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REVIEW ARTICLE

A Review on Phytochemical Characterization of Kwatha–Ayurvedic Polyherbal Formulation

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Abstract

A paradigm shifts from the conventional approach of a single drug-based system to polyherbal formulations is recently observed in the modern pharmaceutical industry. The rising demand has led to a decrease in the quality and efficacy of herbal medicines. In order to ensure the sustained demand and therapeutic efficacy, it is vitally important to devise proper methods of standardization from the raw drugs to finished polyherbal formulations. However, there is no consensus regarding how these herbal medicines should be standardized. The phytochemical standardization by multiple marker-based fingerprint profiling along with preliminary screening and quantification of marker compounds can assure the reproducibility of the activity of the polyherbal formulations to an extent. The clinically pertinent scientific data to support the asserted synergistic therapeutic effects of Ayurvedic poylyherbal formulations is inadequate. In this review, we discuss the phytochemical standardization and pharmacological studies of kwatha, a major form of herbal remedies in Ayurvedic pharmacology, and the underlying concept of synergism. Kwatha (decoction) is the backbone of self-administered herbal preparations in India. Since the majority of the active principles of plants are water-soluble, herbal decoctions that are usually prepared in water formulate the potent and effective Ayurvedic medicines.

Keywords: Ayurveda, Kwatha, Pharmacological, Phytochemical, Polyherbal Formulation

1. Introduction

The Ayurvedic Materia Medica is a rich heritage of herbal practices with therapeutic uses of over 600 medicinal plants[1]. In Ayurveda, a person's constitutional type needs to be determined prior to the treatment. Drugs are recommended depending on the body type of the patient and on what disease or disturbance of the *doshas* they are laid low with. Ayurveda maintains that a definite relationship exists between illness and an individual's metaphysical state[2]. In Indian traditional medicine, combined plant extracts and plant formulations are selected instead of individual ones. Herbals are included as one of its most potent therapeutic components and are recorded in the literature like Vedas and Samhitas. Ayurvedic herbals are usually prepared in various dosage forms, the majority of which are polyherbal formulations[3,4]. The herbs are usually processed to enhance their absorption as they cannot be easily assimilated in their raw state. The form in which a herb is consumed can have a significant impact on its potency and effect.

Ayurvedic pharmacology uses herbal remedies in many forms like fresh juice (*svarasa*), herbal paste (*kalka*), herbal powder (*churna*), decoction (*kwatha*), distilled waters (*arka*), hot infusion (*phanta*), cold infusion (*hima*), herbal jams and jellies (*paka, leha, avaleha*), medicated wines (*arishta, asava*), tinctures, gugguls (resin extract of *Commiphora mukul),* alkaline extracts (*ksara*), medicated ghee (*siddha ghrita*), medicated oil (*siddha taila*),minerals (*rasasastra*)[5]. Kwatha (decoction) is one amongst the major form of the formulations used in Ayurveda to treat various ailments and diseases. Generally, one part of coarsely ground drug (dry herb) by weight is taken and 16 parts water by volume is added to it. It is then reduced to 4 parts of the original volume of water. Kwatha which is used as internal medicine is directed to be reduced to 8 parts[6].

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Continuous advancement of chromatographic and spectroscopic methods has enabled the separation, identification and structural determination of biologically active compounds[7]. The inherent variability and complex nature of the phytoconstituents along with the use of multiple herbal drug combinations, it is difficult to establish a proper standardization profile for the traditional medicines. The constituents responsible for the alleged therapeutic effects are not thoroughly explained or unknown. Thus the standardization of herbal drugs should encompass the whole process from identification of the raw drugs to its clinical studies[8]. In the current work, phytochemical and pharmacological studies, especially on Ayurvedic formulation kwatha is reviewed. Biomarker standardization along with the synergistic interaction between the phytochemical components was also covered in accordance.

2. Phytochemical Standardization

Standardization of the polyherbal formulations is crucial in order to ensure the safety, efficacy, and quality of traditional medicines used for various ailments. It is impossible to do standardization for a polyherbal formulation without knowing its bioactive ingredients[9]. The major phytochemical standardization methods, such as preliminary phytochemical analysis, fingerprint profiling, and marker compound quantification of kwathas is covered in depth. It has been found that the standardization of only a handful of kwathas was done thoroughly during the extensive search in original and review articles for the current work. The bioactive components of the majority of these kwathas are not yet standardized.

Standardization of varuna kwatha churna and its comparison with the marketed samples of the formulation was carried out on the basis of TLC fingerprinting along with pharmacognostic standardization[10]. Stand ardization of Pathyashadangam kwatha was done by TLC, HPTLC and HPLC methods. TLC fingerprinting of Pathyashadangam kwatha along with raw materials and standards confirmed the presence of all ingredients in the formulation by the similarity in bands and Rf values. The mobile phase Toluene: Ethyl Acetate: Formic acid (2.5: 2.0: 0.5) was suitable for the HPTLC characterization of the kwatha. Andrographis kwatha prepared by only using *A. paniculata*, one of the seven ingredients of the kwatha and three batches of Pathyashadangam kwatha methanol extracts were analysed using HPLC[11,12]. A standard Ciruvilvadi kashayam was made and compared with four marketed samples using phytochemical parameters like comparative fingerprinting by HPTLC and HPLC quantification using Piperine and Gallic acid as standards[13]. The presence and absence of ingredients in a polyherbal formulation Bhunimbadi Kwatha Churna were analysed using 1H-nuclear magnetic resonance to establish their spectral signatures. A comprehensive monograph on quality standards of Bhunimbadi Kwatha Churna was proposed including the HPTLC fingerprinting of methanolic extract of the formulation and its ingredients[14,15]. HPTLC standardization of ethanolic and hydroalchoholic extracts of Panchavalkala Kwatha Churna was done along with LC-MS characterization of methanolic extracts. A monograph on quality standards for Panchaval kala Kwatha Churna was proposed from the data obtained[16].

GC-MS analysis of the Ayruvedic formulation Katakakhadiradi kashayam and Patolakaturihinyadi kwatham was performed and presence of various known bioactive molecules was found along with a few compounds that are not yet characterized[17,18]. The presence of compounds like ergosterol, imidazole and piperine with various biological and supportive therapeutic activities in Patolakaturihinyadi kwatham clearly underpin the medicinal value of the formulation. The GC-MS analysis of Kulathadi kashayam showed the presence of the bioactive molecules like benzoic acid, tetratricontane and octadecanoic acid in abundance[19]. The mechanism of action of the different bioactive molecules in the Ayurvedic medicine is far from being analysed properly due to its complexity. Chemical profiling of Amruthotharam kashayam using a rapid LC-ESI MS method was done and a reverse phase HPLC chromatogram was developed as a chemical fingerprint. The structural identification of the compounds was carried out and some flavanoids, along with phenolic acids like gallic acid, chebulic acid, quinic acid and protocatechuic acid, were identified for the first time in the kwatha[20]. The use of modified dosage forms is proposed to overcome the major concerns of kwatha like shelf life and large dosage of administrations. Lodhradi kashaya made by traditional method and the contemporary spray drier method was validated through FTIR spectroscopy. The Absorbance pattern in the IR spectra indicates that there is no significant variation found between the two[21]. The phytochemical standardization studies on different kwathas are listed in Table 1.

3. Multiple Marker-based Standardization

Polyherbal formulations consist of more than one active component. Identification and quantification of the maximum possible number of potential markers

for a polyherbal formulation are essential to assure its quality. Moreover, the quantification of these marker compounds in the raw materials, along with their formulation, could be used to study the chemical modifications during the Ayurvedic processing. Isolation of the marker compounds from the kwatha is a complex and tedious process. Compared to the other polyherbal formulations like churna, arishta and derived Ayurvedic products, systematic investigation of biomarker-mediated kwatha standardization is much less. The presence of andrographolide in the Pathyashadangamkwath was confirmed by HPLC analysis of the formulation along with the andrographolide standard. The similarity in spectral index and retention time of Pathyashadangamkwath with standard andrographolide furtherindicated thatandrographolide is a potential biomarker for the kwathastandardization[12].

In a new simple UV- spectrophotometric method, spec troscopic fingerprint was developed for the quantitative determination of Glycyrrhetinic acid a major content in the polyherbal formulation Pratishyayghnakwath[48]. Biomarker-based estimation of berberine and gallic acid was used for the standardization of Punarnavashtakkwath using HPTLC. The purity of the bands of berberine and gallic acid in the formulation was confirmed by the comparison of absorption spectra with the standards[49]. Multiple marker based standardization of Phalatrikadikwathachurna using gallic acid, picroside-I and ellagic acid as biomarkers was done by using HPTLC. Phytochemical evaluation of the conventionally prepared kwatha with that of the two market sample of the formulation was also studied. Compared to the genuine sample, the amount of marker compounds was found to be significantly lower in the market samples[50]. Phytochemical standardization of a polyherbal formulation Ayaskrti was carried out along with the quantitative estimation of gallic acid by using HPTLC method. Gallic acid quantification in three batches of ethyl acetate extracts of a polyherbal formulation Ayaskrti was carried out by integrating the different peak area with the standard[51]. Quantitative phytochemical standardization of marketed sample of Balaguloochyadikashayam was carried out with ephedrine as the marker compound[34].

4. Pharmacological Studies

Dhanwantaramkashayam is an Ayurvedicpolyherbal formulation with strong antioxidant activities. The effect of Dhanwantaramkashayam on oxidative radical scavenging activity and lipid metabolism in diabetic rats was analysed. The formulation exerted significant anti hyperlipidemic and antioxidant activity. The results showed that the formulation increased the activity of antioxidant enzymes and reduced different lipid levels[52]. Divya Sarva Kalp Kwatha is a polyherbal formulation used for improving liver function. The hepatoprotective effect of Divya Sarva Kalp Kwatha was studied by using both in-vitro and in-vivo systems in CCl4 induced liver toxicity. Wistar rats and human hepatocytes (HepG2) were used as the study model. HPLC and LC-MS-QToF techniques were used to identify the metabolites present in the kwatha. A total of 68 metabolites were present in the kwatha. The potent hepatoprotective of the Divya Sarva Kalp Kwath was reconfirmed by the study[53]. Balaguluchyadi kashayam is widely used for curing chronic inflammatory conditions. The anti-inflammatory effect of polyherbal formulation was analysed based on its effect in the production of pro-inflammatory cytokines and inhibition of monocyte-macrophage differentiation. The results showed that the formulation reduced the monocyte differentiation and blocked the production of TNF-α and IL1β[54].

Anti-arthritic effect of three kashayams viz. Balaguluchiadi kashayam, Punarnavadi Kashayam and Gugguluthiktam Kashayam in collagen induced arthritic rats was validated and its molecular mechanism on TLR-4 signal transduction pathway was also elucidated. The major inflammatory mediators of Rheumatoid arthritis were downregulated on treatment with the formulations and confirmed the clinical efficacy of these kwathas[55]. The in-vitro experiment of Rasna saptak kwatha results showed that the drug is permeating through the skin. The hydroalcoholic extract of the kwatha showed better inhibition than the aqueous extract in the carrageenan edema model studied for the anti-inflammatory effect[56].

The antipyretic effect of Nagaradi Kwatha, Arishta and Ganavati, are compared with the paracetamol as reference standard in Wistar Albino Rats and found that Arishta form of Nagaradi medicine had better antipyretic effect[57]. The anti-inflammatory, anti-lipase and antioxidant activity of Ayurvedic poyherbal formulation Varanadi Kashayam was studied. The treatment with kwatha effectively reduced the THP-1 monocyte differentiation into macrophages along with the production of proinflammatory cytokines in LPS-stimulated macrophages. The anti-lipase activity of five fractions of kwatha was analysed by using porcine pancreatic lipase as enzyme and p-Nitrophenyl palmitate was used as the substrate. The free radical scavenging activity of ethyl acetate fraction of the kwatha was with IC50 values close to quercetin and BHT standards. The anti-obesity action of the formulation on high fat induced obese rats was also studied. Histological, gene expression and biochemical studies were conducted to substantiate the potential activity[58–60].

Table 1. Phytochemical standardization of different kwathas

Kwatha	Medicinal importance	Standardization	Scientific observation	References
Pathyashadangam Kwath	Headache & Migraine	HPLC	HPLC fingerprint of three batches of Pathyashadangam Kwath was developed and compared with that of the gallic acid used as the standard marker.	[22]
Chaturbhadra Kwatha	Gastrointestinal tract (GIT) disorders	TLC	Physio-chemical parameters were determined along with microbial limit test. TLC fingerprint was also developed.	[23]
Drakshadi Kashayam	Anemia & Jaundice	GC-MS	GC-MS profile of the Drakshadi kashayam was developed and a total of 25 compounds were found in the formulation.	[24]
DBC and DMV (combination of 34 and 22 plant materials respectively)	Diabetes mellitus	Preliminary analysis, TLC & HPTLC	Chromatographic profiling of phytochemical constituents using TLC and HPTLC techniques along with the determination of phenoilic compounds in the polyherbal formulation was comparatively evaluated.	[25]
HAF (Hydro-alcoholic polyherbal formulation)	Antioxidant	HPTLC	HPTLC fingerprinting of the polyherbal formulation showed the presence of potential polyphenolic compounds.	[26]
Nirgundi Kashaya	Fever & Pain relief	GC-MS	GC-MS studies showed the presence of a total of 30 compounds in the formulation. Comparative analysis of Nirgundi Taila and Nirgundi Kashaya was also done.	[27]
Trivrittadi Kwatha	Skin disorder	HPTLC	HPTLC profiling of the polyherbal formulation Trivrittadi kwatha was studied.	[28]
Dhatryadi Kwatha	Vitiligo	HPTLC	HPTLC fingerprinting of Dhatryadi kwatha was developed along with the standardization of physiochemical parameters.	[29]
Manjisthadi Kwatha	Psoriasis	HPTLC	Physiochemical analysis and HPTLC profile of the Manjisthadi kwatha were developed in the study.	[30]
Darvyadi Kwatha	Excessive vaginal discharge	HPTLC	Heavy metal analysis and test for microbial limits were carried out and found to be in limit. HPTLC fingerprint of the Darvyadi kwatha was developed. Physio-chemical parameters of the formulation were also studied.	[31,32]
Kutajastaka Pravahi Kwatha	Diarrhoea	HPTLC	Phytochemical standardization was done using HPTLC technique. Heavy metal analysis and test for microbial limits were studied.	[33]
Balaguloochyadi Kashayam	Arthritis	HPTLC	Microbial standardization and quantitative marker based standardization, viz. ephedrine was developed using HPTLC technique.	[34]
Kathakakhadiradi Kashayam	Diabetes	Preliminary analysis	Preliminary phytochemical screening and physio-chemical parameters were studied.	[35]
Kulathadi Kashayam	Amenorrhoea	GC-MS	A total of 21 compounds was identified from the preliminary GC-MS analysis of the Kulathadi kashayam.	[19]
Dhanyapanchaka Kwatha	Pain &Diarrhoea	HPTLC	Physio-chemical parameters of Dhanyapanchaka Kwatha and its raw drugs were compared. HPTLC profile of the formulation was developed.	[36]

Shirishadi Kwath	Allergic Rhinitis	Preliminary analysis & HPTLC	Preliminary phytochemical analysis was done and HPTLC fingerprint of the polyherbal formulation was developed for the first time.	[37]
Maharasnadi Kwatha	Arthritis	HPTLC	The quality of the commercially available polyherbal formulations of Maharasnadi kwatha was analysed. HPTLC profiling showed variation in number of bands and their area percentage. Qualitative estimation and microbial load was also studied.	[38]
Pathadi kwatha	Poly Cystic Ovarian Disease	HPTLC	Phytochemical standardization of Pathadi kwatha was done and HPTLC fingerprint was developed.	[39]
Erandamooladi Kwatha	Enema therapy	HPTLC	HPTLC fingerprint for the alcohol and chloroform extracts of the formulation was developed. Physio-chemical parameters of the formulation were also analysed.	[40]
Amruthotharam Kashayam	Indigestion, Fever	TLC & HPLC	HPLC chromatogram of the formulation was developed with tannic acid as the standard. The physio-chemical parameters were analysed and the TLC fingerprint was also developed. Estimation of total phenolics, flavanoids and tannins were done.	[41]
Vara Asanadi Kwatha	Obesity	TLC & HPTLC	HPTLC fingerprint of the formulation was developed. The qualitative analysis showed the presence of flavanoids and tannins.	[42]
Vasaguduchyadi kwatha	Liver diseases	HPTLC	The phytochemical standardization of the kwatha was done using HPTLC method. Gallic acid is used as the standard marker.	[43]
Panchatiktaka Guggulu Kwath	Neurological disorder	HPTLC	Marker based quantitative estimation of the phyto constituents in the formulation was carried out using HPTLC technique. Guggulusterone z, quercetin and berberine were used as the standards. The HPTLC fingerprint was developed.	[44]
Brihatpanchamoola Kwatha	Digestive and Musculoskeletal diseases	HPTLC	The phytochemical comparison of the Brihatpanchamoola kwatha prepared using the stem bark and root bark were evaluated using HPTLC analysis.	[45]
Bhunimbadi Kwath	Fever	Gas chromatography	Gas chromatographic method was developed for the estimation of ethanol in the polyherbal formulation Bhunimbadi kwath.	[46]
Dhanyapanchak Kwatha, Guduchyadigana Kwatha & Stanyajanana Kashaya	Peri-natal care	TLC & HPLC	UV-visible spectrophotometric analysis, TLC and HPLC fingerprint for the three polyherbal formulations were developed and compared in the study.	[47]

Hepatoprotective activity of Vasaguduchyadi kwatha was studied using induced hepatotoxicity in albino rats by antitubercular drugs and paracetamol[61,62]. Punarnavashtak kwath is a formulation for hepatic disorders and asthma. Hepatoprotective activity of the kwatha was evaluated against induced hepatotoxicity by CCl4 in rats. 25.37% viability was shown by the HepG2 cells exposed to CCl4. Comparative analysis of the effect was done using the standard drug silymarin[63].

A traditional Ayurvedic formulation that could improve the quality and yield of the stem cells in vitro and could be used for mesenchymal stem cells culturing was identified for the first time. The effects of Dhanwantramkashaya on human Wharton jelly mesenchymal stem cells increased the proliferation rate and delayed senescence of the stem cells[64]. A new wound healing gel was prepared by the combination of Ayurvedic formulations viz. Pachavalkala Kwatha, Nimba Kwatha and Kumari Swarasa[65].

Antidepressant activity of Mamsyadi Kwatha along with its individual components viz. Jatamansi, Ashwa gandha and Parasika Yavani was evaluated. The behavioural despair test was used to study antidepressant activity of the formulation in Swiss albino mice[66]. Hepatoprotective effects and antioxidant activity of traditional Ayurvedic formulation Punarnavashtak kwath was evaluated against hepatotoxicity induced by ethanol. Silymarin was used as a reference standard and antioxidative effect on hepatocytes by the kwatha protected the cells from liver damage by ethanol[49].

Chinnodbhavadi kwath is a classical formulation used for gastric problems and hyperacidity. The gastric mucosal injury induced by aspirin in rats was inhibited by Chinnodbhavadi kwath and protects from gastric ulceration[67]. The anti-cancer activity of Vasaguluchyadi kashayam on hepatocelluar carcinoma in male rats was evaluated. The studies found that the kwatha provides protection against DEN and phenobarbitone induced hepatocellular carcinoma and in turn prevents the malignancy[68]. The diuretic activity of Veerataru Kwatha and toxicological studies of Darvyadi Kwatha Churna was studied in albino rats. The various biochemical parameters of male and female rats were altered after the treatment with the formulations[69,70]. Table 2 shows the pharmacological activities of the different kwathas in the literature.

5. Synergistic Interactions in Polyherbal Formulations

The conventional approach of the pharmaceutical industry was to develop a single drug based medicines for treatment. However, in recent times there is a paradigm shift towards medicines with multiple active components viz. polyherbalism. In the traditional system combined extracts of plants are preferred over a single drug based treatment. In Ayurveda majority of the formulations are polyherbal formulations. The concept of synergism underlying the polyherbal formulations is highlighted in the classical Ayurvedic text Sharangadhara Samhita. The Ayurvedic approach towards the drug formulation is unique in a way that the herbs are selected accordingly to a particular disease and combining them in a particular ratio to increase the therapeutic efficacy and reducing toxicity. The main drugs are for curing the disease and other herbs are used along with it to prevent the side effects of the active ingredients in drug formulation[5].

The phytochemical profiling of the active components of individual medicinal plants is well established. The desirable therapeutic effects are not achieved in the practical use as they are secondary metabolites present in minute quantity. Recent scientific studies revealed that while combining these potential plants into a multi drug formulation, greater therapeutic effect was obtained over their individual effect and also with the sum of their individual effect[4]. Unveiling this synergistic interaction between the individual components underlying in a polyherbal formulation is difficult. In spite of a few scientific data available to support the synergistic basis of multi drug formulation, substantial evidences are less and the clinical relevancy of these studies needs to be further determined[85]. In the present study, it was observed that even though phytochemical and pharmacological studies of different kwathas were carried out and mentioned about the possibilities of synergistic interactions, the level of evidences remains low in majority of these studies. Further studies need to be carried out more scientifically to ascertain the inherent potentials of polyherbal formulations.

The comparative quantitative evaluation of the phyto chemical and pharmacological activity of the phyto constituents present in the individual plants with that of the respective polyherbal formulations provides the initial findings as reported in a few works. In order to find the mechanism behind the synergistic activity the active components behind the mechanism should be pointed down or the effect on available marker compound could be studied quantitatively. If we select a biomarker that is already characterized in an individual plant basis, we cannot ensure that it is the active component while in a polyherbal formulation. Even though a number of compounds could be co-interacting in a polyherbal formulation we will be able to identify the

Table 2. Pharmacological activities of the different kwathas

Kwatha	Popular use	Biological activity	Study model/ Study design	Scientific observation	References
Devadarvadi Kashaya	Diabetic mellitus	Antidiabetic	Case study	Assessed the serum biochemical parameters and blood glucose level reduced significantly from P value (<0.0001), PPBS level also reduced.	[71]
Rasna Saptak Kwath	Rheumatoid arthritis	Anti-arthritic	Review	The review focused on the pharmacological activities of the source plants of Rasna Saptak Kwath.	[72]
Lodhradi Kashaya	Diabetes mellitus	Antidiabetic	Review	The review considered Lodhradi kashaya as a potent medicine for diabetes mellitus and other diabetic complications.	[73]
Sukumara Kashayam	Menstrual pain, Constipation.	Antioxidant	Assay	DPPH, FRAP and Hydrogen peroxide scavenging activity. Methanol fraction indicated antioxidant activity.	[74]
Katakakadhiradi Kashayam	Diabetes, Urinary Aailments.	Antioxidant, Hepatoprotective activity	Assay, Albino rats	ABTS, DPPH and FRAP. Good antioxidant activity as compared to ascorbic acid. Carbon tetrachloride induced liver injury; alkaline phosphatase and GOT activity in liver are reduced by the drug.	[75,76]
Panchatiktha Kwatha	Fever and Skin disorders	Antipyretic	Review	The review tried to justify the activity of the raw drugs present in the formulation with the help of scientific evidences.	[77]
Kulathadi Kashayam	Amenorrhoea	Antioxidant	Assay	FRAP assay, DDPH assay and Hydrogen peroxide Scavenging Activity assay.Ethanolic solution showed antioxidant activity.	[78]
Kushtakhnani Kwatham	Skin diseases	Antibacterial & Antioxidant	Assay	Antibacterial activity using Agar well diffusion method and antioxidant assay using Hydroxyl radical, Hydrogen peroxide and DPPH.	[79]
GSPF kwath	Diabetes mellitus Type II	Antidiabetic	Case study	Gymnemasylvestre containing polyherbal formulation found to be effective in regulating both hyperglycemia and hyperlipidemia.	[80]
Patoladigan Kashayam	Skin diseases	Anticancer	Review	The possibilities of using the formulation to minimize the adverse effects of chemotherapy like nausea, vomiting, anorexia etc. were discussed.	[81]
Sarvakalp Kwath	Liver disorder	Hepatoprotective activity	Albino rats	Hepatic damage induced by carbon tetrachloride; Aqueous extract of Sarvakalp Kwath offered significant hepatoprotection	[82]
Indukantham kashayam	Immunity enhancement	Antioxidant	Assay	Antioxidant activities were estimated by DPPH.	[83]
Pathadi Kwatha	Poly Cystic Ovarian Disease	Anticancer	Case study	Effect on follicular growth, ovulation and other symptoms. Statistically effective in regularizing menstruation.	[84]

major component by different phytochemical methods. Isolation of the different active components and the individual and combined biological studies could ensure whether the pharmacological activity is the same as that in the formulation. It could in turn validate the polyherbal formulation and its potential activity. The comparison of different combination of the individual source plants helps us to find out which combination is resulting in the enhanced or reduced activity[86,87]. The study of combinations can only be done for the formulations that have a limited number of individual source plants. It will be difficult to study a polyherbal formulation with a large number of source plants. Thus, to study the synergistic activity of complex polyherbal formulations, there are significant challenges to develop a standard methodology.

6. Conclusion

Kwatha is the mainstay of the Ayurvedic formulations and with the growing interest towards the herbal medicines as an alternative to the synthetic drugs, the purity, safety, therapeutic efficacy and the reproducibility of the activity needs to be ensured. The standardization, pharmacology and clinical trials of the polyherbal formulations will validate the aforementioned objectives. The standardization methodologies will provide a comprehensive specification to a particular polyherbal formulation that would be used to determine the purity and authenticity of the formulations like kwathas. The complete fingerprinting profiles and pharmacological studies for the majority of the formulations are still need to be standardized.Significant contributions from the researchers and scientists are required to develop a meticulously designed methodology for the standardization of all the polyherbal formulations and also to shed light on the underlying principles of synergistic interactions.

7. Conflict of Interest

The authors report no conflicts of interest.

8. Acknowledgement

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9. References

1. Saroya AS. Herbalism, phytochemistry and ethnopharmacology. Enfield: Science Publishers; 2011.
2. Shah B, Seth AK. Textbook of pharmacognosy and phytochemistry. New Delhi: Elsevier; 2010.
3. Jayakumar RV. Herbal medicines for type-2 diabetes. Int J Diabetes Dev Ctries. 2010; 30(3):111. https://doi.org/10.4103/0973-3930.66501
4. Parasuraman S, Thing G, Dhanaraj S. Polyherbal form ulation: Concept of Ayurveda. Pharmacogn Rev. 2014; 8(16):73. https://doi.org/10.4103/0973-7847.134229. PMid:25125878. PMCid:PMC4127824
5. Pole S. Ayurvedic medicine: The principles of traditio nal practice. China: Elsevier; 2006. https://doi.org/10.1016/B978-0-443-10090-1.50027-X
6. Murthy KR, Sarngadhara Samhita. Varanasi: Chowkhamba Orientalia; 1984.
7. Phillipson JD. Phytochemistry and pharmacognosy. Phytochemistry. 2007; 68:2960–72. https://doi.org/10.1016/j.phytochem.2007.06.028. PMid:17761200
8. Folashade O, Omoregie H, Ochogu P. Standardization of herbal medicines –A review. Int J Biodivers Conserv. 2012; 4(3):101–12. https://doi.org/10.5897/IJBC11.163
9. Rajani M, Kanaki N. Phytochemical standardization of herbal drugs and polyherbal formulations. In: Ramawat K, Merillon J, editors. Bioactive molecules and medicinal plants. Berlin: Springer; 2008. p. 349–69. https://doi.org/10.1007/978-3-540-74603-4_19
10. Waghmare PR, Kochar NI, Chandewar AV. Quality control of Varuna kwatha churna, an Aurvedic formulation and its comparative study with marketed formulations. Int J Pharm Sci Res. 2011; 2(7):1861–8.
11. Abraham A, Mathew L, Sarala S, Twincy Y. Standard ization of a classical Ayurveda formulation Pathyasha dangam kwath by thin layer chromatography. Int J Herb Med. 2017; 5(6):75–8.
12. Abraham A, Samuel S, Mathew L. Phytochemical analysis of Pathyashadangam kwath and its standard ization by HPLC and HPTLC. J Ayurveda Integr Med. 2018; 11(2):153–8. https://doi.org/10.1016/j.jaim.2017.10.011. PMid:30446379. PMCid:PMC7329714
13. Purushothaman H, Davis A, John M, Kalarikkal M, Bhaskaran A, Eldho, B. *et al.* Marker assay guided standardization of an Ayurvedic concentrated polyherbal decoction "*Ciruvilvadi kasayam*" and its application in industrial quality control. Anc Sci Life. 2017; 37(1):24–30. https://doi.org/10.4103/asl.ASL_188_17

14. Kumar SK, Priyadarshini P, Ravishankar B, Yashovarma B. Quality standards for Bhunimbadi kwatha churna. J Ayu Med Sci. 2016; 1(1):19–33. https://doi.org/10.5530/jams.2016.1.4
15. Kumar SK, Priyadarshini P, Ravishankar B. HPTLC and 1H-NMR as fingerprints to spot ingredients of a polyherbal medicne Bhunimbadi kwatha churna. J Ayu Med Sci. 2017; 1(2):63–71. https://doi.org/10.5530/jams.2016.1.10
16. Narayana SK, Priyadarshini, Puneeth, Prabhu SN, Ballal M. Chemical fingerprints for Panchavalkala kwatha churna. J Ayu Med Sci. 2018; 3(2):356–68. https://doi.org/10.5530/jams.2018.3.16
17. Jessica A, Rao MR, Anthony J, Prabhu K, Johnson WM, Balasubramanian BS, *et al.* The GC-MS study of one Ayurvedic preparation Katakakhadiradi kashayam. Int. J Pharm Sci Rev Res. 2015; 39(2):216–24.
18. Rao MR, Kumar NS, Jones S, Elizabeth AA, Prabhu K, Ravi A, *et al.* Phytochemical and GC MS analysis of an Ayurvedic formulation, Patolakaturohinyadi kwatham. Int J Pharm Sci Rev Res. 2015; 34(2):6–12.
19. Phillips S, Rao MR, Prabhu K, Priya M, Kalaivani S, Aparna R, *et al.* Preliminary GC-MS analysis of an Ayurvedic medicine "Kulathadi kashayam". J Chem Pharm Res. 2015; 7(9):393–400.
20. Sulaiman CT, Balachandran I. Chemical profiling of an Indian herbal formula using liquid chromatography coupled with electro spray ionization mass spectrometry. Spectrosc Lett. 2014; 48(3):222–6. https://doi.org/10.1080/00387010.2013.872670
21. Singh VK, Reddy KR. Validation of lodhradi kashaya (Ayurvedic formulation) made by traditional method and contemporary spray drier method through FTIR spectroscopy. Int J Pharm Analyt Res. 2015; 4(3):386–90.
22. Abraham A, Mathew L, Samuel S. HPLC analysis of Pathyashadangam kwath, a classical Ayurvedic polyherbal formulation. Mater Today Proc. 2020; 25(2):115–21. https://doi.org/10.1016/j.matpr.2019.12.165
23. Mangal AK, Tomer R, Jindal H, Prasad SB, Sharma H, Sharma BS, et al. Development and standardization of Chaturbhadra kwath churna: An Ayurvedic formulation. JDRAS. 2019; 4(4):192–7. https://doi.org/10.5005/jdras-10059-0090
24. Narayanan G, Prabhu K, Rao MR, Kannan K, Sundaram RL, Dinakar S, *et al.* Gas chromatography-mass spectrometry analysis of one Ayurvedic medicine, Drakshadi kashayam. Drug Invent Today. 2019; 11(10):2652–6.
25. Dinakaran SK, Chelle S, Avasarala H. Profiling and determination of phenolic compounds in poly herbal formulations and their comparative evaluation. J Tradit Complement Med. 2019; 9(4):319–27. https://doi.org/10.1016/j.jtcme.2017.12.001. PMid:31453128. PMCid:PMC6702236
26. Srivastava AK, Kaushik D, Lal VK. High-performance thin-layer chromatography fingerprinting analysis of bioactive compounds in hydro-alcoholic extracts of polyherbal formulation. Drug Invent Today. 2018; 10(5):805–10.
27. Chinivar P, Uchangi P, Narayana SK. GCMS study of taila (medicated oil) and kashaya (decoction) kalpana of Nirgundi (*Vitex negundo* Linn.). J Ayu Med Sci. 2018; 3(2):390–5. https://doi.org/10.5530/jams.2018.3.10
28. Rao R, Thakar AB, Bhatt NN, Harisha CR, Shukla VJ. Pharmacognostical, physicochemical and hptlc evaluation of Trivrittadi kwatha: A polyherbal formulation. Int J Ayurveda Pharma Res. 2017a; 5(1):6–10.
29. Rao R, Thakar AB, Bhatt NN, Harisha CR. Shukla VJ. Pharmacognostical, physicochemical and high performance thin layer chromatography evaluation of Dhatryadi kwatha. Int J Green Pharm. 2017b; 11(1):S112–5.
30. Odedra J, Thakar AB, Bhatt NN, Harisha CR, Shukla VJ. Pharmacognostical, physicochemical, and high performance thin layer chromatography evaluation of Manjisthadi kwatha in the management of psoriasis. Int J Green Pharm. 2017; 11(1):57–61.
31. Tiwari AK, Dwivedi N, Tripathi MK. Scientific evaluation of Darvyadi kwatha churna: –A classical Ayurvedic compound formulation. Indian J Nat Prod Resour. 2015; 6(4):314–9.
32. Tiwari AK, Dwivedi N, Tripathi MK, TripathI SP. Standardization and quality control of Darvyadi Pravahi kwatha –An Ayurvedic formulation. Indian J Tradit Know. 2016; 15(4):654–8.
33. Tiwari AK, Dwivedi N, Tripathi MK, Tiwari A, Ahirwar PK, Tripathi SP. Standardization of Ayurvedic polyherbal formulation, Kutajastaka Pravahi kwatha. Res J Pharmacognosy and Phytochem. 2016; 8(3):103–8. https://doi.org/10.5958/0975-4385.2016.00019.4

34. Khan T, Mallya R, Gohel A. Standardization of marketed Ayurvedic formulation, Balaguloochyadi kashayam–physicochemical, microbial evaluation and ephedrine content. J Appl Pharm Sci. 2016; 6(12):184–9. https://doi.org/10.7324/JAPS.2016.601226

35. Azeez A, Abdalla FM, Tomy S, Suresh R, Johnson B. Pharmocognostic and phytochemical evaluation of polyherbal formulation Kathakakhadiradi kashyam. Int J Pharm Sci Rev Res. 2016; 37(1):96–100.

36. Tripathi M, Dwivedi N, Tiwari A, Ahirwar P, Tiwari A, Pathak S. Scientific evaluation of Ayurvedic compound formulation Dhanyapanchaka kwatha churna. Int J Pure Appl Biosci. 2014; 2(5):88–93.

37. Shrawan SK, Dhiman KS, Harisha CR, Vaghela DB, Shukla VJ. Pharmacognostical and physicochemical evaluation of Shirishadi kwath yavakut (sky) –A promisable Ayurvedic compound formulation - in allergic rhinities (*Vataja pratishyaya*). Int J Sci Invent Today. 2014; 3(2):94–103.

38. Radha A, Sebastian J, Prabhakaran M, Kumar DS. Observations on the quality of commercially manufactured Ayurvedic decoction, Maharasnadi kwatha. Hygeia J D Med. 2014; 6(1):74–80. https://doi.org/10.15254/H.J.D.Med.6.2014.124

39. Vasudevan A, Dei L, Harisha CR, Shukla VJ. Pharma cognostical and phyto-chemical standardization of Pathadi kwatha: A polyherbal formulation. J Pharm Biomed Sci. 2013; 27(27):558–63.

40. Lohith BA, Kumar SK, Girish, KJ. Standardization of Erandamooladi kwatha churna –A compound formulation used in medicated enema therapy (Basti karma). Global J Res Med Plants and Indigen Med. 2013; 2(10):709–15.

41. Sulaiman CT, Anandan EM, Balachandran I. Using biochemical parameters to standardize an Ayurvedic formulation. J Trop Med Plants. 2012; 13(2):133–5.

42. Ramachandran AP, Prasad SM, Samarakoon SM, Chandola HM, Harisha CR, Shukla VJ. Pharma cognostical and phytochemical evaluation of Vara Asanadi kwatha. Ayu. 2012; 33(1):130–6. https://doi.org/10.4103/0974-8520.100330. PMid:23049198. PMCid:PMC3456851

43. Kalpu K, Harisha CR, Shukla VJ, Prajapati PK, Ravishankar B. Pharmacognostical and phytochemical standardization of Vasagduchyadi kwatha a poly herbal Ayurvedic formulation. Pharma Sci Monit. 2012; 2291–303.

44. Gopinath V, Kokila L, Lavanya R, Brindha P. Quality control studies on Panchatiktaka Guggulu kwatha curanam. J Pharm Res. 2011; 4(1):229–32.

45. Vyas M, Yadav P, Shukla VJ, Patgiri BJ, Prajapati PK. Pharmaceutical evaluation of Brihatpanchamoola kwatha prepared by root bark and stem bark. Int J Pharm Biol Arch. 2010; 1(5):436–41.

46. Nehete JY, Shewale VV, Deshmukh VN, Narkhede MR. Gas chromatographic ethanol determination in Bhunimbadi kwath. Res J Pharm Technol. 2009; 2(4):876–7.

47. Santhosh MK, Shaila D, Rao IS. Standardization study of kwatha churnas. J Chem. 2004; 1(5):251–5. https://doi.org/10.1155/2004/729261

48. Roshan A, Jain UK, Patel A, Verma NK, Kumar CS. UV-spectrophotometric method development for estimation of glycyrrhetinic acid in Pratishyayghna kwath. Pharm Lett. 2013; 5(2):242–6.

49. Shah VN, Shah MB, Bhatt PA. In vivo and in vitro antioxidant and hepatoprotective effects of classical Ayurvedic formulation Punarnavashtak kwath against ethanol induced hepatotoxicity. Phcog J. 2010; 2(16):43–52. https://doi.org/10.1016/S0975-3575(10)80049-5

50. Bagul MS, Rajani M. Quantification of ellagic acid, gallic acid and picroside-I from Phalatrikadi kvatha churna by HPTLC. J Nat Remed. 2006; 6(1):53–61.

51. Katekhaye SD, Bhutani KK. Standardization of a polyherbal Ayurvedic formulation: Ayaskrti. Indian J Tradit Know. 2011; 10(4):589–93.

52. Renganathan S, Srivastava A, Pillai RG. Dhanwantaram kashayam, an Ayurvedic polyherbal formulation, reduces oxidative radicals and reverts lipids profile towards normal in diabetic rats. Biochem Biophys Rep. 2020; 22. https://doi.org/10.1016/j.bbrep.2020.100755. PMid:32368622. PMCid:PMC7186759

53. Balkrishna A, Sakat SS, Ranjan R, Joshi K, Shukla S, Joshi K, *et al.* Polyherbal medicine DivyaSarva-Kalp-Kwath ameliorates persistent carbon tetrach loride induced biochemical and pathological liver impairments in Wistar rats and in HepG2 cells. Front Pharmacol. 2020; 11:288. https://doi.org/10.3389/fphar.2020.00288. PMid:32269524. PMCid:PMC7109321

54. Devi SJ, Kumar BP, Bashi MB, Chinchu JU, Merlin T. Evaluation of anti-inflammatory effects of polyherbal decoction, Balaguluchyadi kashayam. Adv Tradit

Med. 2020:1–13. https://doi.org/10.1007/s13596-020-00448-4

55. Aswathi IS, Krishnan S, Peter J, Sabu V, Helen A. Scientific validation of Anti-arthritic effect of Kashayams –A polyherbal formulation in collagen induced arthritic rats. J Ayurveda Integr Med. 2019. https://doi.org/10.1016/j.jaim.2018.02.139. PMid:30660454
56. Pandey S, Chaudhary A. In vitro drug release and study of anti-inflammatory effect of Rasna Saptak Kwath as topical drug. J Ayurveda Integr Med. 2018; 9(2):S14. https://doi.org/10.1016/j.jaim.2018.02.060
57. Liju B, Seema M, Mandugaru R. Comparative antipyretic effect of Nagaradi kwatha, Ghana vati and Arishta in Wistar albino rats. J Ayu Med Sci. 2016; 1(2):86–9. https://doi.org/10.5530/jams.2016.1.11
58. Chinchu JU. Kumar PB. In-vitro anti-lipase and antioxidant activity of polyherbal Ayurvedic medicine Varanadi kashayam. Int J Pharm Sci Res. 2018; 9(12):5373–81. https://doi.org/10.13040/IJPSR.0975-8232.9(12).5373-81
59. Chinchu JU, Mohan MC, Devi SJ, Kumar BP. Evaluation of anti-inflammatory effect of Varanadi kashayam (decoction) in THP-1-derived macrophages. Ayu. 2018; 39(4):243–9. https://doi.org/10.4103/ayu.AYU_53_18. PMid:31367148. PMCid:PMC6639814
60. Chinchu JU, Mohan MC, Kumar PB. Anti-obesity and lipid lowering effects of Varanadi kashayam (decoction) on high fat diet induced obese rats. Obes Med. 2020; 17. https://doi.org/10.1016/j.obmed.2019.100170
61. Kotecha KN, Ashok BK, Shukla VJ, Prajapati PK, Ravishankar B. Hepatoprotective activity of Vasaguduchyadi kwatha –A compound herbal from ulation against paracetamol induced hepatotoxicity in Albino rats. Pharma Sci Monit. 2015; 6(4):157–67.
62. Kotecha KN, Ashok BK, Shukla VJ, Prajapati PK, Ravishankar B. Hepatoprotective activity of Vasagudu chyadi kwatha - A compound herbal formulation against antitubercular drugs (isoniazid + rifampicin + pyrazinamide) induced hepatotoxicity in albino rats. Pharma Sci Monit. 2015; 6(3):73–84.
63. Shah VN, Shah MB, Bhatt PA. Hepatoprotective activity of Punarnavashtak kwath, an Ayurvedic formulation, against CCl4-induced hepatotoxicity in rats and on the HepG2 cell line. Pharm Biol. 2011; 49(4):408–15. https://doi.org/10.3109/13880209.2010.521162. PMid:21391842
64. Warrier SR, Haridas N, Balasubramanian S, Jalisatgi A, Bhonde R, Dharmarajan A. A synthetic formulation, Dhanwantharam kashaya, delays senescence in stem cells. Cell Prolif. 2013; 46(3):283–90. https://doi.org/10.1111/cpr.12026. PMid:23692087. PMCid:PMC6495558
65. Palak V, Prajapati PK, Shukla VJ. An herbal wound healing gel prepared with Pachavalkala kwatha, Nimba kwatha and Kumari swarasa with their physicochemical parameters. Int J Phytother Res. 2013; 3(2):49–60.
66. Shreevathsa M, Ravishankar B, Dwivedi R. Anti depressant activity of Mamsyadi kwatha: An Ayurvedic compound formulation. Ayu. 2013; 34(1):113–7. https://doi.org/10.4103/0974-8520.115448. PMid:24049416. PMCid:PMC3764868
67. Nariya M, Tomar R, Shukla V, Ravishankar B, Jain S. The inhibition of gastric mucosal injury by Chinnod bhavadi kwath (decoction) –An Indian Ayurvedic formulation in rats. J Exp Integr Med. 2013; 3(3):243–7. https://doi.org/10.5455/jeim.200413.or.067
68. Sanjay M, Ariharasivakumar G, Kumar KT, Kokila MT, Devan S, Jeevanantham A. Evaluation of anti-cancer activity of Vasaguluchyadi kashayam on DEN and phenobarbitone induced hepatocelluar carcinoma in male rats. World J Pharm Res. 2012; 7(17):1037–50.
69. Patel BR, Ashok BK, Ravishankar B. Study on the diuretic activity of Veerataru kwatha in albino rats. Ayu. 2011; 32(3):395–7. https://doi.org/10.4103/0974-8520.93922. PMid:22529658. PMCid:PMC3326890
70. Yasmin D, Choudhuri MS, Uddin R. Toxicological studies of Darvyadi kwatha churna using albino rats. S J Pharm Sci. 2011; 4(2):29–34. https://doi.org/10.3329/sjps.v4i2.10438
71. Mishra S, Parida N, Temhunna S, Mishra L, Patel AK. The anti-diabetic (hypoglycaemic) effect of Devada rvadi Kashaya –A polyherbal compound drugs –A pilot study. Int J Health Sci Res. 2018; 8(8):121–8.
72. Pandey S, Chaudhary AK. A review on Rasna Saptak kwath: An Ayurvedic polyherbal formulation for arthritis. Int J Res Ayurveda Pharm. 2017; 8(1):4–11. https://doi.org/10.7897/2277-4343.08126
73. Singh VK, Reddy, KR. Review on Lodhradi kashaya: all-rounder remedy for diabetes mellitus patients. Indian J Tradit Know. 2017; 16(1):100–6.
74. Nirupa P, Rao MR, Prabhu K, Kaliaselvi VS, Kumaran D, Sivaram E, *et al.* Antioxidant study of one Ayurvedic

medicine, "Sukumara kashayam". Int. J Pharm Sci Rev Res. 2017; 42(1):35–41.

75. Sundari SK, Valarmaathi R, Akilandeswari S, Begum TN, Rafiullah MM. Hepatoprotective activity of Kadhaka Kadhiradi Kashayam. Anc Sci Life.2000; 19(3&4):135–8.
76. Jessica SA, Rao MR, Anthony J, Prabhu K, Kavimani M, Balasubramanian BS, *et al.* Antioxidant study of one Ayurvedic preparation Katakakhadiradi kashayam. J Pharm Sci Res. 2017; 9(9):1427–9.
77. Nagarajan K, Shanmugham UK, Natarajan K, Thiyagarajan B, Sekkizhar G. Panchatiktha kwatha churnam - an Ayurvedic medicine. Int J Curr Res. 2017; 9(10):59686–93.
78. Sharada NS, Rao, MR, Priya M, Prabbu K, Kalaivani VS, Kumaran D, *et al.* The study of antioxidant activities of an Ayurvedic medicine Kulathadi kashayam. Pharm Lett. 2016; 8(6):245–248.
79. Jalaj AV, Williams BC, Suja RM. Pharmacological analysis of the polyherbal formulation "Kushtakhnani kwatham". World J Pharm Pharm Sci. 2016; 5(4):1016–21.
80. Mahajan S, Chauhan P, Subramani SK, Anand A, Borole D, Goswamy H, *et al.* Evaluation of "GSPF kwath": A Gymnema sylvestre-containing polyherbal formulation for the treatment of human type 2 diabetes mellitus. Eur J Integr Med. 2015; 7(3):303–11. https://doi.org/10.1016/j.eujim.2015.01.003
81. Verma R, Akhand KK. Review on possibilities of Patoladi gan kashayam as an adjuvant therapy to combat adverse effects of chemotherapy/radiotherapy and cancer. Carib J Sci Tech. 2015; 3:711–7.
82. Yadav A, Kumar S. Hepatoprotective effect of Sarvakalp kwath against carbon tetrachloride induced hepatic injury in albino rats. Biomed. Pharmacol. J. 2014; 7(2):659–63. https://doi.org/10.13005/bpj/538
83. Sruthi CV, Vendamirtham S, Sindhu A. PA02.20. A comparative study on the total phenolic content and antioxidant property of two Ayurvedic formulations-Indukantham gritham and Indukantham kashayam. Anc Sci Life.2013; 32(Suppl 2):S65. https://doi.org/10.4103/0257-7941.123886. PMCid:PMC4147538
84. Patel KD, Dei L, Donga SB, Anand N. Effect of Shatapushpa taila matra basti and Pathadi kwatha on poly cystic ovarian disease. Ayu. 2012; 33(2):243–6. https://doi.org/10.4103/0974-8520.105245.PMid:23559797. PMCid:PMC3611647
85. Zhou X, Seto SW, Chang D, Kiat H, Razmovski-Naumovski V, Chan K, *et al.* Synergistic effects of Chinese herbal medicine: a comprehensive review of methodology and current research. Front Pharmacol. 2016; 7:201. https://doi.org/10.3389/fphar.2016.00201
86. Williamson EM. Synergy and other interactions in phytomedicines. Phytomedicine. 2011; 8(5):401–9. https://doi.org/10.1078/0944-7113-00060. PMid:11695885
87. Yang Y, Zhang Z, Li S, Ye X, Li X, He K. Synergy effects of herb extracts: Pharmacokinetics and pharmaco dynamic basis. Fitoterapia. 2014; 92:133–47. https://doi.org/10.1016/j.fitote.2013.10.010. PMid:24177191