Pharmacopoeial Standard Development and Quality Assessment Studies of Asu. Drug Roughan-E-Qaranfal or Laung Taila (Clove Oil)

*[1]Pawan Kumar Sagar,
[1]Murugeswaran R,
[2]Rampratap Meena,
[1]Mageswari S,
[1]Meera Devi Sri P,
[3]M A Rasheed N and
[1]Asiya Khanum

[1]Regional Research Institute of Unani Medicine, Chennai

[2]Drug Standardization Research Institute, Ghaziabad

[3]Central Research Institute of Unani Medicine, Hyderabad

Abstract

Roughan-e-Qaranfal or Laung Taila (Clove Oil) is useful for treatment of Dyspepsia, Indigestion or Acidity (Su-e-Hazam), Ozostomia, Oral Sepsis or relieving tastelessness and Bad breath, Toothache, Muscular pain (Waj-ul-Asnam), Analgesic (Musakkin-e-Alam), Hepatitis or Weakness of Liver (Zof-e-Kabid), Weakness of Stomach (Zof-e-Meda), Respiratory disorder, Asthma, Breathing difficulty etc. Three batch study samples of these compound formulations were prepared in the Pharmacy, DSRU, CRIUM, Hyderabad by employing authenticated standard methods. The quality assessment physico-chemical research data were investigated and the presence of Arachis oil, Cotton seed oil, Sesame oil and Mineral oil were found negative in clove oil samples. The TLC/HPTLC studies of petroleum ether extracts of the drug samples showed various spots along with their R*f* values at UV-254nm, UV-366nm and in visible light by applying derivatizing agents, i.e. Iodine vapors and 5% methanolic sulphuric acid reagent. The quality control and assurance studies results revealed the absence of hazardous and toxic contamination. The R*f* values of separated spots also show the indication of enhanced presence of bioactive phytochemical constituents.

Keywords: Acidity, Hepatitis, Indigestion, Roughan-e-Qaranfal or Laung Taila.

Introduction

Herbal medicines are complex chemical mixtures obtained from plant and used in healthcare in both developed and developing countries. In the present era, universal trend has been shifted from synthetic to herbal medicine i.e., return to Nature (Sharma et al., 2008). Ayurveda, Siddha and Unani are the plant based system of medicines. As per Unani system of medicine, the drug Roughan-e-Qaranfal or Laung Taila (Clove oil) is frequently recommended for Ozostomia or Oral sepsis (Bakhr-ul-Fam), Toothache or Muscular pain (Waj-ul-Asnam), Weakness of the Stomach (Zof-e-Meda), Hepatitis or Weakness of Liver (Zof-e-Kabid), Dyspepsia (Sue-Hazm), Flatulence in the stomach and Colic (Nafkh-e-Shikam Qulanj) problems. As per Ayurvedic System of Medicine, it is used for respiratory disorder (Svasa), vomiting (Chardi), bloating or gaseous distension of abdomen (Adhmana), wheezing, breathing difficulty (Hikka), cough, cold (Kasa), chronic respiratory disorder (Ksaya or Kshaya), excessive thirst (Trsna or Trushna), indigestion or acidity (Amlapitta), bleeding disorder (Pittasran ashana), asthma (Shwasa) and improving digestion strength (Deepana & Paachan) and taste (Ruchya)(Kabiruddin, 1967; Anonymous, 1989; Anonymous, 2006, Anonymous, 2007(a)

* Author for Correspondence; Email: pawansagarkr93@gmail.com

The drug Roughan-e-Qaranful or Laung Taila (Clove oil) is the fixed essential oil obtained from dried flower buds of *Syzygium aromaticum* (Linn.). Merr. & L. M. Perry. Syn. *Eugenia aromatica* Kuntze, *Eugenia caryophyllata* Thunb. of Myrtaceae family. It is a light brown colored viscous liquid with spicy aromatic odour and astringent sensation of taste. The oil was reported to contain bio-active phytochemical constituents of nearly 36 components with a high concentration of eugenol (88.58%), eugenol acetate (5.62%), β-caryophyllene (1.39%), less concentration of 2-heptaanone (0.93%), ethylhexanoate (0.66%), humuleno (0.27%), α-humulenol (0.19%), calacorene (0.11%) and calamenene (0.10%) (Pulikottil and Nath, 2015; Chaieb et al., 2007). Eugenol (4-allyl-1-hydroxy-2-methoxybenzene), a phenolic non-nutrient compound, is one of the major components with a molecular weight of 164.20 and β-caryophyllene, the other major constituent of clove oil which has a molecule weight of 204.35 (Lee et al., 2002). The Siddha Pharmacopeia of India reported the presence of active constituents such as Caryophyllene oxide, caryphylla-3(12),6-dien-4-ol, caryophylla-3(12),7(13)-dien-6 α-al, eugenol (77.1% of volatile oil), acetophenone, 2-hydroxy,4,6,di-methoxy-5-methylaceto phenone, β-caryophyllene, eugenol acetate, derivative of β-caryophyllene, α-humulene and its expoxide, benzyl salicylate, α-cardinol, γ-decalacetate, fenchone, hexanol, 2-hexanone, methyl palmitate, α-murolene, palustrol, propyl benzoate, α-thujene, β-selinene and eugenine.

Various *in-vivo* and *in-vitro* pharmacological activities were carried out and reported on the plant *Syzygium aromaticum* and its oil content such as antibacterial, antifungal, antioxidant, antistress activity, anti-inflammatory, anticancerous, antiviral, analgesics activity, dental care activity, Mosquito repellent, insecticidal activities and Neuroprotective activity (Wenkhede, 2015; Kessab and Baijomy,2014 ; Kumar et al, 2011). However, one of the impediments in the acceptance of herbal medicines is the lack of standard quality control profiling. Due to the complex nature and inherent variability of the chemical constituents, it is difficult to establish quality control parameters. Hence, this study was designed to standardize the drug Roghan-e-Qaranfal and to arrive at the scientific data for the development of pharmacopeoia in order to gain global acceptance.

Material and Methods

Procurement of the Plant Material

Fresh reddish brown flower buds of Qaranfal or Laung (Clove) - *Syzygium aromaticum* Linn (QF1, QF2 and QF3) were collected from Pharmacy, CRIUM, Hyderabad (procured from authenticated national raw drug vendors of India). The collected raw drug samples were botanically and pharmacognostically identified by the Survey of Medicinal Plant Unit and Drug Standardization Research Units,

CRIUM, Hyderabad. The fresh dried flower bud samples were used for the study after complete assurance of preliminary identity, purity and quality assessment and pharmacognostical test. Analysis of quality control parameters like microbial load, heavy metals and aflatoxins detections were carried out using standard methods

Physico-chemical Screening

Physico-chemical screening of raw drug samples and Roughan-e-Qaranfal (Clove oil) was carried out under the following parameters like foreign matter, moisture contained at 105°C, petroleum ether soluble extractive value and volatile oil content as per the standard methods (Anonymous 1998, Anonymous 2005).

HPTLC Fingerprint of Petroleum Ether Extract

2.5g of each sample was extracted with 30 ml of petroleum ether separately by refluxing on water bath for 30 minutes. The extracts were filtered through Whatman No. 1 filter paper and concentrated to 5 ml in a standard flask separately. HPTLC was performed on 10 cm x 10 cm TLC plates pre-coated with silica gel 60 F_{254} (E. Merck). The samples were applied on plates by using applicator. Linear ascending development to a distance of 8 cm with toulence: Ethyl Acelate (9:1) (v/v) as a mobile phase was performed in a twin-trough glass chamber. The plates were dried in air and visualized under 254 nm and 366 nm for ultra violet detection. Further, the same plates were exposed to I_2 vapours and derivatized with 5% methanolic sulphuric acid spray reagent and densitogram was recorded, Figure 1.

Results and Discussion

The drug Roughan-e-Qaranfal or Laung Taila (Clove oil) has been frequently and widely used since ancient times due to its important miraculous therapeutic and medicinal values. The physico-chemical parameters, High Performance Thin Layer Chromatography (HPTLC) analysis and Quality control parameters of the oil were studied. The mean value of the aromatic oil yield is 45.54%. The physico-chemical parameters data and the quality control parameters data are given in Table 1, 2,3,4,5 and 6. It is inferred from the data that the drug is free from any foreign adulterated materials, microbial load, aflatoxin, pesticide and toxic heavy metals.

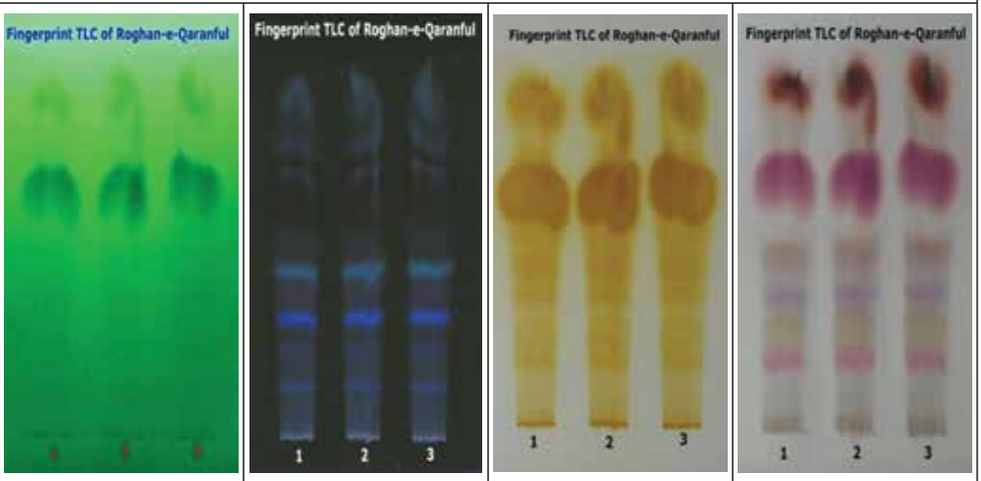
The HPTLC Studies of petroleum ether extracts of Roughan-e-Qaranfal drug samples is shown in Figure -1. The chromatogram profile shows three spots at Rf values 0.24, 0.32 and 0.71, (Dark green) under Uv 254 nm and six spots at Rf values 0.14 (Light blue), 0.26(Light blue), 0.32(Blue), 0.46(Indigo), 0.71(Reddish pink) and 0.76 (Light blue) under UV 366nm. After exposure to Iodine vapours,

it shows four spots at Rf values 0.19, 0.32, 0.46 and 0.71, (Dark brown) and five spots at Rf values 0.19(Pink), 0.32(Brown), 0.38(Purple), 0.49(Brown) and 0.71(Dark pink), under visible region after derivatisation with 5% methanolic sulphuric acid and heating the plate at 105ºC for five minutes.

Conclusion

The research study data and comparative quality screening assessment data will provide the preliminary referral supportive information for the development of pharmacopoeial standard.

Petroleum ether extract



UV 254 nm	UV 366 nm	Exposed to Iodine vapors	Derivatized with 5% Methanolic sulphuric acid

Fig 1: Solvent system: Toluene: Ethyl acetate (9:1 ratio)

Table-1: Oil Yield

Parameters Analyzed	Method applied	Use of solvent	Sample QF1	Sample QF2	Sample QF3	Mean Values
Aromatic oil Yield (%)	Soxhlet hot extraction at 35°C to 45°C temp. for 12 hours and distilled at 45°C to 55°C temp. for 5 hours.	Petroleum Ether (40°C to 60°C)	43.94%	44.36%	48.34%	45.54%

Table 2: Physico-chemical parameters of Roghan-e-Qaranfal

Parameters Analyzed	Sample 1 RQF1	Sample 2 RQF2	Sample 3 RQF3	Mean value
Petroleum ether (40-60°C) Extractive, %, w/v.	100.0	100.0	100.0	100.0
Acid value	2.235	2.745	2.765	2.581

Parameters Analyzed	Sample 1 RQF1	Sample 2 RQF2	Sample 3 RQF3	Mean value
Iodine value	5.023	5.244	5.352	5.206
Peroxide value	14.889	15.127	17.906	15.974
Unsaponifiable matter	1.756	1.821	1.856	1.811
Refractive Index	1.5375	1.5390	1.5396	1.5387
Weight per ml.(gm.)	0.7022	0.7056	0.7035	0.7037
Arachis oil	– ve	–ve	–ve	–ve
Cotton seed oil	–ve	–ve	–ve	–ve
Sesame oil	–ve	–ve	–ve	–ve
Mineral oil	–ve	–ve	–ve	–ve

Table-3: Estimation of Microbial Load

S. No.	Parameters Analyzed	Results			WHO & API / UPI Part-II Limits
		Sample RQF1	Sample RQF2	Sample QF3	
1.	Total Bacterial Count	Nil	Nil	Nil	10^5 cfu/gm.
2.	Total Fungal Count	Nil	Nil	Nil	10^3 cfu/gm.
3.	*Salmonella Spp.*	Absent	Absent	Absent	Nil
4.	*Staphylococcus aureus*	Absent	Absent	Absent	Nil
5.	*Escherichia coli*	Absent	Absent	Absent	Nil

Table 4: Estimation of Aflatoxins

S. No.	Parameters Analyzed	Results			WHO & API / UPI Part-II Limits
		Sample - RQF1	Sample - RQF2	Sample - RQF3	
1.	B1	Not detected	Not detected	Not detected	0.5 ppm.
2.	B2	Not detected	Not detected	Not detected	0.1 ppm.
3.	G1	Not detected	Not detected	Not detected	0.5 ppm.
4.	G2	Not detected	Not detected	Not detected	0.1 ppm.

Table 5: Estimation of Pesticide Residues

S. No.	Parameters Analyzed	Results			WHO & API / UPI Part-II Limits (mg/kg)	Relative Retention time (as per GLC applied method)
		Sample RQF1	Sample RQF2	Sample RQF3		
1.	Aldrin	N/D	N/D	N/D	0.05	0.68
2.	Dieldrin	N/D	N/D	N/D	0.05	0.87
3.	DDE (all isomers)	N/D	N/D	N/D	1.0	0.81-0.87

S. No.	Parameters Analyzed	Results			WHO & API / UPI Part-II Limits (mg/kg)	Relative Retention time (as per GLC applied method)
		Sample RQF1	Sample RQF2	Sample RQF3		
4.	Azinphos-methyl	N/D	N/D	N/D	1.0	1.17
5.	Chlorfenvinphos	N/D	N/D	N/D	0.5	1.00
6.	Endrin	N/D	N/D	N/D	0.05	0.91
7.	Chlorpyrifos	N/D	N/D	N/D	0.2	0.70
8.	Chlorpyrifos-methyl	N/D	N/D	N/D	0.1	0.60
9.	Cypermethrin	N/D	N/D	N/D	1.0	1.40
10.	DDT (all isomers)	N/D	N/D	N/D	1.0	0.95-1.02
11.	Deltamethrin	N/D	N/D	N/D	0.5	1.54
12.	Diazinon	N/D	N/D	N/D	0.5	0.52
13.	Dichlorvos	N/D	N/D	N/D	1.0	0.20
14.	Ethion	N/D	N/D	N/D	2.0	0.96
15.	Fenitrothion	N/D	N/D	N/D	0.5	0.96
16.	α- Endo sulfan	N/D	N/D	N/D	3.0	0.82
17.	Fenvalerate (all isomers)	N/D	N/D	N/D	1.5	1.47-1.49
17.	Heptachlor	N/D	N/D	N/D	0.05	0.61
18.	Hexachlorobenzene	N/D	N/D	N/D	0.1	0.45
19.	Lindane (gamma-HCH)	N/D	N/D	N/D	0.6	0.49
20.	Malathion	N/D	N/D	N/D	1.0	0.67
21.	Parathion methyl	N/D	N/D	N/D	0.2	0.66
22.	Permethrin	N/D	N/D	N/D	1.0	1.29-1.31
23.	Phosalone	N/D	N/D	N/D	0.1	1.18
24.	Pirimiphos methyl	N/D	N/D	N/D	4.0	0.66

Note: Where N/D = Not detected

Table 6: Estimation of Heavy Metals

S. No.	Parameters Analyzed	Results			WHO & API / UPI Part-II Limits
		Sample - RQF1	Sample - RQF2	Sample - RQF3	
1.	Arsenic	Not detected	Not detected	Not detected	3.0 ppm.
2.	Cadmium	Not detected	Not detected	Not detected	0.3 ppm.
3.	Lead	Not detected	Not detected	Not detected	10.0 ppm.
4.	Mercury	Not detected	Not detected	Not detected	1.0 ppm.

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सारांश

एएसयू औषधि रोग़न-ए-क़रंफल या लौंग तेल का भेषज मानक विकास और गुणवत्ता अध्ययन

*[1]पवन कुमार सागर, [1]मुरूगेश्वरन आर., [2]रामप्रताप मीना, [1]मगेश्वरी एस, [1]मीरा देवी श्री पी, [3]एम ए रशीद एन और [1]आसिया ख़ानम

रोग़न–ए–क़रंफल या लौंग का तेल अपच, बदहजमी या अम्लता (सू–ए–हज़म), ओजोस्टोमिआ, मौखिक पूति या स्वादरहितता से छुटकारा और सांस की बदबू, दांत दर्द, मांसपेशियों में दर्द (वज–उल–असनाम), दर्दनाशक (मुसक्किन–ए–अलम), यकृत शोथ या यकृत की कमज़ोरी (ज़ोफ–ए–कबिद), पेट की कमज़ोरी (ज़ोफ–ए–मैदा), श्वसन संबंधी विकार, अस्थमा, सांस लेने में कठिनाई इत्यादि के उपचार के लिए उपयोगी है। इस यौगिक मिश्रण के नमूने तीन बैच में प्रमाणिक मानक विधियों के तहत औषधि मानकीकरण इकाई – केन्द्रीय यूनानी चिकित्सा अनुसंधान संस्थान, हैदराबाद की फार्मेसी में तैयार किये गये। औषधि का गुणवत्ता मूल्यांकन भौतिक रासायनिक अनुसंधान डाटा के आधार पर किया गया और दूसरे तेल के नमूने जैसे अराकिस तेल, कपास के बीज का तेल, तिल का तेल और खनिज तेल की जांच के आधार पर देखा गया। औषधि में ये सभी तेल अनुपस्थित पाए गए। औषधि के पेट्रोलियम ईथर उद्धरण का अध्ययन के पश्चात् यू.वी. 254 एन.एल., यू.वी. 366 एन.एल. और दृश्य प्रकाश में विभिन्न बिन्दु एवं चिन्ह उनके आर. एफ. मूल्य पाए गए। गुणवत्ता अध्ययन एवं प्रतीति अध्ययन के परिणाम से खतरनाक व ज़हरीले पदार्थों की अनुपस्थिति का पता चला। विभिन्न आर.एफ. बिन्दुओं में से माना गया कि औषधि में अनेक प्रकार के जैवसक्रिय और पादपकीय रसायन उपस्थिति हैं।

शब्द कुंजीः अम्लता, यकृत शोथ, बदहजमी, रोगन–ए–करंफल, लौंग का तेल