INTERNATIONAL JOURNAL OF UNANI AND INTEGRATIVE MEDICINE

E-ISSN: 2616-4558
P-ISSN: 2616-454X
https://www.unanijournal.com
IJUIM 2023; 7(2): 28-33
Impact Factor (RJIF): 6.3
Peer Reviewed Journal
Received: 28-03-2023
Accepted: 09-05-2023

Uzma Siddiqui
Department of Ilmul Advia, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Shamshad Alam
Department of Ilmul Advia, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Corresponding Author:
Uzma Siddiqui
Department of Ilmul Advia, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Standardization of Unani polyherbal formulation (Zanjabeel, Luk-e-maghsool, suddab and Marzanjosh)

Uzma Siddiqui and Shamshad Alam

DOI: https://doi.org/10.33545/2616454X.2023.v7.i2a.238

Abstract
Background: Standardization of herbal drugs is very important for quality control. It improves the safety and efficacy of drug to provide the best possible medicine to the society. Unani polyherbal Formulation comprising of Tukhm-e-Suddab (*Ruta graveolens*), Zanjabeel (*Zingiber officinale*), Luk-e-maghsool (*Laccifer lacca* / *Coccus lacca*) and Marzanjosh (*Origanum vulgare* L.) mentioned by Hakim Najmul Ghani in his treatise *khazainul Advia* for the treatment of obesity was selected for standardisation. This formulation was used in a powdered form. The main aim of this study was to standardize UPF, on the basis of organoleptic characters and physico-phytochemical analysis.
Methods: The drugs were cleaned, dried in shade and powdered by passing through sieve # no. 80 as per the method described in National Formulary of Unani Medicine. This Safoof formulation was evaluated using physicochemical tests: extractive value, alcohol and water soluble, ash value, LOD at 105°C, pH and TLC. Statistical analysis used: Mean ± SEM.
Result: Organoleptic characters of the formulation are brown in colour, aromatic, tangy taste and coarse texture. Physicochemical parameters displayed water soluble extractive (14.96 ± 0.145), alcohol soluble extractive (20.30 ± 0.264), total ash (7.83 ± 0.166), acid insoluble ash (2.16 ± 0.600), water soluble ash (5.66 ± 0.928), LOD at 105oC (9.73 ± 0.260), pH of 1% and 10% solution were 8.23 ± 0.033 and 6.23 ± 0.145 respectively. Phytochemical qualitative analysis displayed presence of alkaloids, carbohydrate, glycosides, tannins, sterol, starch, phenols, saponin, fixed oil and amino acid.
Conclusion: The standardization of this formulation was done and the data obtained would be used as a standard for future reference.

Keywords: Standardization, Luk-e-maghsool, Marzanjosh, Suddab, Zanjabeel, thin layer Chromatography, UPF (Unani Polyherbal Formulation)

Introduction

People are becoming more aware of the negative consequences of synthetic modern medication, and there has been a shift in the global trend of choosing herbal therapy instead of synthetic medicine. It is crucial to standardise herbal formulations in order to evaluate the quality, purity, and effectiveness of medicines. In order to support their acceptance in the modern medical time, the quality evaluation of herbal formulations is crucial [1]. The absence of strict quality control standards for herbal medicines and their formulations is one of the biggest issues, the herbal sector is now dealing with. The World Health Organisation (WHO) recognises the role and significance of medicinal plants for the provision of public health services in developing countries. As a result, guidelines have been developed to aid member nations in developing national policies on traditional medicine and in researching their potential benefits, including evaluation, safety, and efficacy [2]. The manufacture of standardised, therapeutically effective formulations plays a significant role and has a lot of potential in Indian traditional medicine, especially the Unani system of medicine (USM). The study of medicinally significant plants and their preparations is necessary. There is a need to explore the medicinally important plants and their formulations. This can be achieved only if the herbal products are analysed and evaluated using sophisticated modern techniques of standardization [3].

Tukhm-e-Suddab (*Ruta graveolens*), Zanjabeel (*Zingiber officinale*), Luk-e-maghsool (*Laccifer lacca / Coccus lacca*), and Marzanjosh (*Origanum vulgare* L.) are polyherbal powder formulations used in the Unani System of Medicine for the treatment of obesity and hyperlipidaemia [4]. According to the literature review, this formulation is a Non Pharmacopoeial Unani F

current study was carried out to establish the quality control standards of Safoof using multiple standardisation parameters like morphological characteristics (colour, aroma, size, form, and taste), fluorescence analysis, physicochemical studies (extractive values, moisture content, ash values, pH values), solubility test (alcohol soluble, water soluble) and thin layer chromatographic studies.

Materials and Methods

Procurement of raw drugs

Ingredients polyherbal powder formulation was procured from the genuine/registered drug seller and further identified and authenticated by pharmacognosist of D/O Ilmul Advia, A.M.U, Aligarh. Unani polyherbal Formulation comprising of Tukhm-e-Suddab (*Ruta graveolens)*, Zanjabeel (*Zingiber officinale)*, Luk-e-maghsool (*Laccifer lacca* / *Coccus lacca*) and Marzanjosh (*Origanum vulgare* L.) [Table- 1 and Fig-1] All the drugs were first cleaned and dried in shade and powdered by passing through sieve # no. 80. The formulation was prepared as per the method described in UPI / National Formulary of Unani Medicine. [4]

Table 1: Ingredients of Safoof

S. No.	Name of the drugs	Botanical Names	Family	Parts Used	Doses
1.	Luk-e- Maghsool	*Laccifer lacca*	Coccedae	Resin	2 gm
2.	Marzanjosh	*Origanum vulgare*	Labiatae	Leaves & flowering tops	9 gm
3.	Tukhm-e-Suddab	*Ruta graveolens*	Rutaceae	Seed	7 gm
4.	Zanjabeel	*Zingiber officinale*	Zingiberaceae	Stem	7 gm

Fig 1: Sample of A. *Laccifer lacca* B. *Origanum vulgare* C. *Ruta graveolens* D. *Zingiber officinale*

Organoleptic evaluation

The Organoleptic evaluation refers to evaluation of the *Safoof* formulation by colour, odour, taste, appearance and texture [5].

Physico-chemical evaluation

The successive extractive values of the test drug in different solvents *viz*. Petroleum ether, Chloroform, Acetone, Ethanol, and distilled water (Aqueous) were determined with the help of Reflux method (Successive method). The heat was applied for 6 hours for each solvent on a heating mantle. [6] Physicochemical analysis such as the total ash, acid insoluble ash, water soluble ash, were calculated according to the methods described by the Afaq *et al*, 1994; Jenkins *et al*., 2008; Anonymous, 1968). water soluble extract, pH of 1% and 10% aqueous solution and loss of weight on drying at 105 °C, moisture content by Dean and Stark Method were also done [7, 8].

Phyto-chemical evaluation

Quantitative analysis: The qualitative analysis of different chemical constituents present in test drug was carried out according to the scheme proposed by Bhattacharjee and Das (1969). Different tests for Alkaloids, Glycosides, flavonoids, tannins, proteins, amino acids, resins etc were also carried out [7, 9] [Table-11].

Thin layer chromatography: The Thin Layer Chromatography is one of the important parameters used for detecting the adulteration and determining the quality of the drugs. Rf values of various spots appeared in different solvent systems were noted in order to set the standard. The Rf value may be used as an indicator of the number of constituents present in test drug. The TLC profile may also be used as the basis for quantitative analysis of the active constituents present in a drug.

Result

Table 2: Organoleptic Description of Test Drug

Parameters	Test drug
Color	Brown
Appearance	Powder
Texture	Coarse
Taste	Tangy
Smell	Aromatic

Table 3: Successive Extractive values

S. No	Petroleum ether%	Diethyl Ether%	Chloroform %	Acetone %	Alcohol %	Aqueous %
1	0.88	0.56	1.02	0.43	0.48	4.55
2	1.27	0.41	1.30	0.68	0.58	5.01
3	1.02	0.28	0.85	0.58	0.61	5.95
Mean± S.Em	1.05 ± 0.114	0.41 ± 0.080	1.05 ± 0.131	0.56 ± 0.072	0.55 ± 0.039	5.17 ± 0.412

Table 4: Ash values

Test drugs	Total ash %	Water soluble ash%	Acid insoluble ash%
1	8	7.5	1
2	7.5	5	2.5
3	8	4.5	3
Mean ± S.Em	7.83 ±0.166	5.66 ±0.928	2.16 ±0.600

Table 5: Percentage solubility of test drug:

Test drugs	Water Soluble Content%	Alcohol soluble Content%
1.	15.2	20.8
2.	15.0	19.9
3.	14.7	20.2
Mean ± S.Em	14.96± 0.145	20.30± 0.264

Table 6: pH values of 1% and 10% solution of powder

S. No	1% Solution	10% Solution
1.	8.3	6.2
2.	8.2	6.5
3.	8.2	6.0
Mean ±S.Em	8.23 ± 0.033	6.23 ± 0.145

Table 7: Percentage of loss on drying and Moisture content of test drug

S. No	Loss on drying%	Moisture content %
1.	9.3	1.02
2.	10.2	0.91
3.	9.7	0.87
Mean ± S.Em	9.73 ± 0.260	0.93 ± 0.044

Table 8: Bulk density and tapped density of test drug.

S. No	Bulk density	Tapped density
1.	0.66	0.40
2.	0.83	0.42
3.	0.82	0.40
Mean ± S.Em	0.80 ±0.048	0.40±0.032

Table 9: Fluorescence analysis of Powder of test drug

S. No	P.D + Chemical reagents	Day light	UV Short	UV long
1	Powdered drug+conc. HNO3	Golden orange	Yellowish green	Brown
2	Powdered drug + conc.H2SO4	Coffee black	Blackish brown	Black
3	Powdered drug + conc. HCL	light Brown	Yellowish Green	Algae green
4	Powdered drug + Dilute HNO3	Straw Yellow	Algae Green	Greenish Brown
5	Powdered drug + Dilute H2SO4	Light Yellow	Light Green	Greenish Brown
6	Powdered drug + Dilute HCL	Light Yellow	Light Green	Muddy
7	P.D + 2%iodine solutions	Reddish brown	Dark brown	Black
8	P.D+ Glacial Acetic acid	Light brown	Green	Greenish brown
9	P.D +Glacial Acetic acid +HNO3	Golden orange	Yellowish Green	Brown
10	Powdered drug + NaOH (10%).	Yellowish brown	Blackish Green	Dark Brown
11	Powdered drug + KOH (10%)	Yellowish Brown	Greenish Brown	Dark Brown
12	Powdered drug +Cuso4 (5%)	Light Green	Light Green	Dark Green
13	Dargendroofs reagent	Yellowish green	Olive Green	Dark green
14	Fehling's Solution	Sea Green	Light Green	Sea green
15	Benedicts Solution	Sea Green	Green	Blue
16	Picric acid	Straw Yellow	Yellowish Green	Greenish Brown

Table 10: Fluorescence analysis of test drug

S. No	Extract	Day Light	UV Long	UV Short
1	Pet ether	Straw yellow	Green	Brown
2	Diethyl ether	Yellowish brown	Green	Brown
3	Chloroform	Golden brown	Greenish brown	Brown
4	Acetone	Olive green	Green	Brown
5	Alcohol	Yellowish brown	Green	Greenish brown
6	Aqueous	Dark brown	Coffee black	Black

(B) Phytochemical Analysis

Table 11: Qualitative Analysis

S.no	Chemical Constituents	Test/Reagents	Test drug
1.	Alkaloid	Dragendorff's Reagent Test	Positive
2.	Carbohydrate	Molisch test	Positive
		Benedict's reagent test	Negative
		Fehling solution test	Positive
3.	Flavonoids	Mg ribbon test	Negative
4.	Glycosides	Baker's yeast test	Positive
5.	Tannins	Ferric chloride test Lead acetate test	Negative Positive
6.	Protein	Biurette test Xanthoproteic test	Negative Negative
7.	Starch	Iodine test	Positive
8.	Phenols	Lead acetate test	Positive
9.	Sterol	Liebermann Burchard reaction	Positive
10.	Saponin	Frothing with NaHCO3	Positive
11.	Resin	Acetic anhydride	Negative
12.	Fixed oil/V. oil		Positive
13.	Amino acid test	Ninhydrin test	Positive

Table 12: Thin Layer Chromatography

Extract	Mobile phase	Treatment	No. of Spots	*Rf* Values
Petroleum ether	Benzene: Chloroform (4:1)	UV Short	5	0.09, 0.20, 0.24, 0.31, 0.35
		UV Long	4	0.18, 0.24, 0.32, 0.45
		Iodine vapour	7	0.09, 0.18, 0.22, 0.44, 0.55, 0.79, 0.89
Chloroform	Toluene: Ethyl acetate (8:2)	UV Short	8	0.06, 0.18, 0.27, 0.38, 0.62, 0.67, 0.75, 0.82
		UV Long	6	0.07, 0.18, 0.40, 0.62, 0.74, 0.85
		Iodine vapour	9	0.09, 0.19, 0.38, 0.41, 0.46, 0.65, 0.69, 0.76, 0.82
Acetone	Chloroform: Methanol (9:1)	UV Short	4	0.18, 0.34, 0.72, 0.86
		UV Long	3	0.22, 0.78, 0.85
		Iodine vapour	10	0.10, 0.22, 0.45, 0.64, 0.69, 0.77, 0.81, 0.85, 0.90, 0.96
Alcohol	Chloroform: Acetone (5:1)	UV Short	3	0.42, 0.76, 0.93
		UV Long	4	0.28, 0.50, 0.59, 0.72
		Iodine vapour	8	0.06, 0.11, 0.27, 0.32, 0.42, 0.72, 0.88, 0.96
Aqueous	n-Butanol: Acetic Acid: water (5:1:4)	UV Short	5	0.35, 0.53, 0.65, 0.78, 0.92
		UV Long	7	0.46, 0.59, 0.68, 0.78, 0.82, 0.87, 0.93
		Iodine vapour	8	0.25, 0.40, 0.50, 0.57, 0.75, 0.79, 0.89, 0.93

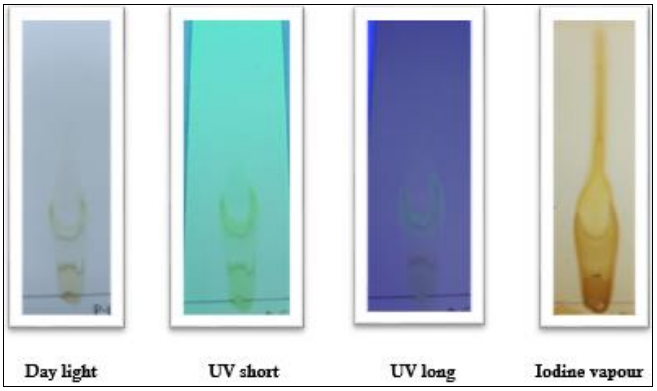

Fig 2: T.L.C Profile of Petroleum Ether Extract of Test Drug. Benzene: Chloroform; (4:1)

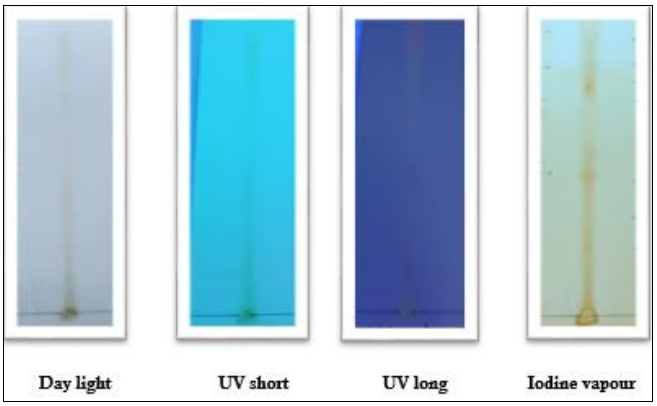

Fig 3: T.L.C Profile of Chloroform Extract of Test Drug Toulene: Ethylacetate; (8:2)

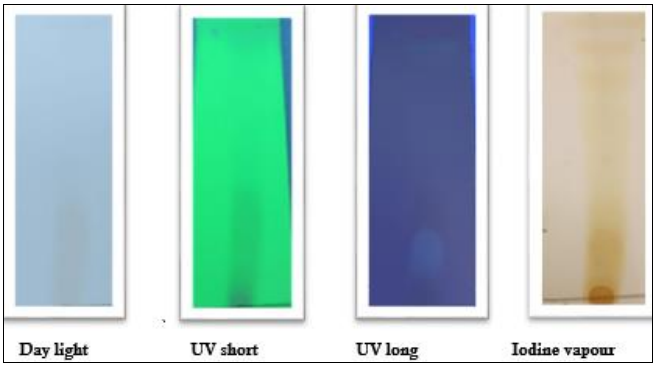

Fig 4: T.L.C Profile of Acetone Extract of Test Drug. Chloroform: Methanol; (9:1)

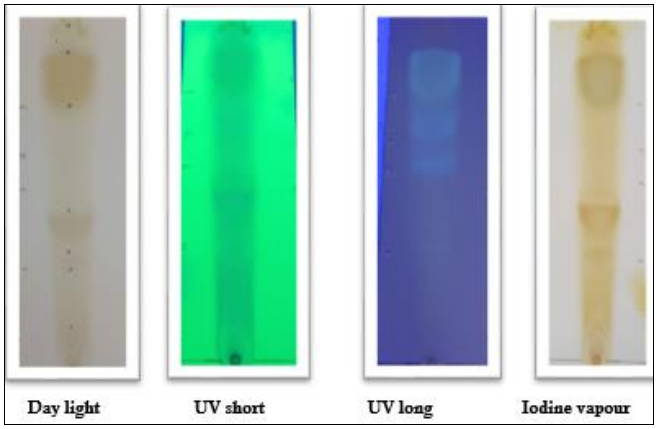

Fig 5: T.L.C Profile of Alcohol Extract of Test Drug Chloroform: Acetone; (5:1)

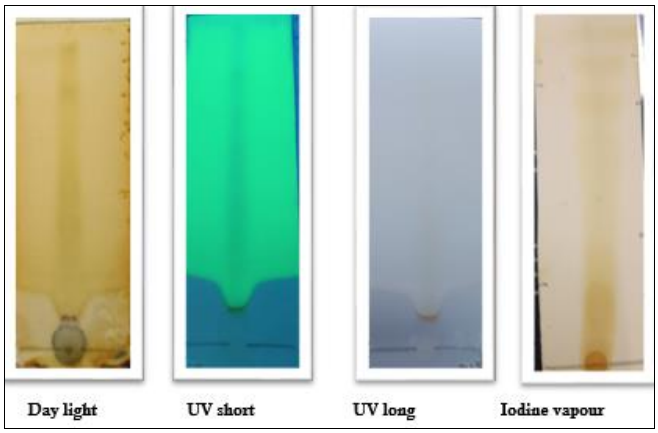

Fig 6: T.L.C Profile of Aqueous Extract of Test Drug. n Butanol: Acetic acid: water (5:1:4)

Discussion

Herbal formulations are considered harmless traditionally and being consumed increasingly by people without prescription. However, some can cause health problems, some are ineffective and some may interact with other drugs. Standardization of herbal formulations is essential in order to assess the quality and purity of drugs, based on the concentration of their active principles. [10] Standardization is an important measure for knowing the quality, purity and for sample identification. It is essential for the correct identity of the materials. Standards for PUF was generated in this work. Finished product of Safoof was brown in

colour as per colour chart (No. PMS 141 of Panton color chart) [11], tangy in taste, aromatic and coarse texture.

The mean values of bulk density and tapped density, were 0.80 ± 0.048, 0.40 ± 0.032 respectively. The mean percentage of loss of weight on drying of Safoof is 9.73± 0.260. It is mentioned that the water content in plant drugs can vary between 6% and 12%. The presence of excessive amount of moisture in plant drugs causes hydrolysis of constituents, growth of bacteria and fungi and biochemical reactions. The pharmacopoeial monographs compulsorily limit for the water content, especially in drugs that have hygroscopic nature, or in which the excessive amounts of water cause deterioration of products [12]. The formulation having less amount of moisture can be expected to be safe for longer time. The ash value is an important parameter in the quality control of herbal drugs. High ash value indicates contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing. The total ash of Safoof was found to be 7.83± 0.166% w/w., which is reasonably low and indicates low contamination. Water-soluble ash is the portion of the total ash content that is soluble in water. It is a good indicator of either previous extraction of water-soluble salts in the drug or incorrect preparation.

Thus, it is the weight difference between total ash and the residue obtained following total ash treatment with water. Safoof's acid insoluble and water-soluble ash values were 2.16±0.600% w/w and 5.66±0.928% w/w, respectively. The pH of the 1% solution was 8.23±0.033, whereas the pH of the 10% solution was 6.23± 0.144. It is somewhat basic in character. Abba *et al*. investigated the relationship between pH and microbial contamination and concluded that a neutral or alkaline pH supports high microbial contamination levels in herbal medicines [14].

The mean percentage of Safoof extractive values that were water-soluble and alcohol-soluble were 14.96±0.145 and 20.30±0.264, respectively.

In petroleum ether, diethyl ether, chloroform, acetone, alcohol, and aqueous, the mean percentage of successive extractive values were 1.05±0.114, 0.41±0.080, 1.05±0.131, 0.56±0.072, 0.55±0.039, and 5.17±0.412, respectively. The extractive value of a medication in a specific solvent is a measure of its purity. Amount of a drug extract in a specific solvent is frequently an appropriate testing tool for a specific ingredient in the medicine. [15]

Alkaloids, glucose, glycosides, tannins, sterol, starch, phenols, saponin, fixed oil, and amino acids were found in the phytochemical qualitative analysis. The therapeutic characteristics of crude pharmaceuticals are mostly attributable to the presence of physiologically active chemical constituents in the drugs, and a smaller percentage of chemical ingredients may result in a reduced therapeutic value.

Even a high proportion may cause an elevated biological reaction that is unpredictable and may be the cause of the adverse effects. As a result, our findings will be useful in forecasting the drug's biological activity and dose response relationship [7, 11]. Thin Layer Chromatography is one of the most essential factors for identifying adulteration and determining medicine quality. The Rf values of numerous spots observed in various solvent systems were recorded in order to establish a standard. The Rf value can be used to estimate the amount of constituents in a test medication. The TLC profile can also be utilised as the foundation for quantitative study of a drug's active components.

The most important barrier in alternative medicine's globalisation is the challenge in ensuring pharmacological homogeneity and quality. This powder dosage form has no pharmacopoeial standards as of yet. Various methods and standards for evaluating powder dose form are stated in various guidelines. Following these rules is required so that data can be created and used to set formulation standards. It can be used for quality control to ensure that medicines are as effective and safe as possible. Further research can be carried out with the use of standards for quantitative estimation and identification of the peak elements.

Conclusion

The present study evaluated a Polyherbal Unani Formulation physicochemically to set its physicochemical standards (loss of weight on drying, pH, total ash, water soluble, acid insoluble ash, extractive values, qualitative data for the presence of organic constituents, and TLC), which may be used as standard monograph for identification and quality control, as well as further evaluation or future research work on standardisation of this formulation.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Satheesh NV, Kumud U, Asha B. Phytochemical screening and standardization of poly herbal formulation for Dyslipidaemia. International Journal of Pharmacy and Pharmaceutical Science. 2011;3(3):235-38.
2. De La Sante OM. Quality control methods for medicinal plant materials, 559, rev.1, Original English, World Health Organisation, 1992, 159.
3. Sriwastava NK, *et al*. Standardization of Ajmodadi churna, a polyherbal formulation. Pharmacognosy Research. 2010;2(2):98-101.
4. Anonymous, National Formulary of Unani Medicine, Government of India, Ministry of Health and Family Welfare (Department of AYUSH), New Delhi, 2011, 105.
5. Protocol for Testing of Ayurveda, Siddha, Unani Medicine, Govt. of India Dept. Of AYUSH Ministry of Health and Family Welfare, Pharmacopeial Laboratory for Indian Medicine Ghaziabad. 2007;40:49-50.
6. Anonymous. British Pharmacopoeia, General Medical Council, Bloomsbury square London. 1968;1227:1276-1277.
7. Afaq SH, Tajuddin Siddiqui MMH.. Standardization of Herbal Drugs AMU Press Aligarh. 1994;8:14-46.
8. Jenkins GI, Kenevel AM, Digangi FE. Qualitative Pharmaceutical Chemistry, CBS Publisher and distributers, Pvt. Ltd., New Delhi, Ed. 6th. 2008;225(229):233-496.
9. Bhattacharjee AK, Das AK. Phytochemical Screening of some Indian Plants, Pointer Publishers, J Crude Drug Res. 1969;9:1408-1412.
10. Yadav NP, Dixit VK. Recent approaches in herbal drug standardization. Int J Integr Biol. 2008;2(3):195-203.
11. Pantone Colour Chart. http://www.american

powder.com/color chart, field color chart and field color family. Accessed on 09-02-18.
12. Júnior JO, Costa RM, Teixeira FM, Barbosa WL. Processing and Quality Control of Herbal Drugs and Derivatives. In: Shoyama Y. (ed) Quality Control of Herbal Medicines and Related Areas. In Tech, Brazil, 2011, 211.
13. Chandel HS, Pathak AK, Tailang M. Standardization of some herbal antidiabetic drugs in polyherbal formulation. Pharmacognosy Research. 2011;3(1):49-56.
14. Abba D, Inabo HI, Yakubu SE, Olonitola OS. Contamination of Herbal Medicinal Products Marketed in Kaduna Metropolis with Selected Pathogenic Bacteria. Afr J Tradit Complement and Altern Med. 2009;6(1):70-7.
15. Jahan N, Afaque SH, Khan G, Ansari AA. Physicochemical studies of the Gum acacia. Nat Prod Radiance. 2008;7:335-7.

How to Cite This Article
Siddiqui U, Alam S. Standardization of Unani polyherbal formulation (Zanjabeel, Luk-e-maghsool, suddab and Marzanjosh). International Journal of Unani and Integrative Medicine. 2023;7(2):28-33