Herbal Immersion Oil for Microscopic Identification of Malaria Parasites

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Abstract

Oils from thirty-two plants were extracted using solvent extraction/steam distillation methods. All oils along with 11 blended oil mixtures were tested for their immersion oil properties for the detection of malaria parasites. The physicochemical properties required for an immersion oil including density, viscosity, refractive index and acid value of five oils namely as Ocimum basilicum, Pogostemon cablin, Papaver somniferum Ricinus communis and Valeriana jatamansi and blended mixture 6 were determined. Stability of above stated plant oils and blended mixture 6, with time showed that all were stable under the observation period while non-dryness of these oils and blended mixture 6, represented very good criteria for the non-drying less than 1% variation in weight. GC-MS analysis of mixture 6 clearly identified compounds namely alpha copane, trans-Caryophyllene, Linalool, Estragole, hexadecanoic acid phenyl methyl ester and 9, 12-Octadecadienoic acid and besides other minor components. No peak of castor oil was detected due to lack of sample character and detection. Summary of in-house validation of blended mixture 6 classified as very good immersion oil (range 67-81%) and good (19-33%) for the examination of malaria parasites while the results of external validation clearly revealed that the developed plant oil mixture 6 possessed very good property as immersion oil for the examination of malaria parasites.

Keywords: Malaria parasites, Immersion oil, Plant oil mixture.

Introduction

Conventional immersion oils typically contain polychlorinated biphenyls (PCBs) which when blended with mineral oil and viscosity adjusting compounds provide generally useful immersion oil having many of the ideal characteristic. In recent years, however, PCB’s have been discovered to be carcinogenic, a hazard to the human environment, and are generally regarded as toxic. Furthermore, PCB’s are difficult to dispose off after use since they are extremely stable and non-biodegradable (Liva, 1986).

Attempts were also made to use oils from natural resources like plants. Cedar wood oil having a refractive index of 1.495 to 1.510 (British Pharmacopeia, 1963) was widely used for many years as immersion oil. Cedar-wood oil was a mixture of organic compounds considered generally safe by the FDA as a food additive preservative and also used as an anti-bacterial, fungicide. However, studies have shown that prolonged exposure to high levels of cedar-wood oil can cause liver and pulmonary toxicity (FAO, 1995). In addition
to this the cost wise cedar wood oil was expensive. In India, Victor et al., (2005) made an attempt to identify cost effective, but qualitative immersion oil for microscopy and photo microscopy. Pure castor oil and refined sunflower oil have been tried along with commercially available immersion oil on GTG banded chromosome preparations. Application of castor oil has been recommended as alternative to synthetic immersion oil. A review of the literature revealed that a limited work has been carried out to explore the possibility of using plant oils as an immersion oil for microscopy in spite of the fact that India has huge flora and fauna of plants. Attempts were made to screen different plants oils and combination of plant oils for their immersion oil property for microscopic identification of malaria parasites.

Materials and Methods

Thirty-two plants based on the physico-chemical properties reported in the literature were collected from various parts of India in consultation with Forest Research Institute, Dehradun. Scientific name, family and common names, raw material used, extraction method of the plants under investigation are given in table 1. Plants were dried in shades and oils were extracted from the plants using solvent extraction (Soxhlet Extraction) and steam distillation methods.

Microscopic examination of blood slides using plant oils as immersion oil

All oils were examined for their immersion oil properties for the microscopic identification of malaria parasites. Stained blood slides were examined under microscope at 100 x objective lens for detecting the malaria parasites. Different malaria parasite namely as Plasmodium falciparum/Plasmodium vivax stages including gametocyte, rings, and schizont were examined by using immersion oil/plant oils.

Although microscopic examination is a manual qualitative approach, attempts were made to grade all plant oils for their performance based on clarity, sharpness and contrast of parasite stages. Based on microscopic examination of malaria parasite, only five plants oils showed good results as immersion oil. Eleven combinations of above five plants oils with different proportions were also prepared and microscopic tested of malaria parasites. The different compositions of five oils were as follow:

Mixture 1 Consisted of fifty percent each of Ocimum basilicum and Papaver somniferum (50:50), Mixture 2: Composed of fifty percent each of Pogostemon cablin and Papaver somniferum (50:50), Mixture 3: 25% Ocimum basilicum and 25% Pogostemon cablin oil and 50% oil Papaver somniferum (25:25:50), Mixture 4: Ocimum basilicum oil was added into Ricinus communis oil at the ratio of 50:50, Mixture 5: The mixture was made from fifty percent of Pogostemon cablin and Ricinus communis oil (50:50), Mixture 6: It consisted of twenty five percent of each four of Pogostemon cablin, Ocimum basilicum, Papaver somniferum and Ricinus communis (25:25:25:25), Mixture 7: Papaver somniferum was blended with Ricinus communis (50:50), Mixture 8: It was a combination of three different oils namely as Ocimum basilicum, Pogostemon cablin and Ricinus communis at the ratio of 33:33:34, Mixture 9: The thirty-five percent of Ocimum basilicum, Pogostemon cablin and Mixture 10: It is composed of twenty-five percent of each of four different oils namely as Ocimum basilicum, Pogostemon cablin, Ricinus communis and Valeriana jatamansi and Mixture 11. Thirty-four percent of Valeriana jatamansi oil was mixed with
thirty-three percent of *Papaver somniferum* and *Ricinus communis* oil.

**Determination of physico-chemical properties of different oils**

**Density**

The density of the microscopically selected plant oils/blended mixtures was measured by specific gravity bottles (relative density bottles) method. Experiments were conducted at room temperature.

**Acid value**

Acid value of different oils was determined as reported earlier (Barkatullah *et al.*, 2012).

**Viscosity**

The Viscosity of the microscopically selected plant oils/blended mixtures were measured at the shear rate by using a D.V-iii ultra-programmable rheometer (Brookfield Engineering Labs, U.S.A). The viscosity was determined by using different spindle no.18, 21 and 27 and different shear rates, ranging from 1.7-4.65s⁻¹. Nzikou *et al.*, (2007) method was used to measure the different oil viscosities.

**Refractive index**

The refractive index of microscopically selected immersion oils/blended mixtures oils were determined by using refractometer based on the principle of the critical angle using diffused daylight.

**Non-dryness**

The non-dryness was determined by performing a test at 30 C for 24 hours in accordance with JIS C 2201 Test of evaporation amount of "electrical insulating oils" (Fujioka *et al.*, 2009) and was evaluated based on the following two levels. Good (0): evaporation amount of less than 1% by mass Poor (x): evaporation amount of 1% by mass or more.

**Stability**

Microscopically selected plant oils/ blended mixtures were kept at 25 and37°C to investigate the stability of oils as an immersion oil. All the five plant oils/ blended mixtures were tested for the microscopic examination of malaria parasite at the intervals of five weeks.

**Gas chromatography- mass spectrometry (GCMS) analysis of plant oils blended mixtures**

GC-MS analysis for the separation and identification of plant oils /blended oils was performed at Department of Chemistry, Forest Research Institute, Dehradun. The sample was analyzed using a Shimadzu GC-2010 gas chromatograph coupled to a QP 2010 mass-selective detector with capillary column BP-20(30m in length, 0.25mm i.d, and 0.25μm in thickness). GCMS conditions were used as described by Dua *et al.*, (2013). Different peaks of gas chromatographic analysis were identified using NIST, WELY and SZTERP software library of mass spectra.

**Validation**

**In –house validation**

Four microscopist from National Institute of Malaria Research, field unit, BHEL, Haridwar were selected to examine the blood slides for malaria parasite using mixture 6. Each microscopist was provided 10 malaria positive blood slides with *Plasmodium falciparum* infection having ring/gametocyte and 10 *Plasmodium vivax* infections with ring, gametocytes and trophozoite stages.
External validation

Each participating institute was provided mixture 6 as immersion oil for the evaluation report. It is suggested to examine minimum of ten blood slide each with Plasmodium falciparum and Plasmodium vivax infection having different stages of parasites for evaluation of particular oil.

Results and Discussion

The maximum % yield was obtained from Junglans regia, (51), followed by Papaver somniferum (45), Michalia champaca (37), Terminella bellerica (33), Aleurites fordii (32) Sapindus mukrossi (31), Pongamia pinnata (31), Moringa oleferia (29), Ceiba pentandra (28), Sterculia foetida (28), Jatropha curcas (28), Ricinus communis (25), Terminelia chebulla (22), Bauhinia purpurea (16), Minusops elengi (15), Bauhinia retusa (15), Pinus roxburghii (15), Pithecolobium dulci (14), Olea europaea (14), Psorlyia coryfolia (8). Cesalpinia boundecella (2), Cymbopogon martini (1.2), Cedrus deodara (1.2), Melaleuca alternifolia (1.1), Cymbopogon citratus (1.1), Eucalyptus citriodora (0.9), Valeriana jatamansi (0.87), Cymbopogon nardus (0.8), Pogostemon cablin (0.53), Mentha piperita (0.19), Ocimum basilicum (0.09), and Pelargonium gravelens (0.09).

A large variation in the extraction yield of oil from the plants may be due to variation in the plant parts, seasonal variation, and environmental factors for the growth of a particular plant.

Based on microscopic examination of malaria parasite only five plants oils namely Ocimum basilicum, Pogostemon cablin, Papaver somniferum, Ricinus communis and Valeriana jatamansi showed satisfactory results as immersion oil for the examination of malaria parasites.

Performance of different blended mixtures was in order of Mixture 6, Mixture 10, Mixture 4, Mixture 5, Mixture 8, Mixture 7, Mixture 11, Mixture 9, Mixture 1 and Mixture 2 respectively. Results revealed that the mixture 6 consisted of twenty-five percent of each four of Pogostemon cablin, Ocimum basilicum, Papaver somniferum and Ricinus communis (25:25:25:25) showed the best oil to be used for microscopic examination of malaria parasites. Figure 1 and 2 represented microscopic identification of Plasmodium falciparum ring and schizont stages of mixture 6 and synthetic immersion oil respectively.

The physiochemical properties required for an immersion oil including density, viscosity, refractive index and acid value of five oils namely as Ocimum basilicum, Pogostemon cablin, Papaver somniferum, Ricinus communis and Valeriana jatamansi and 11 blended mixtures were are summarized in table 2. Density, refractive index, viscosity and acid values from Ocimum basilicum, Pogostemon cablin, Papaver somniferum, Ricinus communis, Valeriana jatamansi oils and blended mixture 1, mixture 2, mixture 3, mixture 4, mixture 5, mixture 6, mixture 7, mixture 8, mixture 9, mixture 10 and mixture 11 ranged 0.916 – 0.967, 1.478 – 1.501, 1.2970 and 0.25 – 20.0 respectively. Refractive index and density of the Ocimum basilicum of present study was similar to the reports of Hussain et al., (2008) who investigated the density and refractive index 0.95-0.97g/cm3 and1.4995-1.5045 respectively. Dev et al (2011) reported basil oil refractive index and density as 1.515 and 0.928 g/cm3 respectively. Pogostemon cablin oil density, refractive index and acid value were 0.919g/cm3, 1.499 and 0.25. Parganiha (2012) found that the density, refractive index and acid value of the Pogostemon cablin were 0.951-0.991, 1.5089, and1.68-3.93 respectively. Similarly, density, refractive
index, viscosity and acid value of *Papaver somniferum* oil were 0.921, 1.478, 65.4 and 20.3 respectively. Ozcan and Atalay (2006) reported refractive index and acid value of the poppy seed oil as 1.4773 and 1.0-3.2 respectively. *Ricinus communis* density, refractive index, viscosity and acid value were 0.916, 1.481, 970, and 0.3 respectively which were similar to earlier reports (Deligiannis et al., 2009), *Valeriana jatamansi* density, refractive index, viscosity and acid value were 0.934, 1.491, 5.6, and 0.35 respectively.

Immersion liquids for light microscopy including Cargille immersion oils, currently comply by the ISO/German DIN (2015) which is specified for synthetic immersion oils. As per literature, no specifications have been given for the immersion oil obtained from the plants. Density, refractive index, acid value and viscosity of blended mixture 6 plant oil were 0.945, 1.488, 33.6 and 2.36 respectively which are slightly vary from the DIN specification due to the fact that mixture 6 is generated from the plants source. It is to point out that British Pharmacopeia has approved cedar wood plant oil as immersion oil and density, refractive index, viscosity and acid values were similar to the values found for mixture 6 oil.

Stability of oils from *Ocimum basilicum, Pogostemon cablin, Papaver somniferum, Ricinus communis, Valeriana jatamansi* and mixture 1, mixture 2, mixture 3, mixture 4, mixture 5, mixture 6, mixture 7, mixture 8, mixture 9, mixture 10 and mixture 11 were represented very good criteria for the non-drying less than 1% variation in weight.

GC-MS analysis of patchouli oil revealed presence of alpha-Copaene (79.13%), trans-Caryophyllene (13.74%) besides 3, 3, 5, 5', 3', 5', 5'-Octamethyl-DI-(.DELT) and 7-Ethylidene-6b 7, 8, a-tetrahydrocyclobut[a]. Luo et al., (1999) identified pogostone (30.99%) in stems, 21.31% in leaves, patchouli alcohol (10.26%) in stems, 37.53% in leaves, deltaguaiene, alpha-guaiene (2.27%) in stems, 6.18% in leaves, seychellene (1.56%) in stems, 1.99% in leaves, alpha-patchouline, aciphyllene, and trans-caryophyllene (4.92%) in stems, 6.75% in leaves as main constituents from leaves and stem of Pogostemonis (Patchouli) while Cheng et al., (2010) identified patchouli alcohol and pogostone as chemical markers. GC-MS study of Patchouli oil carried out by Micheal (1992) and Daniel (2006), Baby et al., (2007) showed presence of 74 compounds namely Patchouli alcohol, 3-octanone, Benzaldehyde, dimethyl phenol, octanoic acid, Pogostol, 4-methyl-pentanoic-acid,b-elemene, epiguaipyridine, Ombuine, nor-patchoulinol, a-bulnesene, b-patchouline, epoxy caryophyllene e, p-vinyl-phenol, seychellene, a-bulnesene oxide, b-pinene, Eugenol, pachypodol, nor-patchoulinol, a- bulnesone, Bulnesol, eugenol, cinnamic aldehyde, patchouli-alcohol, patchouli pyridine, a-guaiene, Cadinene, g-patchoulene, Patchouli pyridine, Methylchavicol, a-guaiene oxide, Camphene, guaiacol, pentanoic-acid, Limonene, a-patchouline, caryophyllene, guaipyridine, phenol, Pinene, a-pine, caryophyllene-oxide, heptanoic-acid, pogostol, p-methoxycinnamaldehyde, anethole, cinnamaldehyde, humulene, pogostone, 1,10-epoxy-alphabetunese, anisaldehyde, cis-2-pentylcyclopropylcarboxylic acid, Apigenin, Limonene, rhamnetin, 1-alpha, 5-alpha-epoxy-alpha-guaiene, Apigenin.
cycloseychellene, nonanoic-acid, seychellene, 1-beta,5-beta-epoxy-alpha-phauguiene, apigenin-7-o-beta-d-(-6'-p-coumaroyl)-glucoside, d-patchoulen, nordemyropatcho ulol,tannin,2-methyl-butyric-acid,apigenin-7-o-beta-glucoside; benzaldehyde, dehydracetic-acid, norpatchoulenol, trans-2-pentylcyclopropylcarboxylic-acid,2-methylhexanoic-acid, azulene, dhelwangan, o-cresol.

Present study revealed that Linalool L and Estragole as chemical marker for the identification of basil oil. Estragol, linalool, methyl eugenol, geraniol, methyl cinnamate, bergamotene, α-cubebene, germacrene D, β-elemene, 1,8-cineole, methyl cinnamate, α-cadinol and limonene are considered as the main constituents and chemotypes of basil from different parts of the world (Koba et al., 2009, Zhang and coworkers (2009), Abdelrahman et al., (2009) and Vani and coworkers (2009). Jirovetz and Buchbauer (2001) found a high level of linalool (71.4%) in O. basilicum essential oil from Bulgaria. According to Gurbuz et al., (2006), linalool (41.2%) was the main compound, identified in the hydro-distilled O. basilicum essential oil from Turkey. Hassanpouraghdam et al., (2010) reported Linalool in leaves as major component in Basil oil.

GC-MS analysis of poppy seed revealed presence of hexadecanoic acid ethyl ester (1.40), 9-octadecanoic acid (3.55), 9,12,15-octadecatrienoic acid, (2-phenyl-1,3-dioxolan-4-YL) methyl E (0.22), hexadecanoic acid phenyl methyl ester (30.73), ethyl(9Z,12Z)-9,12-Octadecadienoate (11.23),9,12-Octadecadienoic acid (Z, Z) (41.59), and Heneicosyl pentafluoro-
propionate (7.82). Ozcan and Atalay (2006) reported linoleic acid high in all the samples, and varied between 52.6 to 71.50% while Rahimi et al., (2011) studied opium poppy seed oils from Turkey where the main fatty acids were linoleic (56.4- 69.2%), oleic (16.1-19.4%) and palmitic acids (10.6- 16.3%). Singh et al., (1990) stated poppy seeds contained up to 50% oil and Indian cultivars have high levels of oleic and linoleic acids. Similarly, some researchers have reported that the linoleic (C18:2), oleic (C18:1) and palmitic acids (C16:0) are major fatty acids in the poppy seed oil (Erinc et al., 2009, Singh et al., (1990), Sener et al., (1999), Bezakova et al., (1994), Bajpai (1999), Bozan and Temelli (2008) and Luthra and Singh, 1989).

Table.1 Scientific name, family and common names, raw plant parts used for extraction and method of extraction the plants under investigation

| S.no. | Scientific name | Family | Common name | Raw material used for oil extraction | Method of extraction |
|-------|-----------------|--------|-------------|--------------------------------------|----------------------|
| 1.    | Cymbopogon martini | Graminae (Poaceae) | Palmarosa | Leaves | Steam Distillation |
| 2.    | Pogostemon cablin | Lamiaceae (Labiatae) | Patchouli | Leaves | Steam Distillation |
| 3.    | Melaleuca alternifolia | Myrtaceae | Tea Tree | Leaves | Steam Distillation |
| 4.    | Cedrus deodara | Pinaceae | Cedar wood | Cone | Steam Distillation |
| 5.    | Pelargonium graveolens | Geraniaceae | Geranium | Leaves | Steam Distillation |
| 6.    | Cymbopogon nardus | Graminae (Poaceae) | Citronella | Leaves | Steam Distillation |
| 7.    | Pinus roxburghii | Pinaceae | Pine | Needle | Steam Distillation |
| 8.    | Ocimum basilicum | Lamiaceae (Labiatae) | Basil | Leaves | Steam Distillation |
| 9.    | Mentha piperita | Lamiaceae (Labiatae) | Peppermint | Leaves | Steam Distillation |
| 10.   | Cymbopogon citratus | Graminae (Poaceae) | Lemongrass | Leaves | Steam Distillation |
| 11.   | Eucalyptus citriodora | Myrtaceae | Lemon gum | Leaves | Steam Distillation |
| 12.   | Jatropha curcas | Euphorbiaceae | Ratanjyot, Physic Nut | Seed | Solvent Extraction |
| 13.   | Pongamia pinnata | Leguminosae, (Fabaceae) | Karanja oil | Seed | Solvent Extraction |
| 14.   | Terminelia chebulla | Combretaceae | Harra | Seed | Solvent Extraction |
| 15.   | Terminellia | Combretaceae | Bahera, | Seed | Solvent Extraction |
| **Plant** | **Family** | **Common Name** | **Part** | **Extraction** |
|-----------|------------|-----------------|---------|---------------|
| belerica   | Sapindaceae| bastard myrobalan Reetha, soapnut | Seed    | Solvent Extraction |
| sapindus mukrossi | Sapindaceae | Mauksari | Seed | Solvent Extraction |
| mimosops elengi | Sapindaceae | Devanagari | Seed | Solvent Extraction |
| bauhinia retusa | Caesalpiniaceae | Orchid tree, purple bauhinia | Seed | Solvent Extraction |
| bauhinia purpurea | Leguminosae | Seed | Solvent Extraction |
| pithecolobium dulci | Fabaceae | Madras thorn, jungle jalebi | Seed | Solvent Extraction |
| ceiba pentandra | Malvaceae | Kapok | Seed | Solvent Extraction |
| moringa oleferia | Moringaceae | Senjana | Seed | Solvent Extraction |
| sterculia foetida | Malvaceae | Wild almond | Seed | Solvent Extraction |
| junglans regia | Juglandaceae | Walnut | Seed | Solvent Extraction |
| michalia champaca | Magnolia | Champaca | Seed | Solvent Extraction |
| cesalpinia bourdecella | Caesalpiniaceae | Gray Nicker, Kantkarej Tung | Seed | Solvent Extraction |
| aleurites fordii | Euphorbiaceae | Tung | Seed | Solvent Extraction |
| olea europaea | Oleaceae | Olive | Seed | Solvent Extraction |
| papaver somniferum | Papaveraceae | Opium poppy | Seed | Solvent Extraction |
| ricinus communis | Euphorbiaceae | Castor | Seed | Solvent Extraction |
| valeriana jatamansi | Caprifoliaceae | Jatamansi | Root | Solvent Extraction |
| psoralea corylifolia | Fabaceae | Babchi | Seed | Solvent Extraction |
Table.2 Physicochemical properties of plant oils and blended mixtures used as immersion oil for microscopic examination of malaria parasites

| S. No | Name of the oils/ Mixtures                  | Density (g/cm³) | Refractive Index (RI) | Viscosity at 5 RPM (cP) | Acid Value (mg/KOH) |
|-------|---------------------------------------------|-----------------|-----------------------|-------------------------|---------------------|
| 1     | Ocimum basilicum (Basil)                    | 0.937           | 1.501                 | 1.2                     | 0.57                |
| 2     | Pogostemon cablin (Patchouli)               | 0.919           | 1.499                 | 9                       | 0.25                |
| 3     | Papaver somniferum (Poppy seed)             | 0.921           | 1.478                 | 65.4                    | 3.49                |
| 4     | Ricinus communis (Castor)                   | 0.961           | 1.481                 | 970                     | 0.3                 |
| 5     | Valeriana jatamansi (Jatamansi)             | 0.934           | 1.491                 | 5.6                     | 0.49                |
| 6     | Mixture 1                                  | 0.927           | 1.492                 | 13.8                    | 3.76                |
| 7     | Mixture 2                                  | 0.916           | 1.488                 | 29                      | 3.36                |
| 8     | Mixture 3                                  | 0.937           | 1.491                 | 19.4                    | 2.76                |
| 9     | Mixture 4                                  | 0.947           | 1.494                 | 31.2                    | 0.48                |
| 10    | Mixture 5                                  | 0.936           | 1.491                 | 102                     | 0.31                |
| 11    | Mixture 6                                  | **0.945**       | **1.488**             | **33.6**                | **1.70**            |
| 12    | Mixture 7                                  | 0.967           | 1.478                 | 206                     | 3.15                |
| 13    | Mixture 8                                  | 0.922           | 1.485                 | 18.6                    | 0.29                |
| 14    | Mixture 9                                  | 0.932           | 1.485                 | 16.8                    | 0.29                |
| 15    | Mixture 10                                 | 0.935           | 1.478                 | 16                      | 0.5                 |
| 16    | Mixture 11                                 | 0.919           | 1.478                 | 24.4                    | 1.45                |

Table.3 Result of In-house validation

| S. No | Examiner Code | Total slides examined | Observations |
|-------|---------------|-----------------------|--------------|
|       |               |                       | Very Good ++++ (%) | Good +++ (%) |
| 1     | NC            | 20                    | 16 (80)       | 4 (20)       |
| 2     | MR            | 20                    | 15 (75)       | 5 (25)       |
| 3     | AR            | 20                    | 16 (80)       | 4 (20)       |
| 4     | AK            | 20                    | 17 (85)       | 3 (15)       |
**Fig.1a & b** Microscopic identification of *Plasmodium falciparum* ring stage using synthetic immersion oil and microscopic identification of *Plasmodium falciparum* ring stage using Mixture 6 immersion oil

![Figure 1a](image1a.png) ![Figure 1b](image1b.png)

**Fig.2a & b** Microscopic identification of *Plasmodium falciparum* schizont stage using synthetic immersion oil and Microscopic identification of *Plasmodium falciparum* schizont stage using Mixture 6 immersion oil

![Figure 2a](image2a.png) ![Figure 2b](image2b.png)
Table 4 Result of external validation

| S. No. | Examiner Code | Total slide examine | Observations |
|--------|---------------|---------------------|--------------|
|        |               |                     | Very Good ++++ (%) | Good +++ (%) |
| 1      | HIM           | 20                  | 17 (85)       | 3(15)       |
| 2      | SG            | 20                  | 16(80)        | 4(20)       |
| 3      | MET           | 20                  | 18(90)        | 2(10)       |
| 4      | GH            | 20                  | 17(85)        | 3(15)       |

HIM: Himalayan Hospital Jolly Grant, Dehradun, SG: Shri Guru Ram Rai Hospital Dehradun, MET: Metro Hospital SIDCUL, Hardwar and GH: Government Hospital Hardwar.

GC-MS analysis of mixture 6 clearly identified presence of alpha copane and trans-Caryophyllene as a chemical marker for the presence of patchouli oil, Linalool L and Estragole as chemical marker for basil oil and phenyl methyl ester, and 9,12-Octadecadienoic acid for the presence of poppy seed oil besides other minor components peaks as stated in results. It is to note that no peak was detected for castor oil due to lack of sample character and detection procedure.

Summary of in-house validation of blended mixture 6 carried out by four microscopists is given in table 3. It is to point out that all four microscopists have classified mixture 6 as very good immersion oil (range 67-81%) and good (19-33%) for the examination of malaria parasites while the results of external validation by four different hospitals/laboratory are given in table 4. Certifications by four Head/Incharge of the hospitals clearly revealed that the developed plant oil mixture 6 possessed very good property as immersion oil for the examination of malaria parasites and may develop as an alternative of synthetic immersion oil.

Present study was aimed to find out plant oil based immersion oil as an alternative to synthetic immersion oil which showed airborne contact dermatitis, burning pruritus and urticarial-like lesions on the face and forearms and carcinogenic, a hazard to the human environment. A blended mixture consisted of twenty-five percent of Pogostemon cablin, Ocimum basilicum, Papaver somniferum and Ricinus communis (25:25:25:25) showed very good results for microscopic examination of malaria parasites.

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