IJBPAS, January, 2014, 3(1): 78-87

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYSICOCHEMICAL CHARACTERIZATION AND SPASMOLYTIC ACTIVITY OF ESSENTIAL OIL OF CUMIN (*CUMINUM CYMINUM* LINN.) FROM RAJASTHAN

SAINI N[1*], SINGH GK[2] AND NAGORI BP[3]

1: Department of Quality Assurance, Lachoo Memorial College of Sci. and Tech., Pharmacy wing, Jodhpur (Rajasthan), Mobile No.00919589796160

2: Department of Pharmacognosy, Lachoo Memorial College of Sci. and Tech., Pharmacy wing, Jodhpur (Rajasthan)

3: Director, Lachoo Memorial College of Sci. and Tech. Pharmacy wing, Jodhpur (Rajasthan)

[*]Corresponding Author: E Mail: saininavdeep079@gmail.com; 919589796160

ABSTRACT

Cumin (*Cuminum cyminum* Linn.) from Rajasthan locally known as 'jira' is a flowering plant in the family Umbelliferae. Cumin is known for its flavour and antioxidant properties. Cumin also act as preservative, provides nutritional and health benefits. The essential oil of *Cuminum cyminum* Linn. was obtained from mature fruits, extracted through hydro distillation for 4-5 hr in clevenger apparatus. The most important chemical component of cumin fruits is essential oil content, ranging from 2.6% to 3.6% which is pale to colourless depending on age and regional variations. Physical characteristics and spasmolytic activity were recorded according to standard procedures. Chemical composition of essential oil of *Cuminum cyminum* L. was analyzed by Gas-liquid chromatography and Thin layer chromatography. The present study results revealed that essential oil of *Cuminum cyminum* Linn. (Fam. Umbelliferae) physicochemical characterization and promising antispasmodic action on excised guinea pig ileum.

Keywords: Essential Oil, Physicochemical Characterization, Gc, Tlc, Jira, Hydro-Distillation, Spasmolytic, Guinea Pig Ileum

INTRODUCTION

Cuminum cyminum L. is an annual plant of the family Umbelliferae. In India cumin is commonly known as 'jeera' or 'jira' **(Table 1)**. Cumin is herbaceous annual plant, with a slender branched stem 30-90cm tall **[1]**. The leaves are 5-10cm long, pinnate or bipinnate, thread-like leaflets. The flowers are small, white or pink, and borne in compound umbels. The fruit a cremocarp, often separated into mericarps, brown with light coloured ridges ellipsoidal, elongated, about 4-6 mm long, 2mm wide, tapering at ends and slightly compressed laterally, mericarps with 5 longitudinal hairy primary ridges from base to apex, alternating with 4 secondary ridges which are flatter and bear conspicuous emergences, seeds orthospermous, odour umbelliferous characteristic, taste, richly spicy **[2, 3] (Table 2)**. Cumin fruit are similar to fennel in appearance, but are smaller and darker in colour. Cumin is native from the East Mediterranean to East India, cultivated almost throughout India. In India Rajasthan and Gujrat states is main cultivator. The medicinal component of the plant is cumin oil extracted from the ripe fruits through hydro distillation for 4-5 hr in Clevenger apparatus **[4]**. Cumin is used as a carminative for stomach disorders, diarrhoea, and spasmolytic. The oil of cumin is especially used as an antioxidant and flavour. Essential oil of *Cuminum cyminum* L is more or less powerful external or internal antiseptic, analgesic, anti-inflammatory, haemolytic, or anti enzymatic action, sedative, stimulants and stomachics **[5]**. The present study reports physicochemical characterization and spasmolytic activity of essential oils extracted from cumin (*Cuminum cyminum* Linn.) collected from Jodhpur, Rajasthan, India. Physicochemical characterization of essential oils was carried out according to the method described by Association of Official Analytical Chemists **[6]** to determine their solubility, yield %, acid value (mg/KOH/g), saponification value (mg/KOH/g), ester value, peroxide value (mEq/kg) and iodine value (g/g). The present paper describes the Chromatographic (TLC and GC) and physicochemical characterisation of the essential oil of the fruits of *Cuminum cyminum Linn.* (Fam. Umbelliferae) **(Figure 1)**.

MATERIAL AND METHOD

Plant Material

All the chemicals, reagents and solvents used for determination of physicochemical parameters of essential oils were of analytical grade and purchased from Puneet Enterprises, Ratlam, Madhya Pradesh, India. Cuminaldehyde was purchased from

Sigma-Aldrich Chemical, St. Louis, MO, USA and acetylcholine from Merck India Ltd., Mumbai, India. The ajwain fruits of *Trachispermum ammi* Linn. Sprague (Fam. Umbelliferae) were collected from the local market of Rajasthan and identified by Prof. (Dr.) S. K. Panday, KNK College of Horticulture, Mandsaur, Madhya Pradesh. Plant material was deposited in the herbarium of the Phytochemistry Research Laboratory, KNK College of Horticulture, Mandsaur, Madhya Pradesh, India.

Extraction of Volatile Oil

At present different extraction techniques like distillation, effleurage, CO_2 extraction, expression and solvent extraction are applied for oil extraction from ajwain. But commonly hydro distillation is used to study the minute quantity of essential oil of *Cuminum cyminum* Linn. (Fam. Umbelliferae). The dried fruits of *Cuminum cyminum* Linn. (Fam. Umbelliferae) were greenish brown to yellowish brown in colour. Extraction of essential oil was carried out by hydro distillation of 500g of the powdered drug with 1000ml of water in clavenger's apparatus for 10hours to obtain essential oil. Light yellowish coloured essential oil (Yield 2.4%) was obtained having characteristic odour and taste. *Cuminum cyminum* Linn. (Fam. Umbelliferae) oil was dried over anhydrous sodium sulphate to remove moisture and stored in refrigerator in dark at 4^oC for further analysis **[9]**.

Physicochemical Characterization

The Physicochemical values like Iodine value, Saponification value, Ester value, Acid value were determined according to the procedure of British Standard Specification **[10]**. Refractive index was determined with Abbe's refractometer. It has been observed that the freshly harvested C*umin* fruits had higher moisture and the same decreased with the passage of time. Low moisture in the *Cumin* extract is advantageous for their shelf-life, enabling preservation for a longer period. The yield of the essential oils, obtained by hydro distillation of C*umin*, varied from 2.1% to 2.6% and the colour from yellowish brown to dark brown. Essential oils were soluble in alcohol, chloroform, carbon tetrachloride and hexane. Acid value (mg/KOH/g) is an important index of physicochemical properties, being indicative of age, quality, edibility and suitability of oils. The acid value 2.45 (mg/KOH/g) was found in the essential oil obtained from freshly harvested C*umin* fruit collected from jodhpur and the highest 20.0 (mg/KOH/g) in the essential oil of C*umin* received from Jain Super Store, New Delhi, India. The high acid value is indicative of oil becoming rancid due to storage of essential oil under improper conditions or adulteration.

Saponification value (mg/KOH/g) gives an idea of the average molecular weight or the chain length of all the acids present. Higher the molecular weight, the lower the saponification value and being inversely related. The saponification value was found higher in the essential oil of C*umin* obtained from Jain Super Store, New Delhi, India. The high saponification value of the oil indicates the presence of high molecular weight fatty acids in it. Ester value is the difference of saponification value and acid value. The peroxide value (mEq/kg) of an oil or fat is used as a measurement of the extent of rancidity reactions. Air, or specially oxygen in the air, can react with the oil and form various peroxide components which eventually affect odor, flavour and quality. Lower the peroxide value, the fresher the oil would be. In general, peroxide levels higher than 10.0 means less stable oil with a shorter shelf life. Iodine value (g/g) indicates the number of double bonds present and, therefore, the degree of unsaturation. The higher the iodine value, the more the double bonds in the molecule as also the oil being more prone to rancidity. The iodine values of essential oils are below 100, which shows the presence of saturated fatty acids and places them in the category of non-drying oils.

The essential oil analysis was carried out in triplicate and the data was statistically analyzed and results were reported as mean±SD. The refractive index of yellowish brown oil of *Cuminum cyminum* Linn. (Fam. Umbelliferae) was found to be 1.4655 at 34ºC and the congealing point was 15.4ºC. The optical rotation was found to be -32.45 to +42.25 while the specific gravity at 20ºC was 0.7455±0.035. Percent moisture, yield % and physicochemical parameters of Cumin Oil were given in **Table 3**.

Chromatographic Analysis

TLC Analysis

Thin layer chromatography plate precoated silica gel 60 F 254 (E. Merck) of 0.2 mm. A known weight of oil (10% solution in chloroform) was loaded in a straight line about 2cm above the lower edge of chromatogram. The mobile phase for neutral lipids was hexane: ether: acetic acid (85:13:2v/v). The mobile phase for polar lipids was hexane: ether: acetic acid (80:20:2v/v) and chloroform: methanol: 30% ammonium hydroxide: water (65:30:5:2.5v/v). The identification reagent was used antimony trichloride. Appearance of red violet spot on TLC plate when kept at 100^{o}C for 10min., confirmed the presence of lipids compounds. The reagent molybdenum blue dragendorff and ninhydrin were also used for the identification of lipids which

showed blue and red violet spot, on thin layer chromatography.

GC Analysis

Gas chromatographic analysis of absolute oil of *Cuminum cyminum* Linn. (Fam. Umbelliferae) was carried out using Shimadzu gas charomatograph Model 17A equipped with Flame Ionization Detector and capillary column (2.5m×0.2mm i.d.) packed with PEG, film thickness was 0.25µm. The temperature of injector and detector was set at 270^{o}C and 300^{o}C, Moisture free carrier gas N_2 was used at 35ml/min. Column oven temperature was 190^{o}C and analysis was done at same temperature without temperature programming **(Table 4)**. The peaks were recorded and identified by comparing their retention times with those of standard cuminaldehyde analysed under the same condition. The injection volume for the seed samples and cuminaldehyde standard was 0.5µl and for the media and cell samples, 5µl.

RESULTS AND DISCUSSION

Gas chromatographic analysis was performed under specific conditions and results thus obtained **(Table 5)** were compared. The compounds are listed along with corresponding peak No. and their % constituents **(Figure 3)**. Cuminaldehyde (peak 5) was 29.2 %. Linalool (peak 6) was 21.7%, α-pinene (peak 8) was 29.2 %, Limonene (peak 7) was 21.7%, 1,8-Cineol (Peak 4) was 6.1% and Linalyl acetate (peak 2) 4.8 % was also found in the oil. Peaks from 1 and 3, 4 remain unidentified due to the unavailability of pure standards.

Antispasmodic Activity

Isolation of Guinea Pig Ileum

Male albino guinea pig weighing 350gm was used in experiments. The animal was exposed to hydro distilled fruit extract of *Cuminum cyminum* Linn. The ileum was dissected out, immersed in Tyroide's solution and cleaned off the mesentery. Respective segments of 2-3cm long were mounted in a 25ml tissue organ bath, filled with a mixture of 95% O_2 and 5% CO_2 and maintained at 37^{o}C. The composition of Tyroide's solution (in mM for l lit) was 9mg KCl, 0.1mg NaCl, 0.1mg $NaHCO_3$, 0.42mg NaH_2PO_4, 0.6mg Glucose and pH value was 7.4.

Anti-Spasmodic Activity Assay Procedure

Initially concentration dependent responses of acetylcholine were recorded (with dose of 0.05ml, 0.1ml, 0.2ml, 0.4ml, 0.8ml, 1.6ml, 3.2ml) using Sherrington's recording drum with a frontal writing lever. Contact time of 60sec. and base line of 30sec time cycle was opted for proper recording of the responses in presence of plane Tyroide's solution as stock-A solution.

Then same concentration dependent responses of acetylcholine (Ach) using same procedure for a mixture of Tyroide's solution+*Cuminum cyminum Linn.* fruits extract (with a concentration of 1mg/ml) as a stock-A_1 solution were recorded.

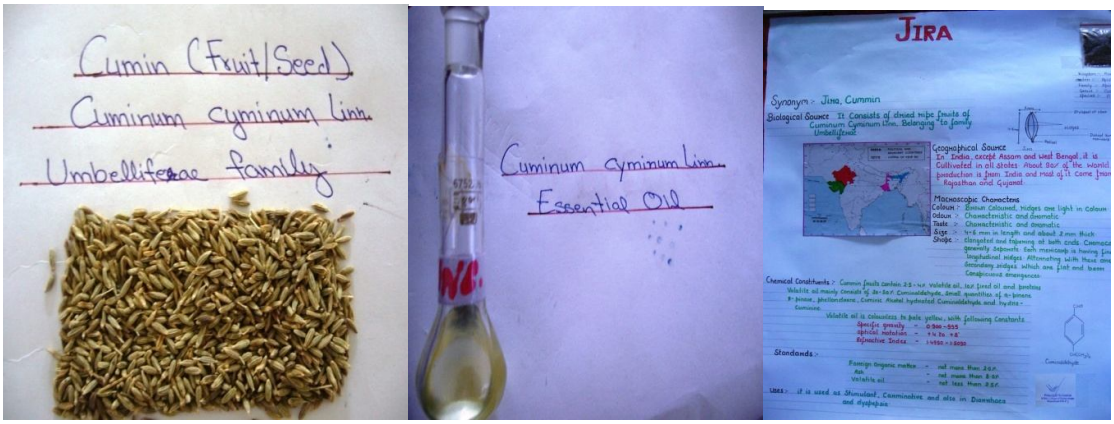
Lastly the same concentration dependent responses of Ach for a mixture of Tyroide's solution + Atropine (as a standard antispasmodic agent) as a stock-A_2 solution were recorded.

OBSERVATIONS AND RESULTS

Effect of Acetylcholine on excised guinea pig ileum reflected an increase in spasmodic activity (response) with an increase in dose as shown in **(Figure 4)**.

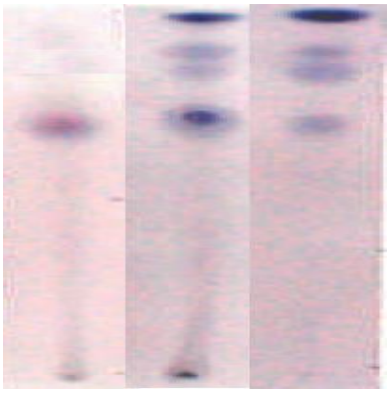
Acetylcholine induced spasm followed by treatment of hydro distilled fruit extract of *Cuminum cyminum Linn.* showed prominent antispasmodic activity as depicted in **Figure 4**. While treatment of anti-cholinergic drug Atropine (which is referred here as standard antispasmodic agent) showed expected receptor blocking action (antispasmodic) on isolated guinea pig ileum as shown in **Figure 4**. Also treatment of hydro distilled extract of *Cuminum cyminum Linn.* showed receptor blocking action (antispasmodic) as that of standard agent on isolated guinea pig ileum as shown in **Figure 4**.

From the present study results, it was observed that acetylcholine (Ach) alone causes contraction of excised guinea pig ileum but when acetylcholine was given in presence of hydro distilled fruit extract of plant *Cuminum cyminum Linn*., there was a marked decrease in contraction of ileum was observed. This revealed that hydro distilled fruit extract of *Cuminum cyminum Linn.* possess a high degree of spasmolytic (anti-spasmodic) activity by blocking cholinergic receptors. From all observations and results obtained for the present study it was concluded that hydro distilled fruit extract of plant *Cuminum cyminum Linn*., exhibits promising anti-spasmodic activity.



(a) **(b)** **(c)**

Figure 1: (a) Cumin Fruits (b) Cumin Essential Oil (c) Herbarium of *Cuminum cyminum Linn.* (Fam. Umbelliferae)



R 1 2

Figure 2: TLC profile of Essential oil of *Cuminum cyminum Linn.* Fruits, Track Ref: Phospholipid, 1 to 2: Test oils and Std. oil

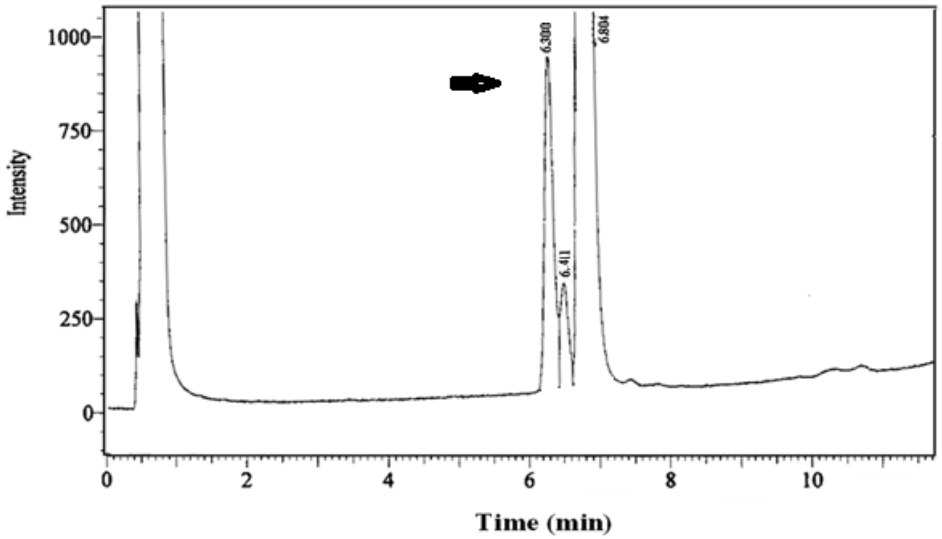


Figure 3: GC Chromatogram of the Cuminaldehyde Standard

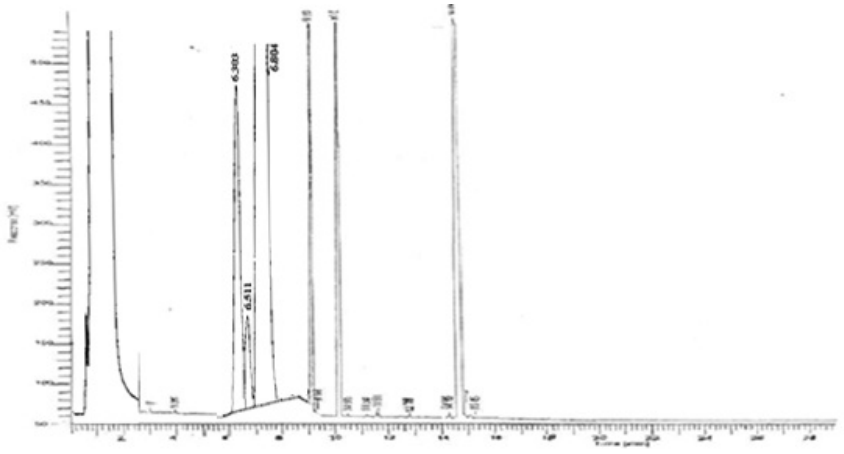


Figure 4: GLC Fingerprint Profile of Essential Oil of *Cuminum cyminum Linn* Fruit

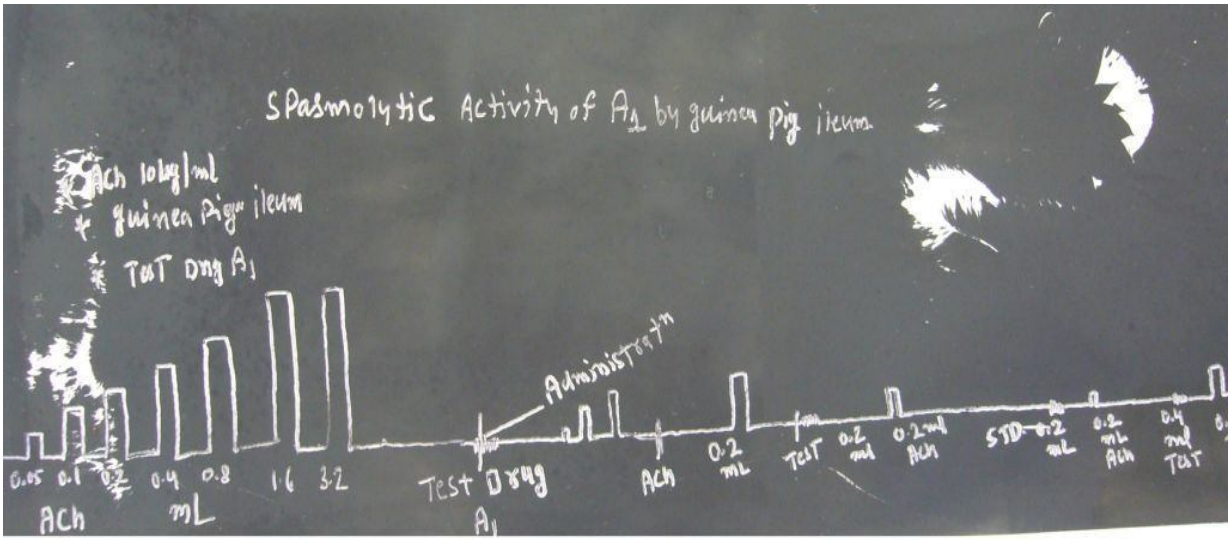


Figure 5: Response Curves of Acetylcholine, Tyroide's solution+ Extract and Tyroide's solution +Standard Drug (Atropine)

Table 1: Vernacular Names of *Cuminum cyminum Linn.* (Fam. *Umbelliferae*) [1]

S. No.	Regional Language	Regional Name
1	Sanskrit	Ajaji, Jiraka, Ajajika
2	Hindi	Jira, Safed Jira
3	Punjabi	Safed Jira, Chitta Jira
4	English	Cumin Seed, Cumin
5	Bengali	Jira, Sadajira
6	Telugu	Jilakarra, Tella Jilakarra
7	Urdu	Zirah, Zirasafed
8	Tamil	Sheeragum, Chirakam, Jirakam
9	Assamese	Jira
10	Marathi	Pandhare jira
11	Oriya	Dhalajeera, Dalajira, Jira

Table 2: Plant Description

S. No.	Physiological Characteristics	Plants Description
1.	Habitat [1]	Egypt, Latin America, North Africa, Rajasthan, Gujrat, West Bengal, Assam
2.	Phytoconstituents [7]	Cuminaldehyde (25%), Cuminyl alcohole(30%), p-cymene (18%), ß-pinene(16%) etc.
3.	Botanical [8]	Annual 25cm Herb, Flower- Small Compound Umbels, Flower Hermaphrodite (have both Male and Female Organs)

Table 3: Physicochemical Parameters of the Essential Oil of Cumin Fruit

	Standard Essential oil	Essential oil at Jodhpur
% Moisture	3.35	4.75
Yield %	-	2
Solubility	A, Cf, Ct, H	A, Cf, Ct, H
Acid value (mg/KOH/g)	1.5	2.45
Saponofication value (mg/KOH/g)	121.50	145.75
Ester value	116.00	141.30
Peroxide value (mEq/kg)	5.65	7.45
Iodine vlue (g/g)	94.25	98.5
Refractive index at 34°C	1.4675±0.25 Mean±SD value	1.4655±0.30 Mean±SD value
Congealing point (16.4°C)	16.4±0.5 Mean±SD value	16.7±0.5 Mean±SD value
Optical rotation	-32.25 ± 0.70 to + 42.25 ± 0.43 Mean±SD value	-32.10 ± 0.36 to + 42.35 ± 0.45 Mean±SD value
Specific gravity at 20°C	0.7455±0.035	0.7525±0.032

Oil received from Jain Super Store, New Delhi, India termed as 'Standard oil', A=Alcohol, Cf=chloroform, Ct=Carbon tetrachloride, H=Hexane

Table 4: Separation Parameters

Amount of test oils applied	0.5µl
Nitrogen gas	35ml/min
Detector temperature	300°C
Column	2.5m ×0.2mm (0.25µ film thickness)
Oven	190°C
Detector	FID

Table 5: Chemical Constituents in Essential Oil of *Cuminum cyminum Linn*. (Fam. Umbelliferae)

Retention time (min)	Constituents
1.2	Unidentified
2.4	Linalyl acetate
4.0	Unidentified
6.2	Linalool
7.83	Unidentified
10.21	Limonene
14.43	α-pinene

Table 6: Dose Response Relationship Observations

Sr. No.	Drug	Dose	Response (cm)	%Decrease in Response
1	Acetylcholine	0.05ml	0.5cm	
2		0.1ml	0.8cm	
3		0.2ml	1.2cm	
4		0.4ml	1.8cm	
5		0.8ml	2.2cm	
6		1.6ml	2.8cm	
7		3.2ml	3.0cm	
8	Test Drug	0.05ml	0.2cm	
9		0.1ml	0.3cm	
10		0.2ml	0.4cm	
11	Acetylcholine+ Test Drug	0.2ml+0.2ml	0.2cm	66.67
12	Acetylcholine+ Std Drug	0.2ml+0.2ml	0.3cm	74.34

ACKNOWLEDGEMENT

The authors express their sense of gratitude towards management of Lachoo Memorial College of Sci. and Tech., Pharmacy Wing, Jodhpur (Rajasthan) for providing all obligatory facilities necessary to carry out present work. Also Prof. (Dr.) G.K. Singh and Prof. (Dr.) B.P. Nagori deserve a special mention for their timely suggestions.

REFERENCE

[1] The Ayurvedic Pharmacopoeia of India, Government of India, ministry of Health and Family welfare, department of Ayush, New Delhi, India, 1(1), 2006, 142.

[2] Treas & Evans Pharmacognosy, International 15th Ed., Saunder Edinburgh, New York, 2002, 258.

[3] Kartikar KR, Basu BD, Indian Medicinal Plants, II, 2nd Ed., Lalit Mohan Basu, Allahabad, India, 1984, 1190-231.

[4] Nadkarnis KM, Indian Material Medica, Bombay Popular Prakashan, 1982, 408-10.

[5] Gruenwald J, Brendler T, Jaenicke C, PDR for Herbal Medicines, 3rd Ed., Medical Economics Company, New Jersey, 2004, 245-6.

[6] Anonymous, 17th Ed., Washington, DC: Association of Official Analytical Chemists; 1997, Official methods of analysis of the Association of Official Analytical Chemists.

[7] Leopold J, Buchbauer G, Albena SS, Evgenii VG and Stanka T, Composition, quality control and antimicrobial activity of the essential oil of cumin (*Cuminum cyminum* L.) seeds from Bulgaria that had been stored for up to 36 years, Int. J. Food Sci. & Tech., 40, 2005, 305-10.

[8] Singh, KK and Goswami TK, Physical Properties of Cumin Seed, J. Agric. Engg Res., 64, 1996, 93-8.

[9] Agrawal S, Volatile oil constituents and wilt resistance in cumin (*Cuminum cyminum* L.), Curr. Sci. India, 1996, 71, 177-8.

[10] Anonymous, British Standard Specification. Methods of analysis of oils and fats. British Standard house, 2 Park Street, London W.I., 1958.