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QUANTITATIVE ESTIMATION OF MENTHOL *(MENTHA ARVENSIS LINN.)* IN THE PREPARATION OF *PUDINA CHURNA* AND *PUDINA ARKA*

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ABSTRACT

Context: The "*Panchavidha Kashaya Kalpana*" is basic processing techniques of Bhaishajya Kalpana. The efficacy of *Kalka, Churna, Swarasa, Taila* and *Arka* is gradually increasing in descending order as said by Arka Prakasha. This efficacy of individual formulation is may be due to various degrees in the concentration of active principle. *Pudina (Mentha Arvensis* Linn.) is easily available and widely used plant drug and also known as mint. Menthol derived from its essential oil has antiseptic, stimulant and diuretic in properties. **Aim:** To compare potency between *Pudina Churna* and *Pudina Arka* with special reference to Menthol. **Methods and Material:** Marked sample of fresh *Pudina* is procured and dried in shade after proper washing. Organoleptic and Physico- chemical parameters such as pH, LOD, WSE, ASE, Total Ash value and AIS were assessed in *Pudina Churna* and Refractive index, specific gravity was done in sample of *Pudina Arka*. In addition to that, the chemical marker – Menthol was quantitatively identified by GC in both the samples. **Results:** All parameters are found within limit of API monograph. Assay of Menthol by GC showed. **Conclusion:** *Pudina Churna* have more concentration of menthol than the *Pudina Arka.*

KEYWORDS: Gas Chromatography, Menthol, *Pudina Churna, Pudina Arka*

INTRODUCTION

The basic principles of preparing various forms of medicaments were thoroughly analysed during this period and they were confined into five basic categories i.e., *Panchavidha Kashaya Kalpana*.[1] Secondary formulations like *Churna, Avaleha, Asava-Arishta, Gutika* etc. are used abundantly. In Ayurvedic field of practice several type of *Kalpana* is being used presently, *Churna Kalpana* plays an important role in pharmaceutics of Ayurveda, owing to many advantages like easy manufacturing and economic than other dosage form. Due to availability of various formulation techniques, good patients' compliance and huge potential powder is popularized in the pharmaceutical market. The dry drug which is finely pulverized and sieved through a fine cloth is taken as the *Churna.*[2] *Arka Kalpana* is correlated with Distillation in modern pharmaceutics practices. *Arka Kalpana* can be defined as a procedure in which volatile oil and active principle of drug are collected, and compound prepared through this procedure is called as *Arka.*[3]

The natural bioactive compound Menthol, which is a well-known active marker present in the *Pudina* (*Mentha Arvensis* Linn.). *Pudina* is mentioned in Nighantu Ratnakar for the first time in 19th century.[4] It is widely used in Ayurvedic formulation preparation. Menthol derived from its essential oil has antiseptic, carminative, refrigerant, stimulant and diuretic in properties.[5]

The efficacy of *Kalka, Churna, Swarasa, Taila* and *Arka* is gradually increasing in descending order as said by Arka Prakasha.[6] This efficacy of individual formulation is may be due to various degrees in the concentration of active principle. Till date no research works have been carried out on quantitative estimation of menthol *(Mentha Arvensis Linn.)* in the preparation of *Pudina Churna* and *Pudina Arka.* considering all these, present work was planned to compare potency between *Pudina Churna* and *Pudina Arka* with special reference to menthol.

SUBJECTS AND METHODS

Procurement of raw drug

Pudina was procured from local market of Vadodara, Gujarat. In the month of November 2020.The sample was identified and authenticated in Pharmacognosy department of Food and Drug Laboratory, Vadodara.

Preparation of samples

Pudina Churna was prepared as per the reference of Sharangdhar Samhita.[7] 3000 g Fresh *Pudina* was taken and sorting was done. After sorting and washing with water, *Pudina* was kept in tray for shade drying. After drying, obtained *Pudina* was converted into fine powder by using mixer machine. It was sieved through 90 no. mess and weighed. Then it was stored in air- tight container.

Pudina Arka was prepared as per the reference mentioned in API.[8] Fresh *Pudina* was placed in a round bottom standard joint flask and 10 times distilled water was added. Distillation apparatus was attached with flask and receiving heads, double surface condenser and receiving flask and enough circulating water was supplied to condense the distillate. The flask was placed on a heating mantle and the temperature was adjusted at 50°C. The distillation was continued until collection of *Arka* was done.

Physico-chemical evaluation

Pudina Churna was analysed through relevant physicochemical parameters such as Loss on drying,[9] Water soluble extractive,[10] Alcohol soluble extractive,[11] Total ash,[12] acid insoluble ash,[13] pH.[14] *Pudina Arka* was also studied by Refractive index,[15] Specific gravity[16] and pH.

Assay of Menthol by Gas Chromatography

(i)Preparation of Standard Solution (S): Accurately weighed 100 mg of standard menthol in a 10 ml Volumetric flask, add Methanol GC Grade to dissolve it up to mark. Sonicate for 5 minutes. Filter it using 0.22 microns syringe filter and it for GC analysis.

(ii) Preparation of Sample Solution (T): Accurately weighed 5 gm of sample in a 10 ml volumetric flask. To it add 10 ml of Methanol GC Grade up to mark. Sonicate it for 5 minutes. Thereafter, Filter the solution with Whatman filter paper No.1 Filter with 0.22 microns syringe filter and use the solution thus obtained for GC analysis.

Chromatographic conditions for estimation of menthol through Gas Chromatography (GC) are as follows: Column: 10% FFAP packed column; Injector: Auto injector; Injection volume: 1 µL; Injector temperature: 240°C; Carrier gas: Nitrogen; Column oven temperature: Initial temperature is 80°C hold for 1 min, increase to 220°C at the rate of 8°C/min and hold for 17

min; Detector: Flame Ionization Detector (FID); Detector temperature: 280°C; Flow Rate: 1 ml/min; Spilt Ratio: 1:25

RESULTS

Organoleptic study

Organoleptic Characters like pungent taste, smooth and fine texture, characteristic odour and dark greenish Colour were found in sample of *Pudina Churna* while Sample of *Pudina Arka* was transparent in Colour and pungent in taste with characteristic odour [Table-1].

Table 1: Showing organoleptic characters of *Pudina Churna* and *Pudina Arka.*

Sr. No	Sample	Organoleptic Characters			
		Colour	Odour	Texture	Taste
1.	*Pudina Churna*	Dark greenish	Characteristic	Smooth and soft	Pungent
2.	*Pudina Arka*	Transparent	Characteristic	Liquid	Pungent

Physico-chemical evaluation

Both samples were tested for relevant physical and chemical parameters. It revealed that 1.2% foreign matter, 12.06% LOD, 11.2% Total Ash, 8.1% pH (10% aqueous solution), 1.003% Acid insoluble ash, 14.7% Alcohol soluble extractive, 27.8% Water soluble extractive, 0.0602% GC of menthol% in Pudina Churna [table-2].

Table 2: Showing Physico-chemical analysis of *Pudina Churna.*

Parameters	Value
Foreign matter	1.8%
Loss on drying	12.06%
Total Ash	11.2%
pH (10% aqueous solution)	8.1%
Acid insoluble ash	1.003%
Alcohol soluble extractive	14.7%
Water soluble extractive	27.8%
Gas Chromatography	0.0602%

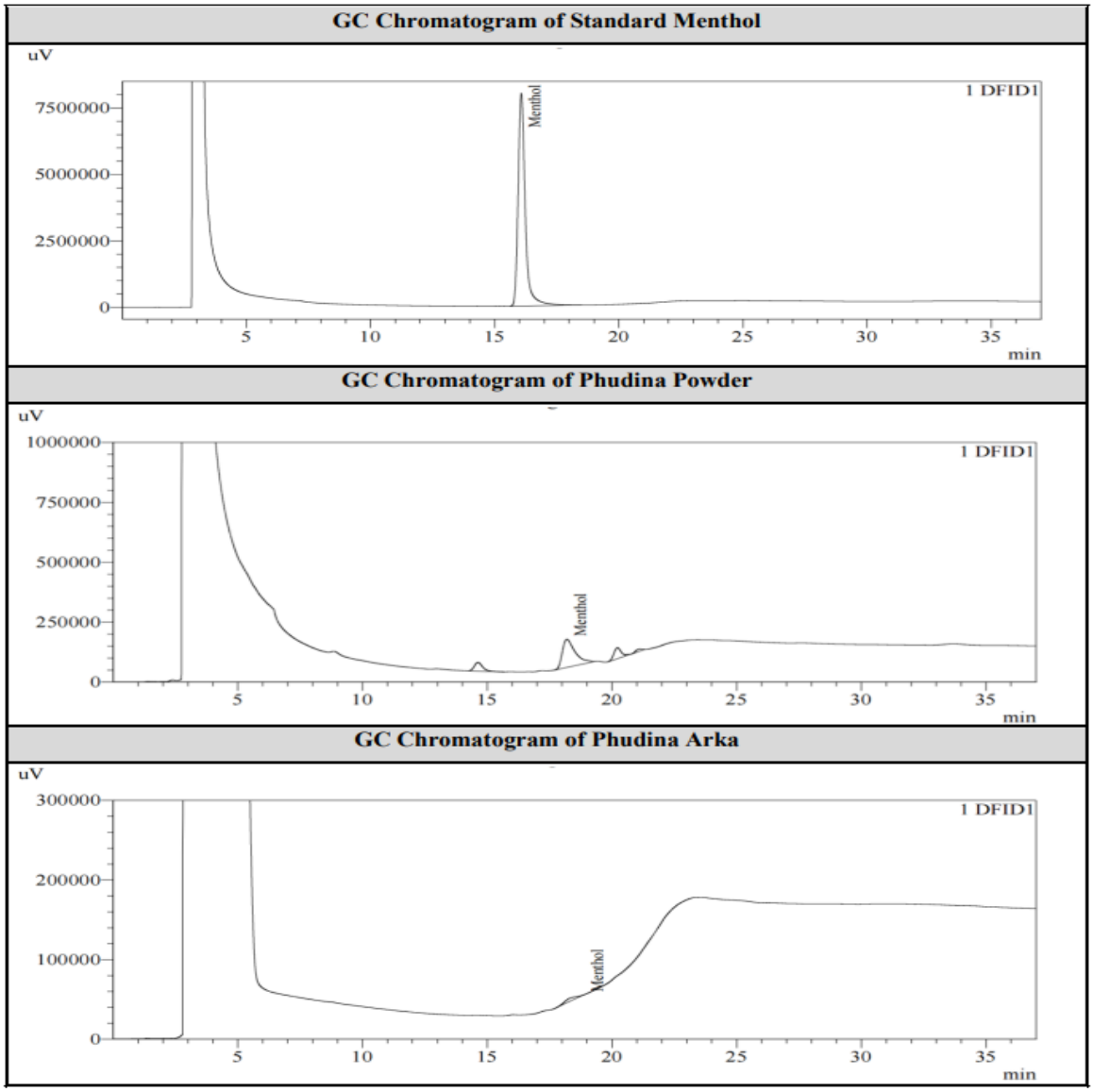
1.002% specific gravity,1.0336% refractive index and 7.21% pH was found in sample of Pudina Arka [table-3].

Table No. 3: Showing Physico-chemical analysis of *Pudina Arka.*

Parameters	Value
Specific gravity	1.002%
Refractive index	1.3306
pH	7.21%

Assay of Menthol by Gas Chromatography (GC)

Quantitative estimation of active component Menthol was done by Gac Chromatography technique. In this, *Pudina Churna* and *Pudina Arka* samples are assessed with marker component Menthol. [Figure-1]



This study showed menthol 0.0602% w/w in *Pudina Churna* sample and 0.00187% w/w in *Pudina Arka*. [table-4].

Table 4: Showing GC result of *Pudina Churna* and *Pudina Arka.*

Parameters	Menthol standard	*Pudina* Churna	*Pudina Arka*
Weight	118mg	5069mg	5021mg
Rt	16.073	18.194	18.864
AUC	159255371	4164380	128132
Menthol%	-	0.0602%	0.00187%

DISCUSSION

Pudina has always been used for a lot of centuries. The name arrives from the Greek myth of the nymph minthe. *Pudina* is also known as mint. In Samhita period, *Pudina* has not mentioned in any Samhita Because *Pudina* introduced after 19th century. So, after that *Pudina* has mentioned in many Nighantus. *Pudina* has mainly *Vata-Kapha shamaka* property and therapeutical.

Pudina has used in *jirna jvara, shoola , agnimandhya, ajirna, aruchi, atisara, shvasa, chhardi, adhmana, hikka, kasa, mutrakruccha, kastartava, mada, visuchika, grahani.*[17] According to Nighantu Ratnakara, *Pudina* possess *Guru Guna* while in other Nighantu *Pudina* Possess *Laghu Guna*. API also considered *Laghu Guna* of *Pudina.*

Menthol crystals are naturally produced through mint essential oil extraction. Menthol is solid constituent of oil of mint, to which its characteristic odor is due and was formerly known as peppermint camphor.[18]

Organoleptic and Physico chemical parameters are carried out to authenticate the samples. pH of *Pudina Churna* and *Pudina Arka* was found respectively 8.1% and 7.21%. which indicates weakly basic nature of the both samples. Moisture content of *Pudina Churna* was 12.06%. Acceptable range of moisture content of *Pudina* is not mentioned in API. Moisture content of *Pudina Arka* is not applicable as it is in liquid form. In *Pudina Churna,* water soluble extractive value was 27.8% and alcohol soluble extractive value was 14.7%. Extractive value plays an important role in evaluation of crude drugs. Total Ash and Acid insoluble Ash of *Pudina Churna* was 11.2% and 1.003% respectively. The percentage of total Ash value and Acid insoluble Ash are within the limits of API.[19] A high ash value is indicative of contamination, substitution, adulteration, inorganic material or carelessness in preparing the drug.[20] Specific Gravity is used to define the weight or density of a liquid as compared to the density of an equal volume of water at a specified temperature.[21] Here, specific gravity of *Pudina Arka* was 1.002% that suggests *Pudina Arka* is denser than the water. *Pudina Arka* is a suspension of the distillate in water.[22] So it has higher specific gravity than water. Refractive index of a liquid media is a dimensionless number that describes how fast light travels through the liquid media The refractive index of *Pudina arka* is 1.3306, meaning that light travels 1.3306 times slower in *Pudina arka* than in a vacuum. Increasing the refractive index corresponds to decreasing the speed of light in the liquid media.

Gas chromatography technique are used particularly for the volatile substance of material. Actives markers are chemically known compounds, which may or may not have therapeutic effect, Gas chromatography use in analytical chemistry for separating and analysing compounds that can be vaporized without decomposition.[23] The choice of the marker has to be justified. Gas chromatography is the process of separating compounds in a mixture by injecting a gaseous or liquid sample into a mobile phase, typically called the carrier gas, and passing the gas through a stationary phase.

In this study Menthol was used as bio-marker as reference of IP.[24] Menthol should not be less than 0.2 % w/w in preparations as per Pharmacopeial standard. In *Pudina Churna* and *Pudina Arka*, menthol contained 0.0602% w/w and 0.00187% w/v respectively. Both the sample couldn't meet the permissible limit of IP. When comparing the Menthol concentrations in both the samples, it is important to note that *Pudina Churna* showed significantly 125 times higher concentration of menthol than *Pudina Arka* prepared from fresh *Pudina*.

Most essential oils are volatile and sensitive in the air conditions (humidity, temperature and velocity). Volatile oils are very sensitive towards the change in temperature. Drying encourages moisture loss from the whole tissue, including gland hairs. Generally, high temperatures influence essential oil quantity and quality in aromatic and medicinal plants not only during drying; reduction in active ingredients continues during storage period.[25]

It seems that exposure of plant material to the high temperature results into great loss of volatile content. This trend the reduction of volatile content of plant by the impact of high temperature drying process has been reported by some scientist,i.e., Fresh *Curcuma* rhizome lost the water content slowly upon storage at Room Temperature (RT) with the average rate of lost was approximately 10% per-week.[26] By the end of the storage, the rhizome was almost dry. Fresh *Curcuma* rhizome contained 1.1% of volatile oil.[27]

Menthol is a volatile oil which is found in *Pudina*. Menthol is not soluble in water. Here, *Pudina Arka* is a suspension of the distillate in water. So, here only suspended particles of Menthol were measured. That's why *Pudina Arka* has less percent of menthol than the *Pudina Churna*. Propable reason for the decrease menthol percentage in *Pudina Churna* may be due to drying process of drug. Propable reason for the decrease menthol percentage in *Pudina Churna* may be due to drying process of drug.

All primary Physico-chemical parameters of *Pudina Churna* and *Pudina Arka* were found as per standards given by Ayurvedic Pharmacopeia. Menthol as marker component in *Pudina* (*Mentha Arvensis* Linn.) is found more (0.0602%) in *Churna* preparation than *Arka* preparation (0.00187%) by Gas chromatography. So, it can be concluded that *Pudina Churna* have more concentration of menthol than the *Pudina Arka.*

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