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Pharmacopeial Standard Development, HPTLC. Fingerprinting And Physicochemical Research Studies of Polyherbal Classical Drug Raughan-E-Muqawwi-E-Dimagh - A Brain Booster Medicated Oil

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ABSTRACT

Medicated Brain booster oil drug Raughan-e-Muqawwi-e-Dimagh a classical formulation used in the treatment of Brain Weakness, Insomnia, Amnesia and other Neurological disorders from since ancient time. Three drugs samples of REMED taken into these studies which 3 Batches were prepared B1,B2 and B3. and developed from applied SOP. on the basis of authenticated classical texts and literature of standard methods. The quality control & quality assurance studies were conducted in accordance to the WHO., AOAC., IPC. and UPC., approved guidelines. The physicochemical data showed Extractive, %, w/v. (100), that the drug samples contained Petroleum ether(60-80°C), Acid value (69.12), Iodine value (23.99), Peroxide value(14.86), Saponification Value (248.29), Unsaponifiable matter,% (2.36),Refractive index (1.4657),Weight per ml.(gm.) (0.9004),Test for presence of Arachis oil, Cotton seed oil and Mineral oil both are found negatives and various bioactive phytochemical screening examined were assessed in REMED. HPTLC. studies of alcohol extracts of different Batches B1.B2 and B3 of REMED. Classical formulation showed various spots at 366nm (UV. region),under exposed to Vinyl - Sulfuric acid reagent. derivatized obtained with equate, best separation using selected suitable solvent system of mobile phase. The quality control studies results revealed the absence of hazardous and toxic contamination from the drug samples. Moreover the obtained research studies data and comparative screening will provide the referential supportive information in the development of pharmacopeial standard monographs, identification of classical formulation, reinvestigation, quality assurance and pharmaco- vigilance and validation of the Quality standard of REMED classical drug providing the

quality medicine to needful public mass of the World.

Keywords : Raughan-e-Muqawwi-e-Dimagh (REMED), Anti-amnesic, Medicated Brain booster oil, development of pharmacopeial standards, physicochemical, comparative screening, quality control and assurance.

I. INTRODUCTION

REMED a Anti-amnesic ,Medicated Brain booster oil based classical formulated of drug used in the treatment of Zof-e-Dimagh (Brain Weakness), Sahart (Insominia), Nisyan (Amnesia) and other Neurological disorders from since ancient time. The Standardization and Validation of ASU herbal Drugs is not an easy challenge as various factors influence the bio efficacy and reproducible therapeutic effects. In order to obtain assured quality based herbal products, care through pharmaco-vigilance and utmost care has to be taken right from the beginning i.e. proper identification of plants, season and area of collection, grading, drying, extraction, purification process and rationalizing the combination in the case of poly-herbal drugs. (Patel *et al.*,2006), The subject of standardization of herbal drugs is massively wide and deep. There are many seemingly contradictory theories on the subject of herbal medicines and its relationship with human physiology and mental function. (Yadav *et al.*,2011) Validation of pharmacopoeial standards by experimentation and observations provides a set of characteristics to a particular herbal medicine. Therefore, Scientific Validation of Unani Formulations is an important tool used in the standardization process. (Kunle, 2012), Historically herbal medicines have played a significant role in the management of both minor and major medical illness (Bahuguna *et al.*,2014). All medicines, either synthetic or plant origin, have to fulfill the basic requirements of safety and efficacy. (EMEA, 2005; Anonymous, 2002). It is also observed that about 80% of the world population is using TM medicinal plants primarily in the developing countries for treating different diseases, due to their safety, efficacy, cultural acceptability and lesser side effect. It is important for herbal formulations to get the quality assurance by the conventional system of medicine, so that they can be justified, accepted and must be beneficial for the ailing masses of the mankind (Naaz *et al.*, 2021). The quality assurance and quality control of herbal crude drugs and formulated products are important in justifying their acceptability in modern system of medicine. Hence it is required to conduct the research on drugs standardization and product validation to provide effective, curable and safe drugs to the needy mass suffering from various ailments.(Sagar *et al.*,2023a &b; 2020)

The research studied drug Raughan-e-Muqawwi-e-Dimagh is commonly known as Anti-amnesic, Medicated Brain booster oil drug REMED frequently used as since ancient time as traditional and integrated medicines in Asian, European and Arabian countries. The drug reported to have Actions like Muqawwi-e-Hefiza(Memory enhancer), Daf-e-Nisyan(Anti-amnesic, Munawwim(Hypnotic/Soporific) and therapeutically used in Zof-e-Dimagh (Brain Weakness), Sahart (Insominia), Nisyan (Amnesia). REMED is golden yellow oil with characteristic of its own smell, viscous liquid formed preparation with agreeable, aromatic odour (Sagar, *et al.*,2015a; Anonymous.1992;1996) REMED was reported bioactive phytochemical constituents as sterol contented and composition - cholesterol max.0.5,Brossicasterol- max.0.1, Campesterol -

max.4.0,) - min.93, Total+ Camp sterol, D-7 Stigma sterol- max.0.5,Beta-stio sterol (≤Stigma sterol sterols mg/kg. Virgin refined and olive oil-min.1,000,Fatty acid composition (%mm of methyl esters) as Myristic acid C 14:0-max.0.05, Palmitic acid C16:0- 7.5 to 20.0, Heptadecanoic acid C 17:0 - max.0.3,Steric acid C18:0- 0.5 to 5.0, Oleic acid C 18:1 - 55 to 83.0, Linoleic acid C 18:2 - 3.5 to 21.0, Arachidic acid C 20:0- max.0.6,Gadoleic acid C 20:1-max.0.4, Behenic acid C 22: 0-max.0.2, Lignoceric C 24:0-max.0.2,Saturated fatty acids in 2 position % - Virgin olive oil -max.1.5 (EU-1.3),Olive oil - max.1.8 (EU-1.5), Crude pomace oil -max. 2.2(EU-2.0), Un-saponifiable material (gm/kg)-Olive oil- max.1.5 gm/kg., Pumace oil- max.30 gm/kg. (Purity standards for olive oil IOOC.- Anonymous 1992; Anonymous 1996; Anonymous 1999;EU. Anonymous 2002; Anonymous 2003).[5-8,12,17] Free acidity%- 1.5 to 2.0(In virgin olive oil), Peroxide value mg O2 /kg.- 15 to 20(In virgin 0.25≤ 2.6(In virgin oil), UV. Absorbency at 270nm. ≤olive oil), UV. Absorbency at 232nm. 0.1(In olive oil),≤ 0.2(In virgin oil) and ≤to 0.90(In virgin oil), H20 and volatiles %- 0.05(In olive oil).≤ 0.1(In virgin oil) and ≤Insoluble impurities% present in used, formula composition ingredient of Olive / Zaitoon seeds . (Sagar, *et al.*,2015a &b ;Anonymous. 2005;2003;Harwood et al.,2000; Kirisakis et al.,1998; Anonymous.1992) Commonly bioflavonoid such as flavanol include catechins epicatechin and procyanidins, and flavonol includes 3-hydroxyflavone backbone glycosides, particularly isorhamnetin-3- O-glucoside, isorhamnetin-3-O-rutinoside, naringenin-7-O-glucoside and kaempferol-3- Orutinoside present in used, formula composition of Badam seeds. (Singh *et al.*, 2022) ω-3 fatty acid, phytoestrogenic-lignans (secoisolari ciresinol diglucoside-SDG), phenols, flavonoids, sterols, proteins, antioxidants as well as various soluble and insoluble fibers present in used, formula composition of Alsi / Katan seeds. (Akter *et al.*, 2021) glycosides, terpenoids and flavonoids phenolics constituents including 3-p-coumaroylquinic, caffeic, ferulic and sinapic acids, kaempferol sophoroside-glucosides and organic acids etc active phytochemical constituents present in used, formula composition of Sarson / Mustard seeds. (Newaz *et al.*, 2018) Kunjad (Sesame oil)- *Sesamum indicum* functional components such as sesamin, sesamolin, sesamol, sesaminol, sesamolin phenol, and other lignan's -like active ingredients protein, Lipids, Rich source of Vitamins-E, Active nutrients minerals- K (525.9 mg/100 g), P (516 mg/100 g), Mg (349.9 mg/100 g), Na (15.28 mg/100 g), Fe (11.39 mg/100 g), Zn (8.87 mg/100 g), and Mn (3.46 mg/100 g), Carbohydrates etc. active phytochemical constituents present in used, formula composition of Kunjad / Sesame seeds. (Wei *et al.*, 2022; Pathak *et al.*, 2014; Ahirwal et al.,2013; Elleuch *et al*,.2007; Anonymous,2003;2000a) The seed oil contains steroid alkaloids and bright natural coloring matter, celapanine, celapanigine, celapagine, celastrine, and paniculatine are the important alkaloids active phytochemical constituents present in used, formula composition ingredient of Malkangani seeds. (Vaibhav, *et al.*,2018) and contained various bioactive phytochemical constituents such as Glycosides phonolic compounds terpenoids., flavonoids, sterols, flavones, flavanols, tannins, trace amount of alkaloids, proteins, triterpene glycosides, saponins, gum, resins have been confirmed in identification tests in the examined REMED classical formulated drug showed in Table -I respectively. (Sagar *et al.*, 2015a; 2015b; 2015c; 2015d; Ahirwal *et al.*,2013)

II. METHODS AND MATERIAL

Ingredients used for preparation: The raw drug formulation is composed of the following mention ingredients for classical formulation REMED :

S. No.	Unani Name	Botanical/ English Name	Part Used	Qty.	Reference
1.	Maweez	*Vitis vinifera* L.	Fruit	250 g	UPI, Part I, Vol. IV, p.96
2.	Kishmish	*Vitis vinifera* L.	Fruit	250 g	UPI, Part I, Vol. III, p.50
3.	Anjeer	*Ficus crica* L.	Fruit	250 g	UPI, Part I, Vol. II, p.11
4.	Maghz-e-Pista	*Pistacia vera* L.	Seeds	125 g	UPI, Part I, Vol. III, p.78
5.	Tukhm-e-Khiyar	*Cucumis sativus* L.	Seeds	125 g	UPI, Part I, Vol. V, p.46
6.	Maghz-e-Tukhm-e-Kharpoza	*Cucumis Melo* L.	Seeds	125 g	Appendix, APR 2008-09
7.	Tukhm-e-Karafs	*Apium graveolens* L.	Seeds	10 g	UPI, Part I, Vol. II, p. 93
8.	Anisoon	*Pimpinella anisum* L.	Fruit	10 g	UPI, Part I, Vol. II, p. 9
9.	Ustukhuddus	*Lavandula stoechas* L.	Inflorescence	20 g	--
10.	Badam	*Prunus amygdalus* Batsch	Seeds	20 g	UPI, Part I, Vol. II, p.17
11.	Babuna	*Matricaria chamomilla* L.	Flower	20 g	UPI, Part I, Vol. II, p.39
12.	Badiyan	*Foeniculum vulgara* Mill.	Fruit	10 g	UPI, Part I, Vol. I, p.15
13.	Mastagi	*Pistacia lentiscus* L.	Gum Resin	20 g	UPI, Part I, Vol. V, p.50
14.	Filfil siyah	*Piper nigrum* L.	Fruit	20 g	UPI, Part I, Vol. IV, P. 38
15.	Sumbul-ut-teeb	*Nardostachys jatamansi* DC.	Root	10 g	UPI, Part I, Vol. I, p.84
16.	Gul-e- Surkh	*Rosa damascene* L.	Flower	105 g	UPI, Part I, Vol. III, p.31
17.	Gul-e-Banafsha	*Viola odorata* L.	Flowers	20g	UPI, Part I, Vol. II, p.41

Drug preparation :

The classical polyherbal formulated drug REM was prepared in there different batches at Laboratory scale as per the ingredients composition and guidelines of NFUM, Part-IV, Ist Edition and NFUM. Part-IV, IInd Edition. The required quantities of all the ingredients were taken of pharmacopoeial quality. All the ingredient were cleaned and dried, ingredients no.1-7 under shade to removed the moisture if any, Soaked the ingredient no.1-7 for 24 hours in 1.5 litre of purified water. Boiled the contented until the water remains half; add ingredient no.8 and mixed thoroughly. Boiled the contented again until water evaporated completely. Allowed the contented to cool to room temperature. Filtered the contented through muslin cloth and kept the filtrate and stored the prepared drug in a tightly closed food grade glass / plastic container free from moisture. (Anonymous, 2022; 2006 and Hkm. Jalaluddin Amrohvi, Qarabadeen Jalali,1897 AD.)

Pharmacopoeial standard parameters:

Identification:

Pharmacopoeial research studies such as organoleptic characters, microscopical, macroscopical and physicochemical, TLC/HPLC., quality control and quality assurance parameters were carried out

1. **Organoleptic Evaluation:** Organoleptic evaluation refers to evaluation of formulation by colour, odour, taste, texture etc., using the sensory organs of our body. The organoleptic characters of the drugs samples were carried out based on the method described by Siddique *et al.* (1995).

2. Powder Microscopy : Since the drug contains more than ingredients (total 8 ingredients), the powder microscopic study is not required. (Wallis, 1987; Johansen, 1940).

Phytochemical screening : Phytochemical screening of REMED has been carried out and confirmed, examined under the standards test methods of Alkaloids, investigated and tested by Dragendorff's and Mayer's tests, Flavanoids, investigated and tested by Shinoda's test, Flavanols, Sterols. and Glycosides, investigated and tested by Molisch test. Triterpenoids investigated and tested by Liebermann-Burchard test. Phenols and Lignans, amino acid, over all mentioned active phytochemical constituents screening have been investigated and found (++) Highly active. Carbohydrates investigated and tested by Anthrone test and Fehling test, and in protein test over all mentioned phytochemical constituents screening have been investigated and found (+) Moderately active in condition. and in Seponins, Steroids investigated and tested by Liebermann-Burchard test and Salkowski reaction, Tannins test over all tested and mentioned phytochemical constituents screening have been investigated and found not deducted or absent. (Sagar *et al.*, 2015c; 2015d; Ahirwal *et al.*,2013)

Physicochemical screening: Physicochemical screening of classical formulated polyherbal drug REM has been carried out under the following parameters like Petroleum C) Extractive, %, w/v., Acid value, Iodine value, Peroxide value,°ether (60-80 Unsaponifiable matter, Refractive Index, Weight per ml. (gm.), Test for presence of Arachis oil, Cotton seed oil, Sesame and Mineral oil (all are negatives) were carried out as per IPC. approved standard methods (Sagar,*et al.*,2015a&b; Anonymous 2006c;2000; 1991; 1989;1987a &b;1986).

3. **TLC/HPTLC finger printing analysis:** The Shacked vigorously 2g drug in 40ml of *Ethanol* in a separating funnel. Allow to separate the Ethanol layer from the oil. Collect the Ethanol extract in a beaker and apply as such on plate precoated with silica gel 60 F254 (E. Merck) (Saxena and Yadav,1983). TLC/HPTLC finger print studies of alcohol extracts of the drug were carried out using aluminum plate precoated with silica gel 60 F254 (E. Merck) with CAMAG Linomat IV sample applicator. The chromatograms of both the extracts were taken using the solvent systems Toluene - Ethyl acetate (9.3: 0.7) for alcohol extracts respectively. The plates were dried at room temperature and observed the spots at various wavelengths. The plates were scanned at 254 nm and to record the finger print spectrum after that same plates were visualized at UV-366 nm and derivatized with spraying of vanillin-sulphuric acid reagent and heated at 105° C till appeared coloured spots. (Sagar *et al.*,2023a&b; Khan *et al.*,2022 ; Sagar *et al.*,2020 and Wagner and Blad, 1996; Sethi, 1996).

4. **Quality assurance and quality control parameters:**

Estimation of microbial load: The microbial load viz. total bacterial count (TBC), total fungal count (TFC), Enterobacteriaceae, *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aurous* were estimated as per standard method (Anonymous.,2008;2006 b&c;2005b;2003;2002;2000b; Anonymous, WHO ,2005a;2002;2000a;1998;1997).

Estimation of Heavy metals: The method used for the analysis of heavy metals like lead, cadmium, mercury and arsenic as per Guidelines of WHO. Heavy metals were analyzed by Atomic Absorption Spectroscopy (Anonymous, 1998) and AOAC (Anonymous, 2005). Details of the Instrument and operating parameters Thermo Fisher M Series, 650902 V1.27 model Atomic Absorption Spectrometer (AAS) was used for the analysis. The operating parameters: Lead and Cadmium: Instrument technique - Flame technique; wavelength (Lead) - 217 nm; wavelength (Cadmium) - 228.8 nm; slit width - 0. 5 mm; lamp current (Pb) - 4.0 mA; lamp current (Cd) - 3.0 mA; carrier gas and flow rate - air and acetylene, 1.1 L/min; sample flow rate - 2 ml/min. Mercury: Instrument technique - Cold vapour technique; wavelength - 253.7 nm; slit width - 0. 5 mm; lamp current - 3.0 mA; carrier gas and flow rate - argon, 1.1 L/min; sample flow rate - 5ml/ min. Arsenic: Instrument technique - Flame vapor technique; wavelength - 193.7 nm; slit width - 0. 5 mm; lamp current - 6.0 mA; carrier gas and flow rate - acetylene, argon, 1.1 L/min; sample flow rate - 5ml/min. The Hallow cathode lamp for Pb, Cd, Hg and As analysis were used as light source to provide specific wavelength for the elements to be determined. (Sagar *et al.*,2023a&b; Anonymous.,2008;2006 b&c;2005b;2003; 2002;2000b; Anonymous, WHO ,2005a;2002;2000a;1998).)

Analysis of Aflatoxins: Aflatoxins B1, B2, G1 and G2 were analyzed as per Official Analytical Methods of the American Spice Trade Association (ASTA), 1997. Aflatoxins were estimated by Kobra cell techniques using Agilent HPLC and CAMAG or Anchrom HPTLC instruments as per the method ASTA (Sagar *et al.*,2023a&b;2020;Anonymous, 1998;1997).

Details of instrument and operating parameters High Performance Liquid Chromatography (Thermo Fisher) and CAMAG or Anchrom HPTLC were used for the analysis of aflatoxins. Column - Ultra C18, 250 X 4.6 mm, 5 µm particles; Mobile phase: Water: Acetonitrile: Methanol (65: 22.5: 22.5); Flow rate: 1 ml/min; Temperature: 35º C; Detector: Fluorescence detector at 360 nm; Injection run: 20 µl (Aflatoxins B1, B2, G1 and G2 mixture and test samples). (Sagar *et al.*,2023a&b; 2020; Anonymous.,2008;2006 b&c;2005b;2003;2002;2000b; Anonymous, WHO ,2005 a;2002;2000a;1998 ;1997).

Analysis of pesticide residue: The method used for the analysis of pesticide residues was as per AOAC (Anonymous, 2005). Pesticide residues were analyzed by Gas Chromatography Mass Spectra (GC-MS) (Instrument- Thermo Scientific, Model - TSQ9000 or Agilent), detector-mass selective detector or Triple Quadrupole mass analyzer detector, column specification-DB-5MS or TG-5MS, carrier gas - helium, flow rate - 1ml/min, column length - 30 m, internal diameter - 0.25 mm, column thickness - 0.25 ìm). The usage of ASU. herbal products along with higher safety margins, WHO has taken necessary steps to ensure quality assurance and quality control parameters with the modern techniques and application of suitable standards. (Sagar *et al.*, 2023 a&b; 2020; Meena *et al.*, 2016) ; Anonymous.,2008;2006 b&c;2005b;2003;2002;2000b; Anonymous, WHO ,2005 a;2002;2000a;1998 ;1997).

III. RESULTS AND DISCUSSION

Organoleptic and identification characteristics of the formulated medicated herbal drug Raughan-e-Mukhtara (REM) indicated that the drug has been brownish yellow oil with its own smell characteristic in colour oily viscous liquid having

aromatic odour, drug samples collected from Pharmacy Section of RRIUM., Chennai, T.N. State (Under CCRUM., Ministry of AYUSH., Government of India. The physico-chemical analysis such as Petroleum ether (60-80^0) Extractive, %, w/v. (100.0), Acid value (69.12), Iodine value (23.99), Peroxide value(14.86), Saponification Value (248.29), Unsaponifiable matter,% (2.36),Refractive index (1.4657),Weight per ml.(gm.) (0.9004), Test for presence of Arachis oil, Cotton seed oil, and Mineral oil have been found negatives. The obtained physicochemical screening data of the drug samples were showed in Table II. respectively. (Sagar, *et al.*, 2015a&b; Wei *et al.*,2022; Anonymous,2008;2007a,b,c & d; Anonymous,2006 b&c; Anonymous,2005a&b; Anonymous,2003; 2000a&b;1998;1997;1991; 1987a&b) In Examined and investigated REM drug samples with IH. standard which have been indicated absence of any mixed foreign adulterated, spurious oily materials and also indicated that the drugs samples is free from any microbial load, aflatoxins Heavy Metals (Pb, As, Cd and Hg) and Pesticide Residues contamination as per WHO/AYUSH permissible limits . HPTLC / Thin Layer Chromatography after applied of above said extraction processed, concentrated the filtrate up to 10 ml (approx.) on water bath and applied the Ethanol extract on precoated aluminum TLC plate of silica gel 60 F_{254}using HPTLC automatic sample applicator. Develop the plate solvent systems Toluene - Ethyl acetate (9.3: 0.7). Allowed the plate to dried in air and examined under UV (366nm). Observed a major fluorescent spots at R_f0.42 (blue). Dip the plate in 1% Vanillin-Sulphuric acid reagent followed by heating at 105^{0}C for 5 minutes and examine under visible light. Observe 05 major spots at R_f 0.36(pinkish purple), 0.42, 0.62, 0.65 (bluish grey) & 0.90(violet). showed in Fig.-1 and Table-III respectively. (Sagar, *et al.*,2023 a&b;2020a,b,&c; 2015a&b; Khan *et al.*,2022 ; Wagner and Blad, 1996 ; Sethi, 1996 and Anonymous,2008;2007a,b,c&d; Anonymous,2006b&c)

The classical formulated TM drug REM is brownish yellow oil with characteristic of its own smell characteristic in colour oily viscous liquid, and having aromatic odour, it's the mixed fixed oil obtained from seeds of Kunjad (Sesame oil)- *Sesamum indicum* functional polar phyto constituents components such as sesamin, sesamolin, sesamol, sesaminol, sesamolin phenol, and other lignan-like active ingredients protein, Lipids, Rich source of Vitamins-E, Active nutrients minerals- K (525.9 mg/100 g), P (516 mg/100 g), Mg (349.9 mg/100 g), Na (15.28 mg/100 g), Fe (11.39 mg/100 g), Zn (8.87 mg/100 g), and Mn (3.46 mg/100 g), as well as in bioactive phytochemical constituents presence, examined, and confirmed as Carbohydrates active fatty acids Free acidity etc. and contained various constituents such as Glycosides phonolic compounds terpenes, terpenoids., flavonoids, sterols, flavones, flavanols, tannins, trace amount of alkaloids, proteins, triterpene glycosides, saponins, have been confirmed in identification of phytochemical and Screening tests in the examined of REM classical formulated drug showed in Table -I respectively. (Sagar *et al.*, 2015c; 2015d; Ahirwal *et al.*,2013) other formulated ingredients both mixed well, addible fixed oil finally obtained with all formulated classical ingredients used in applied process of SOP formula composition as per authenticated classical texts or literature basis. (Wei *et al.*, 2022; Pathak *et al.*, 2014; Elleuch *et al*,.2007), Herbal based medicated oil REM has been based or a medium which has been prepared by used various raw ingredients in different forms, During processed the medicine used various formulated ingredients based compositions of herbal raw drugs in a particular formula along with herbal medicated oil Tukkm-e-Kunjad (Sesame seeds) used as a fixed oil, preparation made with the ingredients in the authenticated formulation as per the composition processed applied.

REM has been a classical formulation widely used in the treatment of Warm-e-Mafasil(Arthritis), Mohallii-e-Warm (Anti-Inflammatory), Daf-e-Alam (Analgesic), Auja (Relief in Nervine Pain), and other Neurological disorders medicated herbal oil based drug as a Indian classical and proprietary medicine since ancient time and most of the popular Unani classical drug sold in the market of the European, Arabian countries, REM has been sold along with the brownish yellow oil with its own smell characteristic in colour oily viscous liquid formed drug. (Anonymous, 2022; 2006 and Hkm. Jalaluddin Amrohvi, Qarabadeen Jalali,1897 AD.; Sagar, *et al.*,2015a&b;Anonymous. 2003; Wei *et al.*, 2022; Pathak *et al.*, 2014 Elleuch *et al*,.2007),

High Performance Thin Layer Chromatography (HPTLC.) finger printing analysis, Quality control, Quality assurance parameters of the brownish yellow oily viscous liquid REM., HPTLC. Studies of alcohol extractive REM concentrated of both B1, B2 and B3 drug samples, prepared and taken from Pharmacy Section of RRIUM., Chennai, T.N State (Under CCRUM., Ministry of AYUSH., Government of India) showed and indicated the similarity of the drug. The R_f values of both the REM samples were showed in Figure-1 and Table III, respectively. Quality control and Quality assurance parameters: Quality Control and Quality Assurance studies of quality parameters were assessed and performed as per IUPAC., IOOC., AOCS, ISO, WHO., IPC., UPC., (API.UPI.) and AOAC. Standard methods, The microbial load (cfu/gm.) and heavy metals (ppm.) toxic contamination estimation were found within the permissible limits due to control of qualifier which indicated the absence of any adulterantive mixed material as well as drugs samples free from hazardous contaminated appearance showed in Table- IV. & V. respectively. The Aflatoxins and Pesticide Residues toxic contaminated estimation were found not detected from the drug samples which is showed in Table-VI. and Table-VII respectively, Freshly Prepared drug also preserved or Store in cool and dry place in tight closed containers free from moisture , protected from light. (Sagar, *et al.*,2023 a&b;2020a,b,&c; 2015a&b; Khan *et al.*,2022 ; Kunle *et al*,2012; Yadav *et al*, 2011 ; Patel *et al*,2006; Wagner and Blad, 1996; Sethi, 1996 and Anonymous,2008;2007a,b,c&d; Anonymous,2006 b&c ; Anonymous,2005a&b; Anonymous,2003; 2000 a&b;1998;1997;1990;1989)

IV. CONCLUSION

The classical formulation REM was standardized for the first time gives rise to various validated standard parameters. The results obtained may provide as a reference standard and could be beneficial for consideration of efficacious polyherbal Unani formulation for future research endeavors and studies for pharmacopoeial standard monograph development work. REM a classical formulation used in the treatment of Warm-e-Mafasil(Arthritis), Mohallii-e-Warm (Anti-Inflammatory), Daf-e-Alam (Analgesic), Auja (Relief in Nervine Pain), and other Neurological disorders, oil drug as mentioned in the authenticated ancient classical Unani, NFUM Vol-IVth. Edition Ist and IInd Classical Pharmacopeial literatures or text basis. The Pharmacopeial Standard development, HPTLC. Fingerprinting and Physicochemical, Phytochemical screening research studies of a polyherbal classical formulated drug Raughan-e-Mukhtara investigated data have been showed in the REM drug samples B1, B2 and B3. The examined samples were authenticated and can be used as the standard Classic Unani formulation. The resulted data of quality control parameters revealed that the drug samples taken for present studies were found free from toxic substances like toxic microbial load, heavy metals, aflatoxins and Pesticide Residues hazardous substance, It is an economic drug and the efficacy of the drug can be used as a traditional alternative medicine, medicated oil drug. Moreover the obtained

research studies data and comparative physicochemical and active phytochemical constituents, HPTLC. Fingerprinting identification, purity, safety screening of investigated drug will be provide the referential supportive information in the development of AYUSH/ IH standard monographs, identification, reinvestigation, quality assurance and pharmacovigilance of the drug, further studies are expected to advance comprehend confirmation the in-vivo detailed mode of action upon animal model of its dynamic active polyherbal phytochemical composition based classical, relief in Nervine Pain and other Neurological disorders medicated oil medicine as potent therapeutics and to completely reveal its preventive and healing potentials.

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Table-I: Test of Pharmacological and Phytochemical test screening:

(Sagar *et al.*, 2015c; 2015d; Ahirwal et al.,2013)

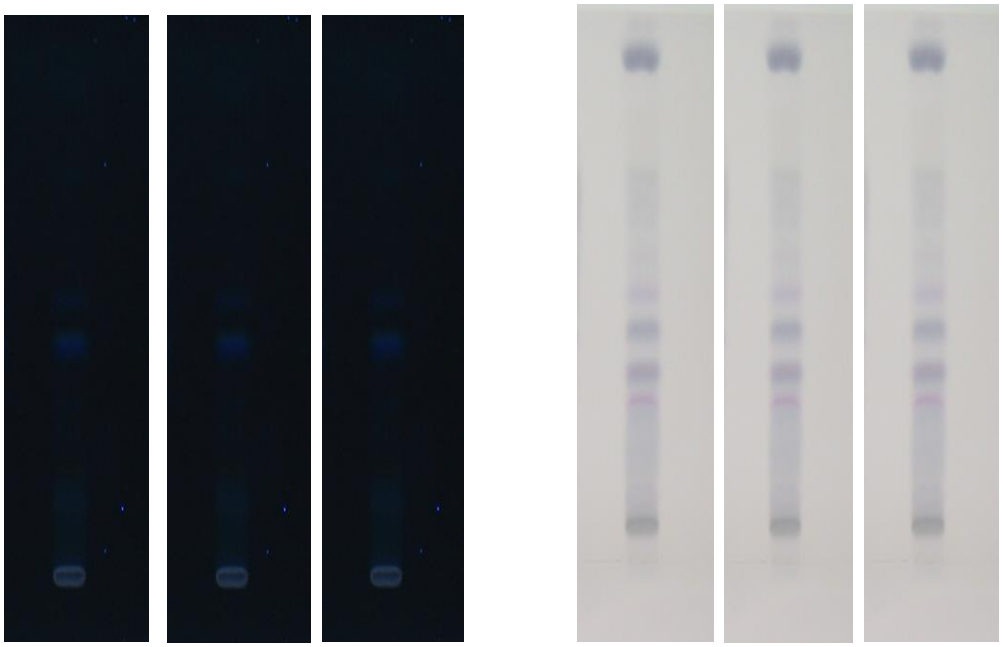
Phytochemical Test	Sample B-1	Sample B-2	Sample B-3
Alkaloids:			
a.- Dragendorff's test	++ve	++ve	++ve
b.- Mayer's test	++ve	++ve	++ve
Carbohydrates:			
a,- Anthrone test	+ve	+ve	+ve
b.- Fehling test	+ve	+ve	+ve
Flavanoids :			
Shinoda's test	++ve	++ve	++ve
Flavanols:	++ve	++ve	++ve
Sterols:	++ve	++ve	++ve
Glycosides			
Molisch test	++ve	++ve	++ve

Triterpenoids			
Liebermann-Burchard test	++ve	++ve	++ve
Phenols	++ve	++ve	++ve
Lignans	++ve	++ve	++ve
Seponins	- ve	- ve	- ve
Steroids			
a.- Liebermann-Burchard test	- ve	- ve	- ve
b.- Salkowski reaction	- ve	- ve	- ve
Tannins	- ve	- ve	- ve
Proteins	+ve	+ve	+ve
Amino acids	+ve	+ve	+ve
Resins	+ve	+ve	+ve
++ Highly active, + Moderately active, ± Trace, - Absent			

Table-II: Physico-chemical parameters:

(Sagar, *et al.*, 2015a&b; Wei *et al.*,2022; Anonymous,2008;2007a,b,c & d; Anonymous,2006 b&c; Anonymous,2005a&b; Anonymous,2003;2000a&b;1998;1997;1991;1987a&b)

Parameter Analyzed	**REMED**			
	Sample, B-1	Sample, B-2	Sample, B-3	Mean Value
Petroleum ether (60-80⁰) extractive (%)-	100.0	100.0	100.0	100.0
Acid value -	68.51	69.18	69.67	69.12
Iodine value -	23.95	23.68	24.34	23.99
Peroxide value -	14.73	15.20	14.66	14.86
Saponification Value -	247.73	248.83	248.31	248.29
Unsaponifiable matter. (%) -	2.29	2.49	2.30	2.36
Refractive index -	1.4656	1.4661	1.4653	1.4653
Weight per ml (g) -	0.8991	0.9026	0.9002	0.9006
Test for presence of Arachis oil -	Negative	Negative	Negative	Negative
Cotton seed oil -	Negative	Negative	Negative	Negative
Mineral oil -	Negative	Negative	Negative	Negative



B-I **B-II** **B-III** **B-I** **B-II** **B-III**

UV366 nm Visible Light

(After derivatization)

Solvent System: Toluene : Ethyl acetate (9.3: 0.7)

Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch – III

Figure 1: TLC/HPTLC Photo of Alcohol Extract

Table-III: *Rf* values of REMED alcohol extract:

(Sagar, *et al.*,2023 a&b;2020a,b,&c; 2015a&b; Khan *et al.*,2022 ; Wagner and Blad, 1996; Sethi, 1996 and Anonymous,2008;2007a,b,c&d; Anonymous,2006b&c; Anonymous,2005a&b; Anonymous,2003; 2000 a&b;1998;1997)

Solvent system	*Rf* Values		
	254nm	366nm	VS reagent
Toluene : Ethyl acetate (9.3 : 0.7)	No Spot Deducted	0.15(Blue)	0.30(Pink)
		0.41(Blue)	0.34(Pinkish purple)
		0.49(Blue)	0.42(Bluish grey)
		--	0.49(Light pink)
		--	0.89(Violet)

Table-IV: Analysis of Microbial load

(Sagar, *et al.*,2023 a&b;2020a,b,&c; 2015a&b; Khan *et al.*,2022 ; Kunle *et al*,2012; Yadav *et al*, 2011 ; Patel *et al*,2006 and Anonymous,2008;2007a,b,c&d; Anonymous,2006b&c; Anonymous,2005a&b; Anonymous,2003; 2000 a&b;1998;1997)

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Total Bacterial Count	700 cfu/gm	10^5cfu/gm
2	Total Fungal Count	300 cfu/gm	10^3cfu/gm
3	*Escherichia coli*	Absent	Absent
4	*Salmonella typhai Spp.*	Absent	Absent
5	*Staphylococcus aurous*	Absent	Absent

Table-V: Estimation of Heavy Metals

(Sagar, *et al.*,2023 a&b;2020a,b,&c; 2015a&b; Khan *et al.*,2022 ; Kunle *et al*,2012; Yadav *et al*, 2011 ; Patel *et al*,2006and Anonymous,2008;2007a,b,c&d; Anonymous,2006b&c; Anonymous,2005a&b; Anonymous,2003; 2000 a&b;1998;1997;1990;1989)

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Lead	3.72ppm	10ppm
2	Cadmium	0.04ppb	0.3ppm
3	Mercury	Not detected	1.0ppm
4	Arsenic	0.09 ppm	3.0ppm

Table-VI: Estimation of Aflatoxins

(Sagar, *et al.*,2023 a&b;2020a,b,&c; 2015a&b; Khan *et al.*,2022 ; Kunle *et al*,2012; Yadav *et al*, 2011 ; Patel *et al*,2006and Anonymous,2008;2007a,b,c&d; Anonymous,2006b&c; Anonymous,2005a&b; Anonymous,2003; 2000 a&b;1998;1997;1990;1989)

S.N0.	Parameter Analyzed	Results	WHO Limit
1	Aflatoxine, B1	Not detected	0.5ppm
2	Aflatoxine, B2	Not detected	0.1ppm
3	Aflatoxine, G1	Not detected	0.5ppm
4	Aflatoxine, G2	Not detected	0.1ppm

Table-VII: Estimation of Pesticide Residues

(Sagar, *et al.*,2023 a&b;2020a,b,&c; 2015a&b; Khan *et al.*,2022 ; Kunle *et al*,2012; Yadav *et al*, 2011 ; Patel *et al*,2006and Anonymous,2008;2007a,b,c&d; Anonymous,2006b&c; Anonymous,2005a&b; Anonymous,2003; 2000 a&b;1998;1997;1990;1989)

S.N0.	Parameter Analyzed	Results	WHO Limit (mg/kg)
1	DDT (all isomers, sum of ρ, ρ'-DDT, α, ρ' DDT, ρ, ρ'-DDE and ρ, ρ'-TDE (DDD expressed as DDT)	Not detected	1.0
2	HCH (sum of all isomers)	Not detected	0.3
3	Endosulphan (all isomers)	Not detected	3.0
4	Azinphos-methyl	Not detected	1.0
5	Alachlor	Not detected	0.02
6	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	Not detected	0.05
7	Chlordane (cis & tans)	Not detected	0.05
8	Chlorfenvinphos	Not detected	0.5
9	Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	Not detected	0.05
10	Endrin	Not detected	0.05
11	Ethion	Not detected	2.0
12	Chlorpyrifos	Not detected	0.2
13	Chlorpyrifos-methyl	Not detected	0.1
14	Parathion methyl	Not detected	0.2
15	Malathion	Not detected	1.0
16	Parathion	Not detected	0.5
17	Diazinon	Not detected	0.5
18	Dichlorvos	Not detected	1.0
19	Methidathion	Not detected	0.2
20	Phosalone	Not detected	0.1
21	Fenvalerate	Not detected	1.5
22	Cypermethrin (including other mixtures of constituent isomers sum of isomers)	Not detected	1.0
23	Fenitrothion	Not detected	0.5
24	Deltamethrin	Not detected	0.5
25	Permethrin (sum of isomers)	Not detected	1.0
26	Pirimiphos methyl	Not detected	4,0