Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

ORIGINAL RESEARCH ARTICLE

A Comparative Quantity Estimation of *Eugenol* in *Tulasi Patra* (Ocimum sanctum Linn.) Powder and *Tulasi Patra Arka*

Author: Pankti D Jayswal[1]

Co Authors: Mayur Mashru[2] **and Rajesh G. Baravaliya**[3]

[1-3]Upgraded Department of Rasashastra and Bhaishajya Kalpana, Government Ayurved College, Vadodara, Gujarat, India

ABSTRACT

Context: Standardization of Ayurvedic formulations is essential in order to assess of quality and purity of drugs, which is based on the concentration of their active principle, physico-chemical standardization and in vitro, in-vivo parameters. WHO also focus attention on the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable analytical standards.**Aim:** To compare the potency between *Tulasi Patra* Powder and *Tulasi Patra Arka* with special reference to Eugenol.

Objective: To study and learn analytical procedures required for the validation of *TulasiPatra* Powder and *Tulasi Patra Arka.*

Materials and Methods: *Tulasi Patra* Powder (TPP) and *Tulasi Patra Arka* (TPA) was evaluated analytically through various parameters.

Observation and results: In TPP Alcohol Soluble Extractive value was 11.22 %, Water Soluble Extractive was 21.38%, Total Ash was 11.63 %Acid Insoluble Ash 1.47 %Loss on drying was10.12 % and pH was 6.21. In TPA Specific gravity was 0.9995 and pH was 6.58. Eugenol percentage in TPP was 1.56% and in TPA 0.11%.

Conclusion: *TulasiPatra* Powder has more Eugenol percentage than *Tulasi PatraArka*.

Key Words *Tulasi, Tulasi Powder, Tulasi Arka, Eugenol, Gas Chromatography*

Received 20th August 21 Accepted 14thSeptember 21 Published 10thNovember 2021

INTRODUCTION

Aim: To compare the potency between *Tulasi Patra* Powder and *Tulasi Patra Arka* with special reference to Eugenol.

Objective: To study and learn analytical procedures required for the validation of *TulasiPatra* Powder and *Tulasi Patra Arka.*

In our daily routine life *Tulasi* (*Ocimum Sanctum* Linn.) is most commonly useful herb. *Tulasi* is known as "The Queen of Herbs" and "Mother Medicine of Nature". *TulasiPatra* have *Katu-Tikta Rasa*, as well as *Laghu*, *Ruksha* and *Tikshna* properties and *UshnaVirya*[1]. It is *Vata-Kaphanashaka* and *Pittavardhaka*,

ORIGINAL RESEARCH ARTICLE

Hikkanashaka, *Kasanashaka*, *Vishnashaka*, *Swasnashaka*, *Parshwashoolhara*, *Durgandhnashaka*[2], and *Vranshodhaka*[3]. *Tulasi* plants have antiallergic and antimicrobial properties. *Tulasi* plant is best antiviral herb of Ayurved. *Tulasi* have Antiasthmatic, Immunomodulator, and Expectorant properties. *Tulasi Patra* are extensively used by Ayurvedic physicians and Ayurvedic Pharma Companies as single drug or in formulations.

Bhaishajya Kalpana is one of the holistic branch of Ayurveda which primarily deals with the various pharmaceutical, nutraceutical formulation and their therapeutic application of plant origin drugs. Among the large number of formulations specified by Acharyas, Secondary dosage forms are derived from primarily through the *PanchavidhaKashayaKalpana* which have great importance. The five basic *Kalpana* comprise of *Swarasa*, *Kalka*, *Kwatha*, *Hima* and *Phanta*. The different pharmaceutical processing of a drug is of great importance in drug preparations. It is accepted fact that the success in the treatment is solely depends on the quality and genuineness of the drug.

According to AcharyaSharangadhara[4], *Churna* means, nicely powdered dry drug, which is filtered through a cloth.

The method by which the volatile oil and active principles of the drug are collected is called *Arka Kalpana* and the compound prepared through this procedure is called as *Arka*. *Arka* is a liquid preparation obtained by distillation of certain liquids or of drugs soaked in water using the *Arkayantra* or any convenient modern distillation apparatus[5]. *ArkaKalpana* is one among the *PanchavidhaKalpana* told in Arka Prakasha[6].

The efficacy of *Kalka*, *Churna*, *Swarasa*, *Taila* and *Arka* is more in descending order[6]. This efficacy of individual formulation is may be due to various degrees in the concentration of active principle. This implies that the author of Arka Prakasha said this on the basis of concentration of drug in formulation.

Eugenol is one type of essential oil which is found in leaves of *Tulasi*. Prominent sources of eugenol are pepper, cinnamon, clove and *Tulasi*[7]. As per Indian pharmacopoeia *Tulasi* contains not less than 0.40 per cent w/w of eugenol. That's why quantitative estimation of eugenol was planned in present study[8].

Essential oils are secondary metabolites that plants produce for protection from pests and predators, attraction of pollinators, or seed dispersal[9]. The oils are located in different parts of the plant such as roots, stems, leaves, flowers, fruits, and even in seeds depending on the plant species[10]. In these plant parts, the essential oil is accumulated in cells, secretory cavities, or glandular hairs[11].Almost every part of a plant can produce essential oil, which can be extracted and exploited in various industrial ways[12].The essential oils are highly volatile[13],transparent, and lipid-soluble liquids[14].

Till date no research works has been carried out on quantitative estimation of Eugenolin the preparation of *Tulasi Patra* Powder (TPP) and *Tulasi Patra Arka* (TPA), considering all these,

ORIGINAL RESEARCH ARTICLE

present work was planned to compare potency between *Tulasi Patra* Powder (TPP) and *Tulasi Patra Arka* (TPA) with special reference to Eugenol.

MATERIALS AND METHODS

Collection of raw materials

Fresh *Tulasi Patra* were collected from Government Ayurvedic Pharmacy attached garden, Rajpipla. The raw material was authenticated in Pharmacognosy Laboratory, Food and Drug Laboratory, Vadodara.

Preparation of Formulations

Tulasi Patra Powderand *Tulasi Patra Arka* were prepared in pharmaceutical laboratory of Upgraded Department of Rasashastra and Bhaishajya Kalpana, Government Ayurved College, Vadodara.

TPP was prepared by Sha.Sa.Ma.6/1 reference andPrincipleisImpact and Attrition. TPA was prepared by AFI Part 2, 2:1 reference andPrincipleis *ArkaPatana* (Distillation).

Preparation of *Tulasi Patra* Powder

Fresh *Tulasi Patra* were collected and manually sorting was done to separate unwanted foreign matters from it and subsequently washed with water. After sorting and washing with water, *Tulasi Patra* were kept in tray for shade drying. After drying, obtained *Tulasi Patra* was converted into fine powder by using a mixer grinder. It was sieved with 85 no. sieve and it was weighed. The final product obtained was packed in airtight LDPE containers.

Preparation of *Tulasi PatraArka*

Fresh *Tulasi Patra* were collected and manually sorting was done to separate unwanted foreign matters from it and subsequently washed with water. After that it was cut into small pieces. Then it was placed in round bottom standard joint flask of 2 l capacity and 1 l of water was added. Distillation assembly was properly attached with distillation and receiving heads, double surface condenser and receiving flask and enough circulating water to condense the distillate i.e. *Arka*. The flask was placed on a heating mantle. The temperature was adjusted when boiling starts and the distillation was continued until 500 ml of *Arka* was collected. The apparatus was heated to 50°C and temperature was maintained during the procedure. First 5 to 7 drops were not collected. *Arka* was collected up to 50% amount of water, then further heating was stopped. The collected *Arka* was stored in air tight bottle and kept it in cool place.

Evaluation through Analytical Parameters

TPP and TPA were analyzed through Organoleptic Characters and relevant physicochemical parameters such as Foreign organic matter, Alcohol Soluble Extractive, Water Soluble Extractive, Total Ash, Acid insoluble Ash, pH, Loss on drying, Specific gravity and other parameters like HPTLC for fingerprinting, Heavy Metals analysis, Microbial limit test, and Assay of Eugenol by Gas Chromatography.

Assay of Eugenol by Gas Chromatography

Preparation of Standard Solution (S):

ORIGINAL RESEARCH ARTICLE

Weigh 100 mg of Standard Eugenol in a 10 mL Volumetric flask, add Methanol GC grade and make up volume up to mark. Filter it using 0.22 microns syringe filter and it for GC analysis.

Preparation of Sample Solution (T):

Weigh 100 mg of Sample in a 10 mL Volumetric flask, add Methanol GC grade to dissolve and sonicate if required and make up volume up to mark. Filter it using 0.22 microns syringe filter and it for GC analysis.

Chromatographic conditions for estimation of Eugenol through Gas Chromatography (GC) are as follows: Column: 5% Diphenyl dimethyl polysiloxane capillary column, Injector: Auto injector, Injection volume: 1 µL, Injector Temperature: 240^0C, Carrier gas: Nitrogen, Column oven temperature: Initial temperature is 80^0C hold for 1 min, increase to 220^0C at the rate of 8^0C/min and hold for 17 min. Detector: Flame Ionization Detector (FID), Detector Temperature: 280^0C, Flow rate: 1 mL/min, Split ratio: 1:25.

RESULTS AND DISCUSSION

Organoleptic characteristic

In organoleptic characters colour of *Tulasi Patra* Powder (TPP)was green and colour of *Tulasi Patra Arka* **(**TPA) was transparent. In TPP and TPA Characteristic Aromatic smell was observed. Taste of TPP was Pungent and Astringent, while Taste of TPA was Pungent. TPP was smooth in touch and TPA was slightly cold in touch. [Table-1].

Table 1 Organoleptic characteristic of TPP and TPA

No	Organoleptic characteristic	*Tulasi Patra* Powder (TPP)	*Tulasi Patra Arka* (TPA)
1	Colour	Green	Transparent
2	Odour	Characteristic	Characteristic
3	Taste	Pungent and Astringent	Pungent
4	Touch	Smooth	Slight cold

Physicochemical analysis

TPP and TPA were analyzed using relevant physicochemical parameters at the Food and Drug Laboratory, Vadodara.

In TPP Alcohol Soluble Extractive value was 11.22 %, Water Soluble Extractive was 21.38%, Total Ash was 11.63 %Acid Insoluble Ash 1.47 %Loss on drying was10.12 % and pH was 6.21. In TPA Specific gravity was 0.9995 and pH was 6.58. [Table-2].

Assay of Eugenol by Gas Chromatography (GC):

Quantitative estimation of active component Eugenolwas done by Gas Chromatography technique. In assay per cent of Eugenol by Gas Chromatography in TPP sample was 1.56 % and in TPA Sample was 0.11%. [Table-3].

Heavy Metal Analysis

Sample of *Tulasi Patra*Powder consist of Lead, Cadmium and Arsenic 0.944, 0.0502 and 0.7069 respectively (In ppm), which are within permissible limit which is mentioned in API.

Table 2 Physico-chemical Analysis of TPP and TPA*****

ORIGINAL RESEARCH ARTICLE

No.	Parameters	*Tulasi Patra* Powder (TPP)	*Tulasi Patra Arka* (TPA)	Permissible limit as per IP[18]	Permissible limit as per API [26]
1	**Foreign organic matter**	Not found	Not found	Not more than 2.0 per cent	Not more than 2.0 per cent
2	**Alcohol soluble extractive**	11.22 %	-	Not less than 3.0 per cent	Not less than 6.0 per cent
3	**Water soluble extractive**	21.38%	-	Not less than 10.0 per cent	Not less than 13.0 per cent
4	**Total Ash**	11.63 %	-	Not more than 15.0 percent	Not more than 19.0 percent
5	**Acid Insoluble Ash**	1.47 %	-	Not more than 5.0 per cent	Not more than 3.0 per cent
6	**Loss on drying**	10.12 %	-	Not more than 12.0 per cent	Not mentioned
7	**pH**	6.21	6.58	Not mentioned	Not mentioned
8	**Specific gravity**	-	0.9995	Not mentioned	Not mentioned

Table 3 Quantitative estimation of Eugenol in TPP and TPA using GC

Parameters	Eugenol Standard	*Tulasi Patra* Powder	*Tulasi PatraArka*
Weight	117.5 mg	102.1 mg	110.1 mg
Rt	12.155	12.043	12.045
AUC	6904140	94725	7154
% Eugenol	-	1.56 %	0.11 %

Sample of *Tulasi Patra Arka* consist of lead and cadmium 0.3463 and 0.0218 respectively (in ppm), which are within permissible limit which is mentioned in API. In TPP and TPA samples mercury was not detected. [Table-4].

Table4 Gas Chromatography of TPP and TPA

No.	Parameters	*Tulasi Patra* Powder (TPP)	*Tulasi Patra Arka* (TPA)	Permissible limit as per IP[18]	Permissible limit as per API
1	**Assay (%) of Eugenol by Gas Chromatography**	1.56%	0.11%	Not less than 0.40 percent (% w/w)	Not mentioned

Microbial Limit Test

The microbes Escherichia Coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella Sp. were absent in sample of TPP and TPA. Total Aerobic Count were $3.1*10^3$ and Yeast and Mold were $4.4*10^2$ which are within Permissible limit of Microbial load as per API.[Table-5].

Table 5 Heavy Metal Analysis ofTPP and TPA

Sr. No	Heavy Metal Content	*Tulasi Patra* Powder (In ppm) TPP	*Tulasi Patra Arka* (In ppm) TPA	Permissible Limits As per API[27]
1	Lead	0.944	0.3463	10 ppm
2	Cadmium	0.0502	0.0218	0.3 ppm
3	Arsenic	0.7069	Not detected	3 ppm
4	Mercury	Not detected	Not detected	1ppm

HPTLC Analysis

In HPTLCanalysis, in sample of TPP when it was scanned at 254 nm 8 band were seen and in 366 nm 6 band were seen. In sample of TPA when it was scanned at 254 nm 3 band were seen and in 366 nm band was not seen. [Table-6].

Table 6 Microbial Limit Test of TPP and TPA

ORIGINAL RESEARCH ARTICLE

Sr. No.	Microbial Test	*Tulasi Patra* Powder (TPP)	*Tulasi Patra Arka* (TPA)	Permissible limit of Microbial load[27]
1	Escherichia Coli	Absent	Absent	Should be Absent
2	Staphylococcus aureus	Absent	Absent	Should be Absent
3	Pseudomonas aeruginosa	Absent	Absent	Should be Absent
4	Salmonella Sp.	Absent	Absent	Should be Absent
5	Total Aerobic Count (cfu/g)	$3.1*10^3$	Nil	10^5cfu/gm
6	Yeast and Mold (cfu/g)	$4.4*10^2$	Nil	10^3 cfu/gm

In *Tulasi Patra* Powder (TPP) and *Tulasi Patra Arka* **(**TPA) Characteristic Aromatic smell was observed because *Tulasi* has essential oils and that oils are made up of a mixture of compounds that give a characteristic flavour and odour[15]. Any type of foreign organic matter was not found in sample of TPP and TPA and both materials were pure due to self-collection and self-preparation of material. Alcohol soluble extractive and water-soluble extractive values are indicating the solubility of active principles of the material in alcohol and water respectively.Extractive values by different solvents are used to assess quality purity and to detect adulteration in material. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage[16].In Sample of TPP and TPA water soluble extractive and alcohol soluble extractive values are 21.38 % and 11.22 % respectively which are within permissible limit which is mentioned in API.

The percentage of Total Ash content in TPP sample was 11.63 % which is found to be within the specified Permissible limits of IP and API. Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards[17].Total ash test is performed to determine the amount of minerals in formulation.Ash values are helpful in determining the quality and purity of crude drugs in powdered form. Ash involves oxidation of the components. It consists of inorganic radicals like phosphates, carbonates and silicates and silica of sodium, potassium magnesium and calcium. A high ash value is indicative of contamination, substitution or adulteration. Calcium oxalate, silica and carbonate content of crude drug affects total ash values.

The percentage of Acid insoluble ash in TPP sample was 1.47 % which is found to be within the specified Permissible limits of IP and API. Acid insoluble ash valuedetermines the inorganic impurities present in the substance reacting with acid.

The pH value of a sample expresses the degree of acidity or alkalinity of a sample solution. pH of TPP was 6.21 and TPA was 6.58 which indicates weakly acidic nature of the samples. pH value of *Tulasi* is not denoted in IP and API.

The percentage of Loss on Dryingin TPP sample was 10.12 % which is found to be within the specified permissible limits of IP. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs[16].Moisture refers to all matter within a sample which can be vaporized, and thus

ORIGINAL RESEARCH ARTICLE

includes not just water but fats, volatile solvents, and alcohols. The specific gravity of TPA sample was 0.9995 which is not mentioned by IP and API.

Gas chromatography (GC) is a chromatography technique that can separate and analyse volatile compounds in gas phase. It is analytical technique that helps to separate the analyse a mixture of organic vaporizable or volatile compounds without their decomposition.

Eugenol is a volatile oil which is found in leaves of *Tulasi*, that's why Gas chromatography was selected for Quantitative estimation of Eugenol.In Assay per cent of Eugenol by Gas Chromatography in TPP sample was 1.56 % and in TPA Sample was 0.11% (Figure 1, 2 &3).

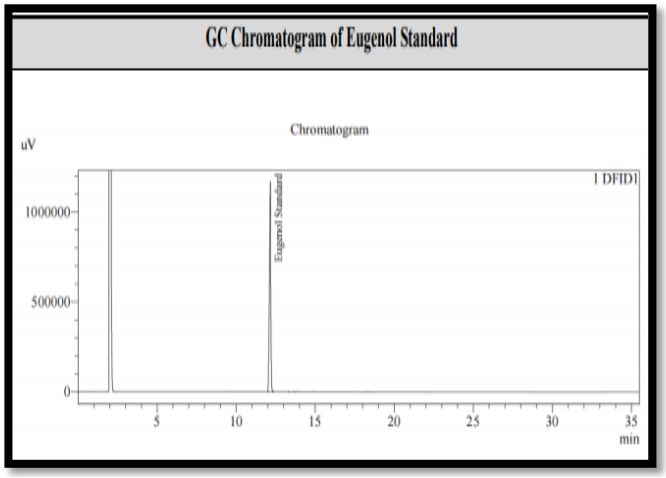


Figure 1 GC Chromatogram of Eugenol Standard

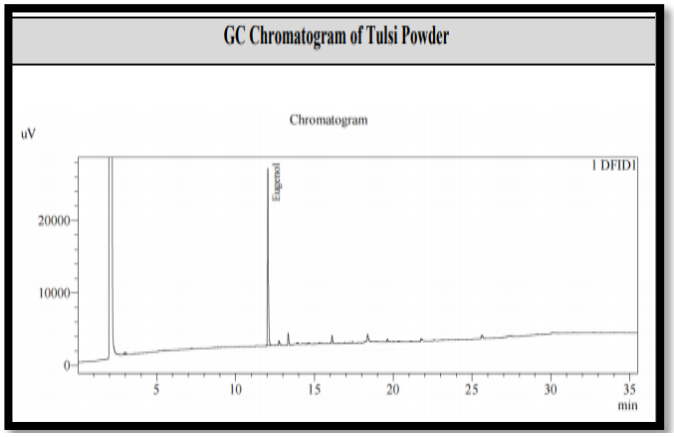


Figure 2 GC Chromatogram of *Tulasi Patra* Powder

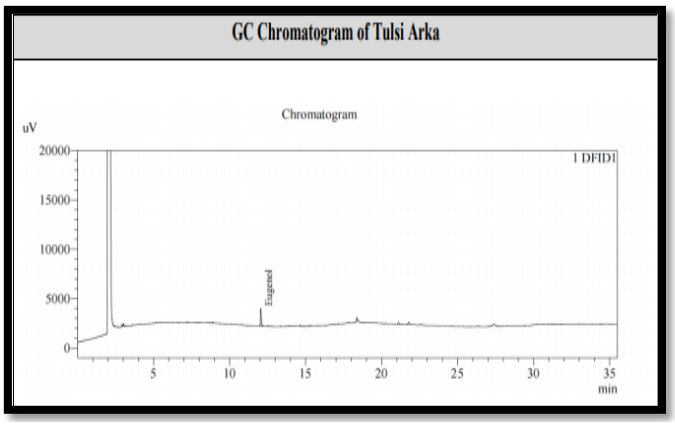


Figure 3 GC Chromatogram of *Tulasi Patra Arka*

Eugenol is a volatile oil which is found in leaves of *Tulasi*. Eugenol is not soluble in water. Here *Tulasi Patra Arka* is a suspension of the distillate in water[17] So, here only suspended particles of Eugenol were measured. That's why *Tulasi PatraArka* has less percent of Eugenol which was very less than permissible limit of IP (Not less than 0.40 percent (% w/w) [18].*Tulasi Patra* Powder contains drug as a whole so it has acceptable quantity of Eugenol 1.56% which was more than permissible limit of IP (Not less than 0.40 percent (% w/w) [18].*Tulasi Patra* Powder contains 14 times more Eugenol than *Tulasi Patra Arka.*

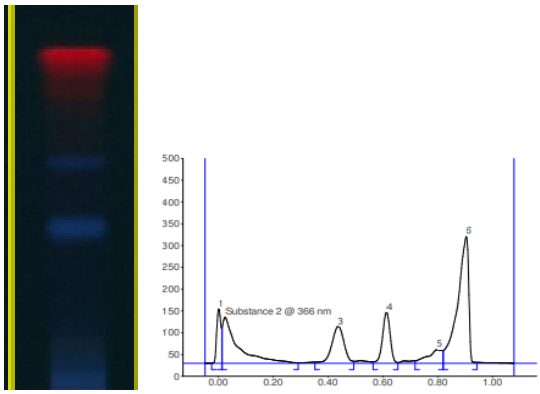
In this research work heavy metals in sample of TPP and TPA are within permissible limit which is mentioned in API. Heavy metals are widespread in soil as a result of geo-climatic conditions and environmental pollution. Therefore, their assimilation and accumulation in plants is obvious. Together with other pollutants, heavy metals are discharged into the environment through industrial activity, automobile exhaust, heavy-duty electric power generators, municipal

ORIGINAL RESEARCH ARTICLE

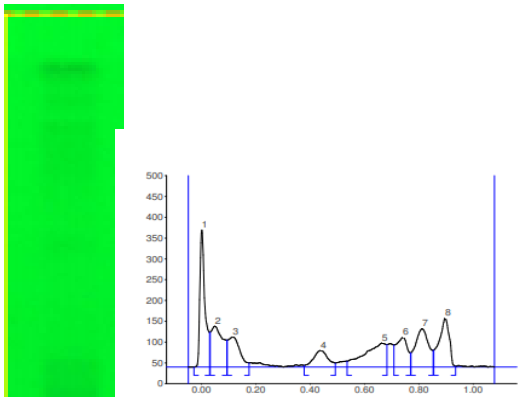
wastes, refuse burning and pesticides used in agriculture[19].

Human beings, animals and plants take up these metals from the environment through air and water. Heavy metals have the tendency to accumulate in both plants and human organs[20].The accumulation of heavy metals can have middle-term and long-term health risks, and strict periodical surveillance of these contaminants is therefore advisable[21]. Lead accumulation results first in reduced functioning of kidney, liver and brain cells and later in complete breakdown of the tissues. Cadmium and its compounds are also toxic to humans[22].Heavy metal may also introduce during the preparation of the raw material for traditional medicine products which covers many steps such as cultivation, harvesting, collecting, cleaning and drying of the medicinal plants[23].The environmental factors are probably contributed in the contamination of such products. It includes the contamination of the agriculture soils and irrigation of water[24].

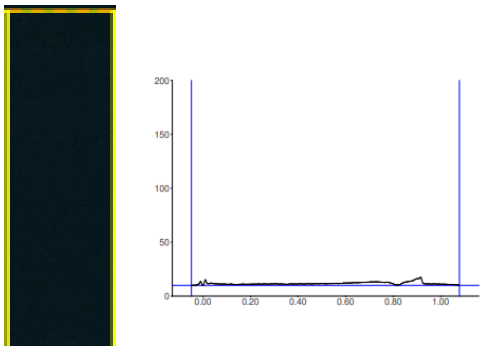
HPTLCanalysis was performed of TPP and TPA sample in which HPTLC plate was scanned at 254 nm and 366 nm. In sample of TPP when it was scanned at 254 nm 8 band were seen and in 366 nm 6 band were seen. Here, *Tulasi Patra* Powder is drug as a whole, so it contains more chemical constituents, so it has more band than *Tulasi PatraArka*. In sample of TPA when it was scanned at 254 nm 3 band were seen and in 366 nm band was not seen. *Arka* is a liquid preparation obtained by distillation of certain liquids or of drugs soaked in water using the *Arkayantra* or any convenient modern distillation apparatus [Table-7] & [Graph No. 1,2,3,4.]. The method by which the volatile oil and active principles of the drug are collected is called *Arka Kalpana* and the compound prepared through this procedure is called as *Arka*. Thus, *Arka* has only volatile oils, so it contains less band in comparison to *Tulasi Patra* Powder.



Graph 1 *Tulasi Patra*Powder 366nm

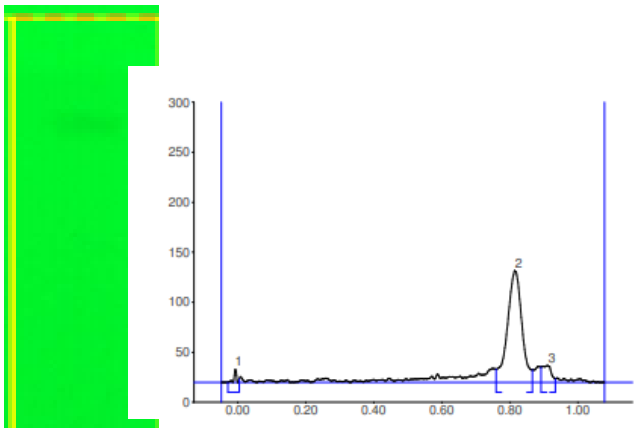


Graph 2 *Tulasi Patra*Powder254 nm



Graph 3 *Tulasi PatraArka*366 nm

ORIGINAL RESEARCH ARTICLE



Graph 4 *Tulasi PatraArka*254 nm

CONCLUSION

In the present study, percentage of Eugenol by Gas Chromatography in *Tulasi Patra* Powder sample was 1.56 % and in *Tulasi Patra Ark a*sample was 0.11%. Thus, it can be concluded that *TulasiPatra* Powder has more Eugenol percentage than *Tulasi PatraArka*.

ACKNOWLEDGEMENT:

Authors are thankful to Food and Drug laboratory, Vadodara and Dr Amit Gohil (head of pharmacognosy department, FDL) for their help in analytical study. Authors are also thankful to Vasu laboratories, Vadodara for carrying out GC study.

ORIGINAL RESEARCH ARTICLE

REFERENCES

1. Bhavaprakasha, Bhavaprakash Nighantu, Pushpadivarga. Ver.62-63. Commentary by Prof. KC. Chuneker. edited by Late Dr. G.S. M.D. (Ayu.). Varanasi: Chaukhambha Bharti Academy, 2010; p. 496.

2. Agnivesha, Charaka Samhita of Acharya Charaka, Dridhabala krit, Vidhyotini Hindi Commentary by Kashinath Shastri and Dr. Gorakhnatha Chaturvedi. Sutrasthan. Ch. 27, Ver. 169. Varanasi: Chaukhambha Bharti Academy; 2014; p. 545.

3. Maharshi Sushruta, Sushruta Samhita, edited by Kaviraj Dr.Ambikadutta Shastri. Sutrasthan Ch.38, Ver. 18. Varanasi: Chaukhambha Bharti Academy; 2014. p. 184.

4. Acharya Sharangadhara, *Sharangadhara Samhita*, '*Jivanprada*' Hindi commentary, commented by Dr. Shreematee Shailaja Shreevastava, Madhyam Khanda Ch.6, Ver. 1. Varanasi: Chaukhamba Orientalis; 2013. p.173.

5. Anonymous, Government of India, AFI. Part 1, 2nd ed. New Delhi: the controller of publications civil lines; 2003. p. 27.

6. Lankapatiravana, Arkaprakasha, Hindi commentary by Indradeva Tripathi, Prathama Shataka Ver. 46. 4th edition. Varanasi: Chaukambha Krishnadas Academy; 2011. p. 9.

7. Anees Ahmed Khalil. Essential oil eugenol: sources, extraction techniques and nutraceutical perspectives. RSC advances 2017; 7: 32669-32681.

8. Anonymous, Indian Pharmacopoeia, Volume III, Herbs and Herbal Products, English commentary by Govt. of India ministry of health and family welfare, The Indian Pharmacopoeia commission Ghaziabad Published 2014, *Tulasi*, p.3275.

9. M. Wink. Plant secondary metabolites modulate insect behavior-steps towards addiction. Frontiers in Physiology 2018; 9:364. View at: Publisher Site | Google Scholar.

10. Baser K. H. Handbook of Essential Oils: Science, Technology and Applications, University of Wien, Vienna, Austria, 2010, 978-1-4200-6315-8.

11. R. Rehman. Biosynthetic factories of essential oils: the aromatic plants, Natural Products Chemistry and Research 2016; 4 (4): 227.View at: Google Scholar

12. B. Adorjan. Biological properties of essential oils: an updated review. Flavour and Fragrance Journal 2010; 25(6): 407–426. View at: Publisher Site | Google Scholar

13. M. Alboofetileh.Antimicrobial activity of alginate/clay nanocomposite films enriched with essential oils against three common foodborne pathogens. Food Control 2014; 36(1):1–7.View at: Publisher Site | Google Scholar

14. A. R. Bilia. Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. Evidence-Based Complementary Alternative Medicine 2014:14. View at: Publisher Site | Google Scholar

15. M. Wink. Plant secondary metabolites modulate insect behavior-steps towards

ORIGINAL RESEARCH ARTICLE

addiction. Frontiers in Physiology 2018; 9:364. View at: Publisher Site | Google Scholar

16. Vidita V. Bhargava. Detection of Heavy Metal Contents and Proximate Analysis of roots of Anogeissus latifolia. Journal of Pharmacognosy and Phytochemistry 2013; 1(6): 61-65.

17. The Ayurvedic formulary of India, Part -1, Second revised edition, Government of India, Ministry of health and family welfare, Department of Indian systems of medicine and homeopathy, Published by the controller of publications civil lines, Delhi; 2003, p. 27.

18. Indian Pharmacopoeia, Volume III, Herbs and Herbal Products, English commentary by Govt. of India ministry of health and family welfare, The Indian Pharmacopoeia commission Ghaziabad Published, 2014, *Tulasi*, p.3275.

19. Jarup L. Hazards of heavy metal contamination. Journal of Ethnopharmacology 2003; 68:167- 182.

20. Bayor MT. Croton membraceous used in herbal formulations for measles in Ghana has potent antimicrobial activities. Journal of Pharmacognosy and Phytotherapy 2009; 1(4):47-51.

21. Abou-arab AAK. Pesticide residues in some Egyptian spices and medicinal plants. Food chemistry 2001; 72:439-445.

22. Baker AJM. The possibility of in situ heavy metal decontamination of polluted soils using crops of metal accumulating plants. Journal of conservation and recycling 1989; 11:41-49.

23. Tong. Environmental lead exposure; a public health problem of global dimension. Bulletin of the World Health Organization, 78(9):1068-1077.

24. Sharma P. Lead toxicity in plants. Brazilian Journal of Plant Physiology 2005; 17(1): 35-52.

25. Alam Abrar. Standardization of Unani Drugs with Modern Analytical Parameters: A Necessary Step, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s): 648-652.