove buds, Phenolic.

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INTRODUCTION:

Carrom seeds and clove buds are traditional spices grown in India in majority of states. Though both the spices belong to different family still they possess almost similar medicinal properties. Carrom seeds and clove buds categorize itself under family Apiaceae[1] and Myrtaceae[2] respectively. Carrom seeds botonical name is tranchyspermum ammi[1] where trachyspermum is genus and ammi is species. Botonical name of clove buds is Syzygium aromaticum[2] where Syzygium is genus and aromaticum is species. Both come under kingdom plantae. The Apiaceae family consists of almost 347 genera and 12816 species. Essential oil extracted from both the spices is a volatile oil having distinguishable organoleptic and physio-chemical characteristics. Both the oil possesses wide medicinal properties followed by wide range of compounds[3] useful in perfume, flavor and fragnance industry. The carrom oil is majorly used in mouthwash[4] due to its good antimicrobial properties compared to other spices. Clove oil in turn is a natural antimicrobial[5,6], antiseptic, and anti-fungal[7] agent. Clove oil is also one of the highest sources of nutrition as it contains manganese, vitamin C and vitamin K[2]. Clove oil are also a natural anesthetic the reason for which they were often used for dental treatment from centuries and are still used in some cultures to remedy toothache[8]. They are highly beneficial in reducing the pain of tooth[9]. Clove oil also possesses antibacterial properties[10], thus they can be used a natural pesticides to kill bacteria. Apart from medicinal usage the seeds and buds of carrom and clove are as such used as spices and herbs in preparing various edible dishes. Traditionally smoke of heated carrom seeds can be sometimes used for treatment of cough. Essential oil derived from carrom oil possesses high antimicrobial[11], anti fungal[12] and insecticidal[13,14] activity. These pesticide properties are basically because of thymol the chief constituents of oil which largely contributes to success of oil[15]. Moreover this oil can also be used as natural antioxidant due to presence of phenolic compound.

EXPERIMENTAL SECTION:

a) *Materials and Methods:*

The fresh seeds and buds of carrom and clove were collected from local market in Maharashtra, India. The carrom seeds were sunlight dried prior to extraction for 24 hour. After drying, seeds were stored away from moisture. In case of clove buds instead of drying and crushing they were directly used for isolation of oil.

b) ***Isolation of essential oil:***

The fresh crushed powder (100g) of carrom seeds and clove buds were hydro-distilled for 4 h at 100 °C using Clevenger apparatus. The time of distillation was decided by checking the aroma profile of oil. Based on density difference oil and water were separated in Clevenger apparatus. Collected oil was further dried using anhydrous sodium sulphate and stored in dark glass bottle until analysis.

c) ***Analysis of oil:***

The gas chromatography (GC) analysis of the essential oil was carried out on capillary column (HP-5 Agilent 19091J-413; 325 C 30 m X 320 µm X 0.25 µm). Temperature programming was 90 -190°C with 10 °C ramp/min and 190 – 290 °C with 5 °C ramp/min. Inlet temperature and detector temperature was 180 °C and 210 °C. Nitrogen was used as carrier gas at 1ml/min. FID section was used. The sample injection volume was 1.0 µl/min diluted in n-hexane, with split ratio of 100:1.

d) ***Physio-chemical analysis:***

Oil obtained from hydro-distillation was subjected to physio-chemical analysis such as refractive index, specific gravity, acid value, moisture. Further Organoleptic properties of oil were also determined such as color, physical appearance, odor, solubility. Result of Physio-chemical and organoleptic analysis of both oil are tabulated in table 1. All the analysis done was based on standard method of pharmacopeia.

e) ***Other analysis:***

Phyto-chemical analysis of both carrom and clove oil was carried out[16,17]. This analysis does not give any brief information about compounds of oil but it do show group of phyto-chemical compound present in oil. More so compound confirmatory test of oil is also done. Results of confirmatory and phyto-chemical analysis are presented in table 2 and 3.

f) ***Identification of the constituents:***

Identification of major volatile constituents of carrom oil and clove oil occurring in GC graph shown below was done by comparing both retention time and area of samples of, extracted oil and standard compound. Both the samples were diluted in same concentration followed by same method of programming for analyzing on GC. Few other compounds, whose standards were not available, were identified by comparing literature data from library. Calibration curve

was used for thymol, p-cymene and eugenol. Confirmation of these specific major volatile compounds present in carrom and clove oil was done by FT-IR analysis. In FT-IR analysis major (i.e. thymol and eugenol) compound were identified by confirming functional group of peaks adhering in FT-IR spectrum with standard frequency.

RESULTS AND DISCUSSIONS:

Physio-chemical analysis followed by organoleptic properties of carrom oil and clove oil are very much similar to standard oil samples. Confirmatory test of both oil confirm presence of aldehyde and phenolic group. Phyto-chemicals analysis shows presence of terpenoids in oil. GC result presented in table, indicates the major volatile compound present in carrom oil and clove oil based on their retention time. From calibration curve it was concluded that carrom seed oil contains 61 % thymol followed by 14% p-cymene where as clove oil contains 78% of eugenol. Also, presence of thymol and eugenol were confirmed by FT-IR analysis. The entire bond present in structure of both thymol and eugenol were confirmed with standard frequency and functional group adhering in their spectrum.

Table No. 1: **Physio chemical and organoleptic analysis of Carrom seed and clove bud oil**

Test	Carrom seed oil	Clove bud oil
Moisture	0.08 %	0.02%
Odour	Spicy	Woody spicy
Solubility	Soluble in solvent but not in water	Soluble in solvent and partially soluble in water
Physical state	Liquid	Liquid
Colour	Yellow brown	Light yellow
Refractive index at 25 °C	1.485	1.579
Specific gravity at 25 °C	0.93	1.04
Acid value	1.32	8.217

Table No. 2: Confirmatory test of oil

Tests	Carrom oil	Clove oil
Aldehyde	Present	Present
Ketone	Absent	Absent
Phenol	Present	Present
Ester	Absent	Absent
Alcohol	Absent	Absent

Table No. 3: Phyto-chemical analysis of oil

Tests	Carrom oil	Clove oil
Terpenoids	Present	Present
Saponin	Present	Absent
Tannin	Absent	Present

Table No. 4: GC results of carrom oil

Sr. no	RT (min)	Constituents
1	10.138	β-pinene
2	11.531	p-cymene
3	12.516	γ-terpinene
4	19.157	Thymol

Table No. 5: GC results of clove oil

Sr. no	RT (min)	Constituents
1	20.978	Eugenol
2	22.436	caryophyllene
3	23.243	Iso eugenol
4	24.875	Eugenol acetate

Table No. 6: FT-IR results of Carrom oil

For Thymol

Sr.no	Bond	Functional group	Frequency, cm^{-1}
1	O-H stretch	Phenol	3462.22
2	C-H stretch	Aromatic ring	2960.73
3	C-C stretch	Aromatic ring	1417.68
4	C-H substituted	Meta disubstituted aromatic	808.17

Table No. 7: FT-IR results of Clove oil

For Eugenol

Sr.no	Bond	Functional group	Frequency , cm^{-1}
1	O-H stretch	Phenol	3510.45
2	C-H stretch	Aromatic ring	2937.59
3	C-C stretch	Aromatic ring	1512.19
4	C-H substituted	Para disubstituted aromatic	794.67
5	C-O stretch	Ether	1265.3
6	=C-H bend	Alkene	1010

Figures:

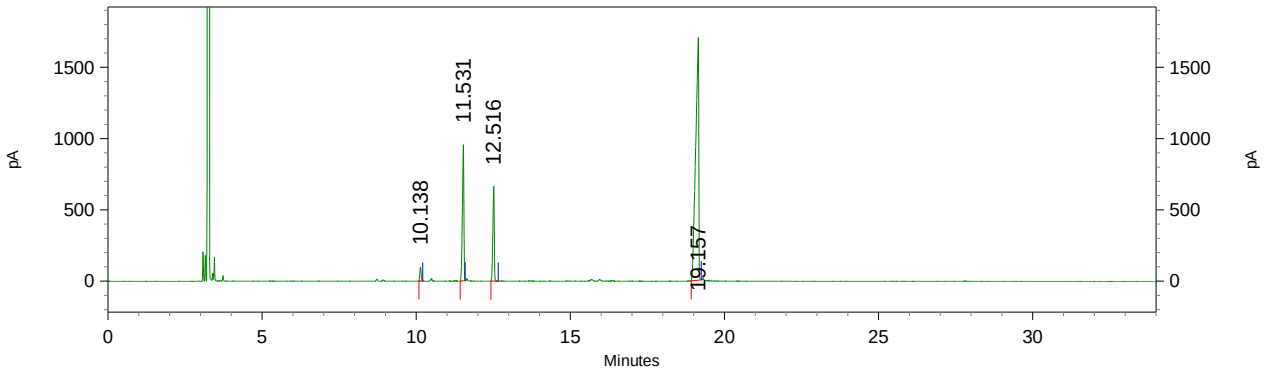

Figure No. 1: GC graph of carrom oil

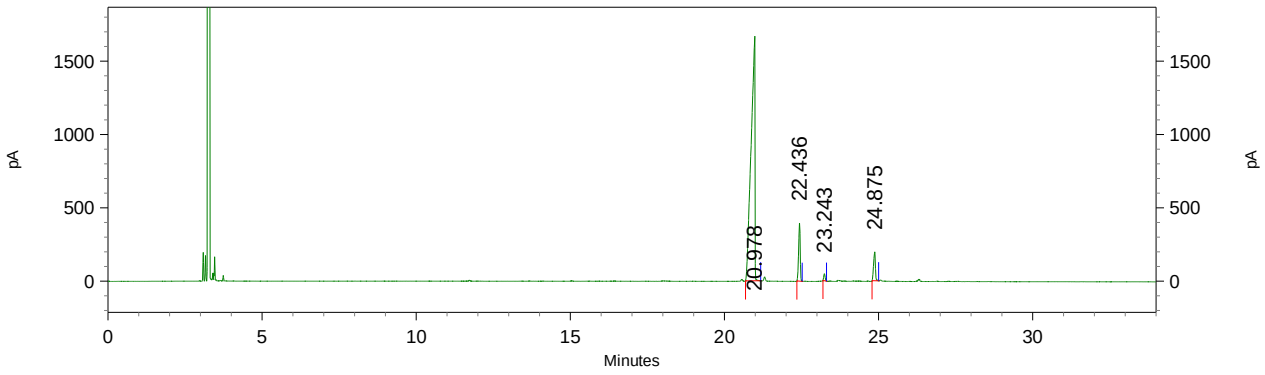

Figure No. 2: GC graph of clove bud oil

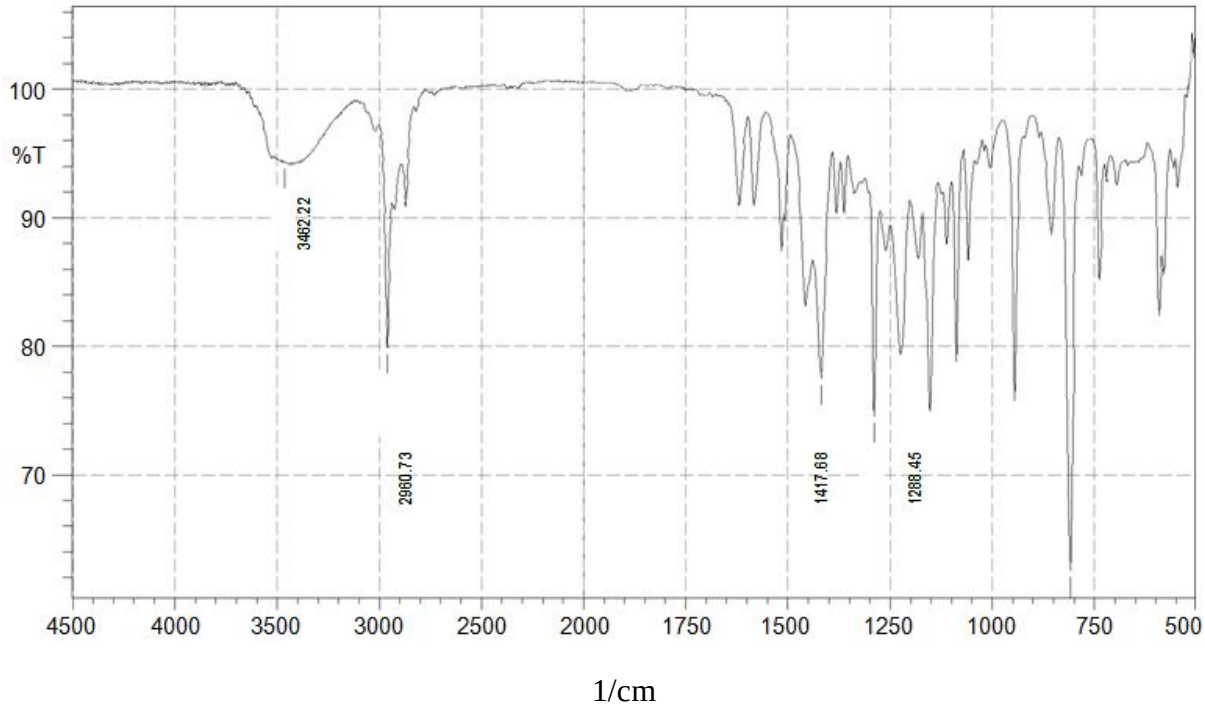

Figure No. 3: FT-IR spectrum of carrom oil

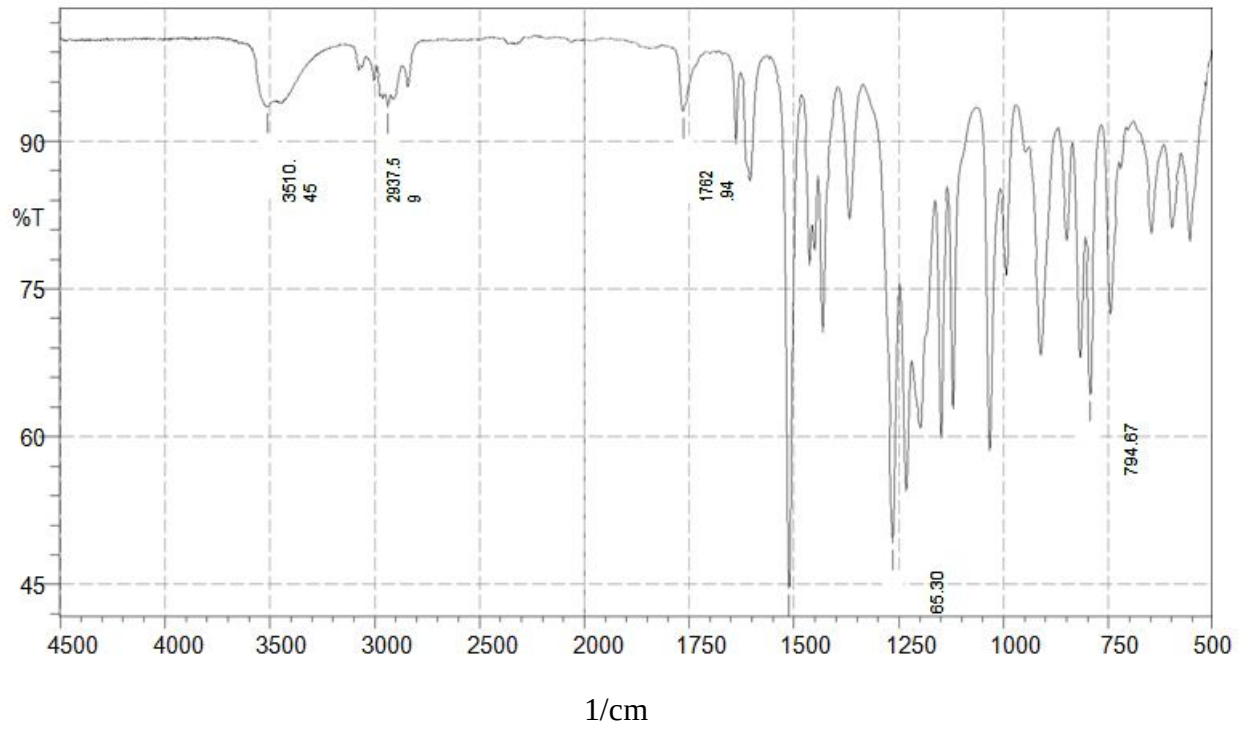

Figure No. 4: FT-IR spectrum of clove bud oil

CONCLUSION:

From the above work it can be concluded that hydro-distillation is efficient technique to extract essential oil from spices considering yield, purity and aroma of oil. Also, carrom oil and clove oil are one of the natural sources to isolate thymol and eugenol.

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