Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 4801-4806
Received: 09-03-2019
Accepted: 13-04-2019

Sachin Palekar
Assistant Professor & Head, Department of Bioanalytical Sciences, Ramnarain Ruia Autonomous College, L. N. Road, Matunga, Mumbai, Maharashtra, India

Anushka Joshi
Assistant Professor, Department of Bioanalytical Sciences, Ramnarain Ruia Autonomous College, L. N. Road, Matunga, Mumbai-19, Maharashtra, India

Correspondence
Sachin Palekar
Assistant Professor & Head, Department of Bioanalytical Sciences, Ramnarain Ruia Autonomous College, L. N. Road, Matunga, Mumbai-19, Maharashtra, India

Standardization of ova Ark: An effort to standardise formulation from grandma's pouch

Sachin Palekar and Anushka Joshi

Abstract
Ova ark is a traditional home remedy, used to get relief from routine gastric problems, in Maharashtra. Although *Ajjowan* is one of the main constituent, several other plants, *Saindhava* and asafoetida are added in the formulation which makes it more efficacious. The current study aims at standardization of the same formulation; using multidisciplinary approach.
All the ingredients were first dried pulverized and the extract was prepared by steam distillation. This was repeated several times with variations in the amount of ingredients, to achieve perfect taste, aroma. Proximate analysis of the formulation was carried out and the formulation was further evaluated using TLC fingerprinting, GC and GC-MS Fingerprinting. Moreover MIC Assay and AAS were also carried out. GC-MS analysis of formulation confirms presence of Thymol and other Terpenoids, which is the active constituent of Ajjowan and other plants used in the preparation respectively. Formulation showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus.* Iron and copper were found to be present by atomic absorption spectrophotometry. In conclusion, this study provides a standardized protocol for preparation and quality control parameters for the Ova ark formulations using conventional and sophisticated approach.

Keywords: Ajjowan, antimicrobial activity, *Carum copticum,* ova ark, standardization, thymol

Introduction
India is the largest producer of medicinal plants and is rightly called the "Botanical garden of the world". According to the World Health Organization (WHO) estimates, more than 80 % of the people in developing countries depend on traditional medicine for their primary health needs [2]. Medicinal plants constitute a source of raw materials for both traditional systems of medicines (e.g. Ayurvedic, Chinese, Unani, Homeopathy and Siddha) and modern medicine. Plant materials are employed throughout the industrialized and developing world as home remedies, over-the-counter drugs, and ingredients for the pharmaceutical industry. As such they represent a substantial proportion of the global drug market [1].
The constituents of a Herbal drug preparation are either of a known therapeutic activity or are chemically defined substances or group of substances generally accepted to contribute substantially to therapeutic activity of drug [1]. The fruit of *Carum copticum* (L.) Sprague ex Turill (Family: Apiaceae) locally known as Ajowan and Nakhwa; in the Middle East, and is primarily grown and used in the Indian subcontinent, but also in Egypt, Iran and many other countries as a spice, flavouring agent and a condiment [3]. Ajjowan is one of the ingredients of the grandma's pouch. Several home remedies of Ajjowan are popular in tribal region of Maharashtra. Traditionally Ajwain extract also known as an ark in Ayurveda is used as a medicine. *Carum copticum* is used in treatment of many gastrointestinal disorders such as indigestion, colic, dyspepsia, and diarrhea [4]. Major active components of Ajjowan are essential oil which is mainly composed of thymol and other terpenoids such as y-terpinene, p-t cymene, α-pinene and β-pinene [4]. The essential oils of *Carumcopticum* have been reported to have antioxidant, anti-cholinergic, antihistaminic activities and analgesic effects [5].
Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles [6]. Standardization involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding Excipients or by mixing herbal drugs or herbal preparations. Botanical extracts made directly from crude plant material show substantial variation in composition, quality, and therapeutic effects. Standardized extracts are high-quality extracts containing consistent levels of specified compounds, and they are subjected to rigorous quality controls during all phases of the growing, harvesting, and manufacturing processes. As a result the term 'standardization' may mean many different things. When the active substances are unknown, marker substance(s) should be established for analytical purposes and standardization.

Marker substances are chemically defined constituents of herbal drug that are important for the quality of the finished product. Ideally, the chemical markers chosen would also be the compounds that are responsible for the botanical's effects in the body [1]. The formulation prepared and studied is an ark or extract prepared in water.

Traditionally, Ajwain ark (Ova ark in Marathi) was prepared by boiling the contents in a container and covering the container with a larger container, such that, the steam formed is trapped by the top container, condensed and falls in a larger pot beneath from the side. Several meetings with Late Smt. Susheela Gokhale, a resident of Palghar District Thane, Maharashtra, who had been preparing the arka since past 50 years, have contributed a lot during this study. Her expertise & experiences helped us in preparing best quality of arka.

In the current study, steam distillation was employed to prepare the formulation, we aimed at standardizing a traditional ova ark formulation from grandma's pouch for it to be natural, nontoxic, cost effective, safe and efficacious.

Material and Methods

Preparation of formulation: Two Ova ark formulations were prepared by varying the composition of raw materials. The traditional method of preparation of Ova ark was modified and formulations were prepared using Steam distillation technique and apparatus. (Table 1)

The plant materials used in the formulation were purchased from a local crude herbal drugs store and the quality of the same was assessed using standard pharmacopoeial tests.

Double distilled water and lab grade chemicals were used for the entire study.

Preparation Procedure: All the plant materials were separately & finely pulverized, weighed accurately on a calibrated balance and mixed in the proportions listed in table1. Water was used as an extracting solvent for plant components. A clean dry stainless steel vessel was kept on hot plate and 9 liters of potable water was added in it, which was reduced to $1/8^{th}$ proportion and was further used for the preparation. The pulverized plants were mixed properly and mixture (91 g) was taken in a clean and dry round bottom flask. 1000 mL of 9L water which was reduced on hot plate was added in it. Further this was connected to distillation unit, temperature of the heating mantle was adjusted to 60ºC and distilled extract was collected in a clean, dry, air tight flask. *Saindhava* was heated at 100ºC in hot air oven. It was then added to the extract, the flask was sealed and was shaken for the dissolution of *saindhava*. The prepared formulation was stored in glass bottles in cool and dry place, away from direct sunlight. Refrigeration of ark was not advised.

Qualitative detection of phytoconstituents like alkaloids, tannins, terpenoids, flavonoids and essential oils was carried out by standard protocols. Further the formulation was subjected to proximate evaluation by assessing the parameters such as pH, viscosity, Total dissolved solid content etc. The presence of Terpenoids in the formulation was qualitatively detected by using thin layer chromatography using Toluene: Ethyl acetate, (9:1 v/v) as solvent system. The presence of terpenoids was confirmed by derivatizing the plates by Vanillin sulphuric acid reagent.

Table 1: Formulation composition, two formulations were prepared by combining different proportions of the same ingredients.

Name of the ingredient	Part used	**Formulation 1** (W / V)	**Formulation 2** (W / V)
Carum copticum (Ova)	Fruits	50 g	25 g
*Anethum graveolens (*Shopa)	Fruits	25g	50 g
Artemisia maritime (Kirmani ova)	Fruits	1.25 g	1.25 g
Embelia ribes (Vavding)	Fruits	2.5 g	2.5 g
Piper nigrum (Miri)	Fruits	2 g	2 g
Holarrhena antidysenterica (Kudabeej)	Seeds	2.5 g	2.5 g
Caesalpinia crista (Sagargota)	Seeds	1.25 g	1.25 g
Ferula asafoetida (Khadahing)	Gum	2 g	2 g
Zingiber officinale (Suntha)	Rhizome	4.5 g	4.5 g
Saindhava lavana (Rock salt)	-	10 g	10 g
Water	-	9 Liter	9 Liter

Sample preparation for GC & GC-MS analysis

10 ml of formulation was taken in a clean, dry separating funnel and 5 mL of hexane was added in it. After shaking the organic layer was collected and used for injecting in GC.

GC analysis

It was performed on GC-QP 2010 ultra, equipped with flame ionization detector. Retention times for comparison with authentic compounds were measured with Rtx-5MS column (30m x 0.25mm i.e. 0.25µm film thickness). The oven temperature was programmed as isothermal at 40 ºC for 1 min, then raised to 80 ºC at 6 ºC/min and held at this temperature for 1 min then raised to 250 ºC at 15 ºC/min. helium was used as the carrier gas at the rate of 1.5ml/min.

GC-MS analysis

The essential oils of the formulation were analyzed on GCMS-QP 2010 ultra-equipped with a Rtx-5MS column (30m x 0.25 mm i.e., 0.25µm film thickness). The oven temperature was programmed as for the previous analysis. Helium was used as the carrier gas at the rate of 1.5ml/min. Compounds were tentatively identified by comparison of mass spectra of each peak with those of authentic samples in the NIST08. LIB library.

AAS analysis

10 ml of sample was evaporated on hot plate and 10 mL of concentrated nitric acid was added in it. Acid was completely evaporated to dryness and 4 ml of per chloric acid was added in it and then reduced to half volume. This was further dissolved in deionized water and the solution was used for AAS analysis of Iron and Copper. AA-7000F was used for the analysis.

Antioxidant activity

1ml of formulation was taken in test tube and 2.5 ml of 0.2M phosphate buffer, and 2.5 ml of 1% $K_3Fe(CN)_6$ were added to it. Further it was incubated at 50 ºC for 2 minutes. The reaction was stopped by adding 2.5 ml of 10% TCA and reaction mixture was then centrifuged at 300rpm for 10

minutes. The supernatant was collected in clean dry test tube and freshly prepared ferric chloride was added to it. The absorbance was measured at 700 nm. Vitamin C was used as a standard.

Minimum inhibitory concentration

MIC of the formulations was performed on the strains of *Escherichia coli* (ATC 1133D) and *Staphylococcus aureus* (ATC 6538P). Half strength nutrient broth was used as media. Test readings were taken on colorimeter at 520 nm over 48 hours. However, the viability of the organisms cannot be predicted by optical density of the MIC tubes. Hence, the spread plate method was used further to check the viability and assess efficacy of the formulation. The plates were incubated and observed to check the effectiveness of the formulation.

Results and Discussion

The preliminary biochemical tests showed that the formulation contains Terpenoids and essential oils only. Tests for other Phytoconstituents like alkaloids, tannins and flavonoids were found to be negative.

pH of the formulation was 4.14 and specific viscosity was 0.2913. The total solid content of the formulation was found to be 0.44g per 10 ml. Pink band of Thymol was detected at Rf 0.75 in all the samples (Table 2). However intensity of the pink band was more in Formulation 1 among all three samples (Figure 1).

Table 2: TLC analysis of the formulations

	Sample	No. of pink bands observed (Terpenoids)	Rf
Track 1	PET ether extract of formulation	1	0.75
Track 2	Toluene extract of formulation	1	0.75
Track 3	N-hexane extract of formulation	1	0.75
Track 4	1st Fraction of formulation	1	0.75
Track 5	2nd Fraction of formulation	1	0.75
Track 6	3rd Fraction of formulation	1	0.75

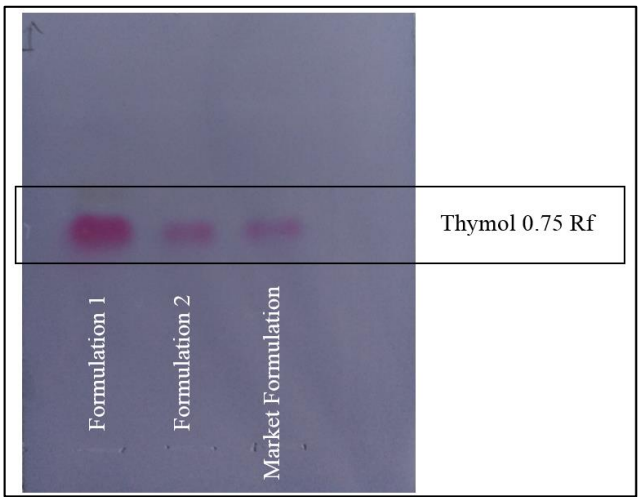


Fig 1: TLC analysis of Thymol from the formulations

The GC fingerprint of *carum copticum* extract, *Anethum graveolens* extract and formulation was compared, which showed similar peaks (Table 3, 4, 5, 6, 7 and Figure 2, 3, 4). Therefore it is evident that the essential oils from both ova and shopa were successfully extracted in the formulation by the method of steam distillation which was standardized.

However to detect the active components of Ova & shopa from the formulation, GC-MS fingerprinting was carried out due to unavailability of standard compounds. GC-MS fingerprint of the formulation analysis showed presence of *thymol* and p-*cymene* which are the active constituents of *Carum copticum*. (Table 8).

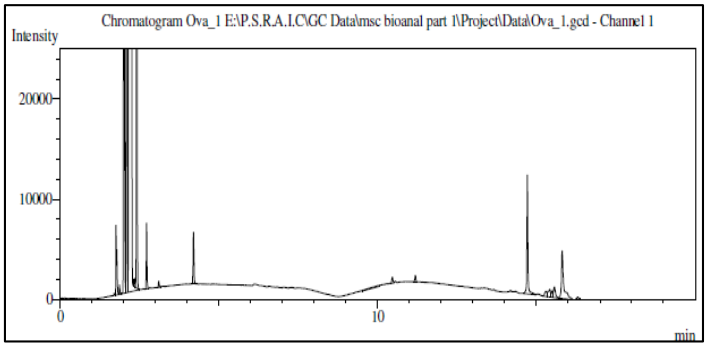


Fig 2: GC analysis of *Carum copticum*

Table 3: GC analysis of *Carum copticum* (Ova)

Peak no.	Retention time (Rt)
1	1.754
2	1.864
3	2.002
4	2.081
5	2.200
6	2.337
7	2.405
8	2.716
9	3.102
10	4.196
11	10.005
12	10.460
13	11.184
14	14.712
15	15.293
16	15.417
17	15.499
18	15.566
19	15.811
20	16.300

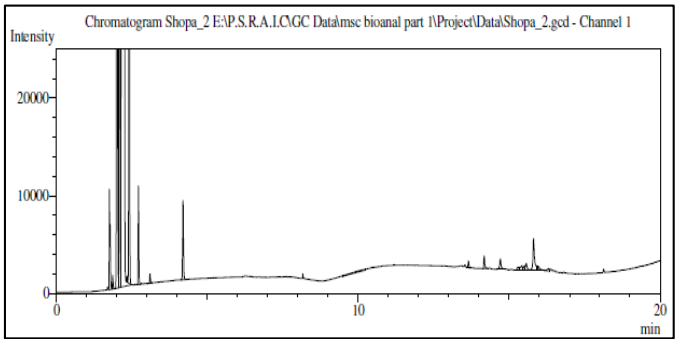


Fig 3: GC analysis of *Anethum graveolens*

Table 4: GC analysis of Anethum graveolens (Shopa)

Peak no.	Retention time (Rt)
1	1.752
2	1.863
3	2.001
4	2.080
5	2.206
6	2.403
7	2.715
8	3.101
9	4.193
10	9.997
11	10.137
12	13.652
13	14.174
14	14.709
15	15.292
16	15.416
17	15.505
18	15.567
19	15.810
20	15.919
21	15.961
22	16.304

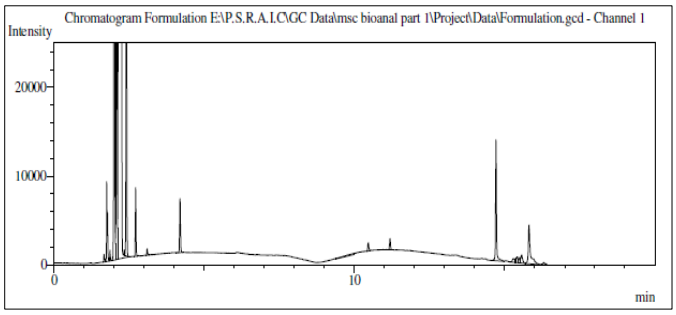


Fig 4: GC analysis of formulation

Table 5: GC analysis of the formulation

Peak No.	Retention time (Rt)
1	1.661
2	1.752
3	1.862
4	2.000
5	2.079
6	2.200
7	2.402
8	2.714
9	3.099
10	4.193
11	9.978
12	10.460
13	11.183
14	14.710
15	15.290
16	15.373
17	15.417
18	15.504
19	15.568
20	15.813
21	16.303

Table 6: Comparison of Retention time of *Carum copticum* (ova) and formulation

No. of similar peaks	Retention time (Ova)	Retention time (Formulation)
1	1.754	1.752
2	1.864	1.862
3	2.002	2.000
4	2.081	2.079
5	2.200	2.200
6	2.405	2.402
7	2.716	2.714
8	3.102	3.099
9	4.196	4.193
10	10.460	10.460
11	11.184	11.183
12	14.712	14.710
13	15.293	15.290
14	15.417	15.417
15	15.566	15.568
16	15.811	15.813
17	16.300	16.303

Table 7: Comparison of Retention time of *Anethum graveolens* (Shopa) and formulation

No. of similar peak	Retention time (Shopa)	Retention time (Formulation)
1	1.752	1.752
2	1.863	1.862
3	2.001	2.000
4	2.080	2.079
5	2.403	2.402
6	2.715	2.714
7	3.101	3.099
8	4.193	4.193
9	14.709	14.710
10	15.292	15.290
11	15.416	15.417
12	15.505	15.504
13	15.567	15.568
14	15.810	15.813
15	16.304	16.303

Table 8: GCMS analysis of formulation

Peak No.	Retention Time	Name
1	0.220	Trichloroethylene
2	1.535	Pentane, 2-methyl
3	1.599	Pentane, 3-methyl-
4	1.655	Hexane
5	1.690	Propyltrioctyltin
6	1.851	Carbonic acid, hexyl 2,2,2-trichloroethyl
7	2.107	Cyclohexane
8	9.172	P Cymene
9	9.987	1,4-Cyclohexadiene, 1-methyl-4-(1
10	13.471	Thymol
11	14.440	Naphthalein,2-ethenyl-
12	14.653	Diphenyl Ether

AAS Analysis confirmed that the formulation contains 0.1221 ppm of Cu and 0.2743 ppm of Fe, which are essential elements in the metabolism (Figure 6, 7).

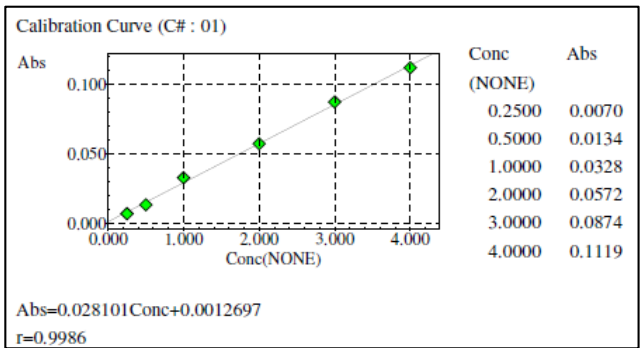


Fig 6: Calibration curve of standard Copper

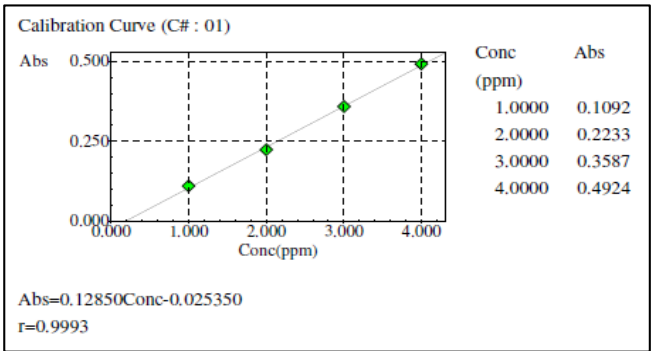


Fig 7: Calibration curve of standard Iron

Antioxidant activity test was evaluated using iron reducing activity of the formulation. The formulation showed the antioxidant activity which is comparable with 6 ppm of vitamin C. (Figure 8)

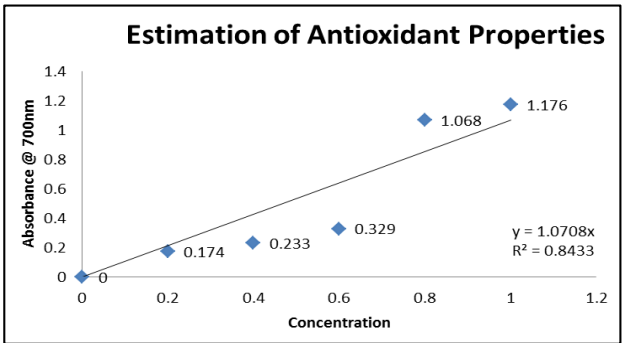
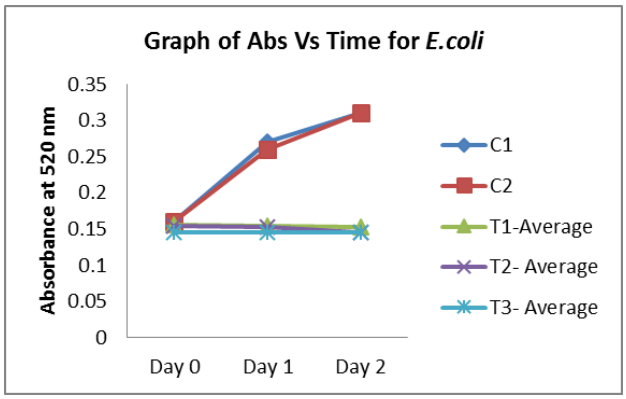


Fig 8: Calibration graph for antioxidant activity of a formulation

To evaluate the antimicrobial activity, the MIC assay was carried out. 2 ml of Formulation1, Formulation 2 and Market formulation inhibits the growth of *E. coli* and *S. aureus*. The results for Formulation 1 and Formulation 2 were comparable with the market formulation thereby indicating that prepared formulations have equivalent therapeutic value as the market formulation. (Table 9, Figure 9). There was considerable reduction in the viable cells of both the organisms. Thus we can say that the formulation is safe & efficacious.

Table 9: Minimum inhibitory concentration test

		Optical density (O.D)		
		Day 0	**Day 1**	**Day 2**
A1 (Saline + media)		0.16	0.16	0.16
A2 (Arka + media)	Formulation1	0.15	0.16	0.15
	Formulation2	0.16	0.16	0.16
	Market formulation	0.16	0.15	0.16
B1 (*E. coli* + Media)		0.16	0.35	0.38
B2 (*S. aureus* + media)		0.15	0.30	0.35



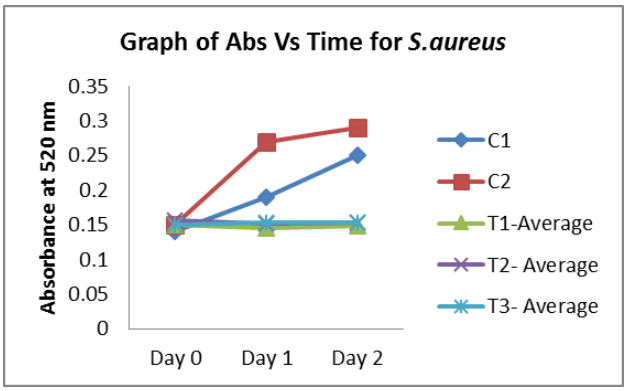


Fig 9: MIC assay results for *E coli* & *S aureus* (Key- C-control, T test)

Conclusions

From the current study it is evident that the ethno medicine or home remedies from grandma's pouch, are beneficial, and hence the effort was made to standardize the formulation. Current study provides the standard operating procedure for preparation of Ova ark. Use of conventional and modern instrumental techniques for analysis, safeguards the quality assurance. Microbiological evaluation of the formulation shows that ark is safe for human consumption and also; it can be used as potential drug against enteric pathogens. The current study provides the SOP for the preparation of ova ark with multidisciplinary approach for analysis.

Acknowledgments

Authors are grateful to

1. Late Susheela Gokhale for providing the authentic recipe for preparation of the ova ark
2. Dr.Ajit Datar, SHIMADZU analytical India Pvt Ltd, Mumbai
3. PS Ramanathan Advance instrumentation Laboratory, Ruia College
4. Dr. Suhas Pednekar, Former Principal, Ruia College for providing the necessary infrastructure
5. Ms. Dhanashree Kulkarni, Former Head, Department of Bio analytical Sciences, Ruia College
6. Teaching and non-teaching staff of Dept. of Bioanalytical Sciences, Ruia College.

References

1. Ahmad F Aqil, Owais M. Modern Phytomedicine. Turning Medicinal Plants into Drugs, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2006. ISBN: 3-527-31530-6.
2. Dr. Pulok K. Mukherjee. Quality control Herbal Drugs: An approach to evaluation of Botanicals, Business Horizons, ISBN 8190078844, 2008

3. Alqasoumi S. Gastric antisecretory and antiulcer effects of ajowan *Carum copticum* in rats, African Journal of Pharmacy and Pharmacology. 2011; 5(5):572.
4. HejazainY[1-2] SH, Sepheri H, Dashti-R MH, Mahdavi SM, Bagheri SM, Morshedi A *et al*. Does essential oil of Carumcopticum affect acetylcholine-induced contraction in isolated rat's ileum?, African Journal of Pharmacy and Pharmacology. 2011; 5(12):14-32.
5. Zomorodian K, Moein MR, Rahimi MJ, Pakshir K, Ghasemi Y, Abedi S, Sharbatfar S. Possible Application and chemical compositions of *Carum copticum* Essential Oils Against Foodborne and Nosocomial Pathogens, Middle-East Journal of scientific Research. 2011; 9(2):239.
6. Sriwastava NK, Shreedhara CS, Aswatha Ram HN. Standardization of Ajmodadi Churna, A polyherbal formulation, A publication of Phcog. Net Pharmacognosy Research A Rapid Publication Journal. 2010; 2(2):98.
7. Rafiul Haquea MD, Ansari SH, Abul Kalam Najmib, Kammran Javed Naquvi. HPTLC analysis method for quantification of thymol content in *Trachyspermum ammi* and polyherbalunani formulation Arq Zeera, International Journal of Pharmacy and Pharmaceutical Sciences, 2012, 4(3). ISSN-0975-1491.
8. Seyed Hassan Hejazian, Houri Sepehri, Mohammad Hossein Dashti-R, Seyed Majid Mahdavi, Seyyed Majid Bagheri, Abbas *et al*. Does essential oil of *Carum copticum* affects acetylcholine-induced contraction in isolated rat's ileum? African Journal of Pharmacy and Pharmacology. 2011; 5(12):1432-1435.
9. Zarshenas MM, Samani SM, Petramfar P, Moein M. Analysis of essential oil components from different *Carum copticum* L. samples from Iran, Pharmacognosy Res. 2014; 6(1):62-6. Doi: 10.4103/0974-8490.122920
10. Katasani Damodar, Srinu Bhogineni, Bala Ramanjaneyulu. Phytochemical screening, Quantitative estimation of total phenolic, flavonoids and antimicrobial evaluation of *Trachyspermum ammi*, Journal of Atoms and Molecules an International Online Journal. ISSN – 2277 – 1247.
11. Gh R Goudarzi1, Saharkhiz MJ, Sattari M, Zomorodian K. Antibacterial Activity and Chemical Composition of Ajowan (*Carum copticum* Benth. & Hook) Essential Oil, J. Agr. Sci. Tech. 2011; 13:203-208.
12. Harborne JB. Phytochemical Methods: A Guide to Modern techniques of plant analysis, Chapman and hall London, 1998.
13. Thimmaiah SR. Standard method of biochemical analysis. Kalyani Publishers, UP, ISBN: 8176630675, 1999.
14. Zhang LL, Lin YM. Antioxidants Tannis. African Journal of Biotechnology. 2009; 8(10):2301-2309.
15. Ranganna S. Handbook of analysis & Quality control for fruit and vegetable products. 2nd Edition, TMH publishing company ltd., ISBN-10:0074518518, 2004.
16. Wagner H, Bladt S, Rickl V. Plant Drug Analysis: A thin layer Chromatography Atlas, Springer; 2nd edition, ISBN-10:3540586768, 2009.