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Best therapeutic activity of *Rasna saptak kwath* may be achieved by its hydro-alcoholic dosage form: A comparative study

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Abstract
Rasna saptak kwath (RSK) is an Ayurvedic polyherbal formulation. In Ayurvedic classics it is recommended to administer this drug in decoction form only, to treat inflammation and pain related to the rheumatoid disorders. The present study was carried to fulfill two aims. First aim was to characterize the Ayurvedic preparation i.e. *RSK* which is a polyherbal formulation comprising of eight ingredients including an adjuvant drug, on the basis of the physicochemical, pharmaceutical, microbiological, spectroscopic and chromatographic parameters to confirm its identity, quality and purity. The second aim was to provide this formulation into some another dosage form for much better therapeutic activity in comparison to decoction form. Both the extracts were compared for standardization and for antioxidant activity. The results revealed that HA extract possess more phyto-constituents. The IC_{50} of HA extract was 33.3µg/ml while that of AQ extract was 480µg/ml. Microbial load and heavy metal analysis confirm their safety issue.

Keywords: Ayurveda, antioxidant, arthritis, FTIR, hydroalcohilc extract, polyherbal formulation, rasna saptak kwath

Introduction
Rasna saptak kwath is apolyherbal Ayurvedic formulation used in the treatment of inflammation and pain related to *Amavata* [1] which may be correlated with Rheumatoid arthritis (RA) [2] in current scenario. It is characterized by polyarthritis (synovitis) that affects the hands and feet, although any joint lined by a synovialmembrane may be involved [3]. The feature of the disease is severe joint pain, swelling, tenderness and redness of the affected joints. In RA the inflammatory cytokines andmatrix metalloproteinases (MMPs), free radicals are also produced in excessive amounts which enhance the inflammatory pathways and interaction with host cells including chondrocytes, fibroblasts and osteoclasts [4]. Drug used in the treatment of rheumatoid arthritis are believed to show their therapeutic activity by several mechanisms, one of them being is believed to be reduction of oxidant damage at place of inflammation by drugs either acting as reactant oxygen intermediates(ROI) scavengers or inhibitors of ROI production by phagocytes [5].
In Indian system of medicine there is concept of *Rasayana*, which is more or less similar to the antioxidant therapy [6]. *Rasna saptak kwath* which is a formulation of eight herbs (Table 1) including *Zingiber officinale* which is used as a adjuvant, contain herbs *viz*. *Rasna (Pluchea lanceolata*), *Guduchi (Tinospora cardifolia), Punarnava (Boerhhia diffusa)* which are used as *Rasayana* in *Ayurveda* [7] while in contemporary science, these herbs of *RS* including *Rasna (Pluchea lanceolata*), *Guduchi (Tinospora cardifolia), Punarnava (Boerhhia diffusa), Aragvadha* (*Cassia fistula*) are reported for antioxidant activity [8]. The different extracts of herbs of *RS* have been reported for anti-inflammatory [9], analgesic [10], anti-arthritic [11] activity related to RA.
So, here is the need of research to know in which extract form, the same drug is giving more consistency of phyto-constituent, best antioxidant effect etc. The choice of media is restricted to two only *viz*. aqueous and hydroalcoholic. The reason behind this, in Ayurvedic classics, along with aqueous extract there is one more extraction method is mentioned i.e. sandhana kalpana (hydroalcoholic extraction) where alcohols are self generated. In this extraction method, extraction of herbs takes place in self generated hydro-alcoholic media. The main aim of the study was to get the best dosage form of same formulation with the focus on the point not to alter with pharmaceutical basics of Indian system of medicine. Hence, the present investigation dealt with the preparation of a standardized extract in two solvent media by mixing all the mentioned drugs in equal proportion and evaluated for its antioxidant activity with phyto-chemical screening.

Table 1: Ingredients of *Rasna Saptak* formulation

Ingredients of *Rasna Saptak*	Scientific name	Part used	Amount taken (gm)
Rasna	*Pluchea lanceolata* Clarke.	Leaf	50
Gokshura	*Tribulus terrestris* Linn.	Fruit	50
Guduchi	*Tinospora cordifolia* (Wild) Miers	Stem	50
Punarnava	*Boerhhia diffusa* Linn.	Root	50
Eranda	*Ricinus communis* Linn.	Root	50
Devdaru	*Cedrus deodara* (Roxb.) Loud	Stem	50
Aragvadha	*Cassia fistula* Linn.	Fruit	50
Sunthi	*Zingiber officinalae* Rosc.	Rhizome (dry)	50

Material and Methods

Procurement of Herbs

All the ingredients of formulations were procured from local market (Goladinanath, Varanasi, Uttar Pradesh, India) except *Eranda and Guduchi*, which were collected from botanical garden of Banaras Hindu University. Plant materials were authenticated in the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University (BHU), Varanasi, India, and voucher specimens were kept in the Museum of the Laboratory of Dravyaguna, BHU, Varanasi, India for future reference. These specimens were provided with a specific code or number and these numbers are, for *Rasna* DG/17-18/144, *Guduchi* DG/17-18/145, *Gokshura* DG/17- 18/146, *Punarnava* DG/17-18/147, *Eranda* DG/17-18/148, *Devdaru* DG/17-18/149, *Aragvadha* DG/17-18/150, *Sunthi* DG/17-18/151 respectively.

Chemicals

Chloroform, methanol, absolute ethanol, toluene, ethyl acetate, acetic acid, potato dextrose agar (PDA) medium (potato 200 g; dextrose 20 g; agar 18 g and distilled water 1000 ml), sucrose, magnesium, potassium, and 2,2-diphenyl-1- picrylhydrazil (DPPH) were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Silica gel G for thin layer chromatography (TLC) was procured from SRL, Mumbai, India. Gallic acid procured from Natural remedies, Bengaluru, India (purity >97%).

Preparation of plant extract

The whole plant material of *RS* [Table 1] was dried and grind to prepare *kwath churna* i.e. coarse powder (mesh size # 60), and then, it was collectively, extracted through two different processes. One of the extractions was done through classical method [12] described in Ayurvedic treasures i.e. through decoction method where the temperature was maintained at 8–85°C. Another extraction was done in hydro-alcoholic media (50:50), i.e., distilled water and absolute ethanol, through soxhlet process at temperature 80 °C [13]. Practical details of both the extraction presented in Table 2 and 3 respectively.

Evaluation of Physico-Chemical Parameters

According to Ayurvedic pharmacopeia of India the prepared *Kwath churna* (coarse powder for decoction) and extract must go for certain physico- chemical parameters evaluation, to establish their strandization. So, the *kwath churna* evaluated on certain parameters. Different physicochemical investigations of the *kwath churna*as well as both the extracts were carried out on all the three batches using standard pharmacopoeial methods which include determination of alcohol soluble extractives, water soluble extractives, total ash, acid insoluble ash, loss on drying and pH determinations [14, 15].

Qualitative phytochemical study

The presence of phytochemicals such as flavonoids, phenolics, saponins, steroids and tannins in the plant was analyzed following standard protocols [16, 17]. The details of reagents used are given in Table 7.

High performance thin layer chromatography (HPTLC) analysis [18]

Silica Gel 60F_{254} pre-coated aluminium plates (Merck KGaA) of 10×10 cm with 0.2 mm thickness were prewashed by methanol and activated at 60° for 5 min prior to chromatography. The extracts were diluted up to a concentration of 1 mg/ml. Two microliters of each extract sample of 8 mm width was applied by auto sampler system-Camag Linomat. HPTLC plates were developed in Camag glass twin-through chamber (20×10 cm) previously saturated with the solvent for 60 min maintained at 60° and 40% relative humidity (RH). The development distance was kept to be 7 cm. Solvent systems consisting of Toluene: Methanol: Ethyl Acetate: Acetic Acid in the ratio of 3:0.5:5.5:1 (v/v).

Fourier transforms infrared (FTIR) spectrophotometric profiling [19]

For FTIR scanning the spectrophotometer (Varian 640 IR) was first calibrated and then the dried extract of *RS* was mixed with KBr (FTIR grade) at a ratio of 1:100 and ground to fine powder and compressed into a pellet form using hydraulic pressure. The pellet was immediately placed in the sample holder of the FTIR and the spectrum was recorded in the range of 4000-400 cm^{-1}. FTIR profiling spectra of two extracts of *RS* were collected.

Thin layer chromatography (TLC) analysis for marker component.

The two extracts were taken for TLC profiling with marker component [20] which gives an idea about the presence of phyto-constituent in extract. Gallic acid was taken as marker component as it is reported in *eranda (Ricinus communis)* which is one of the ingredients of *RS* [21]. Solvent systems [22] consisting of Toluene: Methanol: Ethyl Acetate: Acetic Acid in the ratio of 3:0.5:5.5:1 (v/v) was used for developing the TLC plates. The extracts were applied on 0.2 mm thick precoated Silica Gel 60F_{254} plates (Merck KGaA) and developed in the above mentioned solvent system. The developed TLC plates were examined under ultraviolet light at 254 nm and 366 nm.

Heavy metal analysis

Heavy metal analysis was performed using Perkin Elmer AAS-200 instrument. As per the protocol, sample digestion was carried out by multi-acid digestion system for lead (Pb), cadmium (Cd), Mercury (Hg), Arsenic (AS), zinc (Zn) [23]. After completion of the digestion process, the filtered samples were analysed by Atomic Absorption Spectrometer (AAS). However, being volatile, mercury (Hg) and arsenic (As) were digested using nitric acid-hydrochloric acid-potassium permanganate system before analysis [24]. The standards of Pb,

Cd, As, Hg, Zn, were purchased from Merck, Germany and utilised for development of the respective calibration curves for these metals.

Microbial limit test

Microbial analysis was carried out as per standard procedure mentioned in Ayurvedic Pharmacopeia of India. It included total bacterial count, total fungal count, and presence of pathogens like *Escherichia coli*, *Salmonella ebony*, *Pseudomonas aeruginosa and Staphylococcus aureus* [25].

Free radical scavenging activity

Free radical scavenging activity of both the extracts of *RS* was measured by recording the extent of bleaching of the purple-colored DPPH solution to yellow [26]. Different concentrations of extracts were added to 5 ml of 0.004% DPPH solution in methanol. After 30 min incubation at room temperature, the absorbance was measured against a blank at 517 nm using a spectrophotometer. Scavenging of DPPH free radical with reduction in absorbance of the sample was taken as a measure of their antioxidant activity IC_{50}, which represented the concentration of *extracts* that caused 50% neutralization of DPPH radicals was calculated from the graph plotting between percent inhibition and concentration.

$I\% = (A_{blank} – A_{sample}/A_{blank}) \times 100$, where, A_{blank} is the absorbance of the control (without test compound), and A_{sample} is the absorbance of the test compound.

Statistical analysis

Results of percentage yield of extraction and physicochemical parameters of different extract of *Rasna saptak* were expressed as mean±standard deviation (SD) of three in-house batches of the formulation. SD is standard deviation for n = 3 observations.

Results and Discussion

The extraction of *Rasna Saptak* was carried out in two different solvent media. Table 2 and 3 is all about extraction procedure adopt and percentage yield. The percentage yield was more in HA extraction. The number of factors play role in extraction process like extraction method as well as solvent based on sample matrix properties, chemical properties of the analytes, interaction, efficiency and desired properties [27]. In conventional extraction, heat is transferred through convection and conduction from the surface, here, the extractability of solvents depends mainly on the solubility of the compound in the solvent, the mass transfer kinetics of the product and the strength of solute/matrix interaction with corresponding heat and mass diffusion rate [28]. In soxhlet extraction because of synergistic effect of water and alcohol and more time exposure of solvent and heat on membrane structure resulting into the higher and faster diffusion or partition rate of the solute from the solid matrix into solvent may be the probable reason for the highest yield.

Table 2: Practical details for Aqueous extract of *Rasna Saptak* formulation

Parameters	Mean ±SD
Quantity(gm)	400±0.061
Water(lit)	3200±0.035
Percentage yield(w/w)	10.4±0.95
Temperature	85ºC±0.022
Total Duration (hrs.)	8±0.31

Table 3: Practical details for Hydro-alcoholic extract of *Rasna Saptak* formulation

Parameters	Mean ±SD
Quantity(gm)	400±0.024
Water(lit)	3±0.012
Temperature	80±0.011
Percentage yield(w/w)	12.2±0.56
Total Duration (hrs.)	15±0.043

Importance of Soaking

Overnight soaking was done before application of heat to allow the imbibition of the menstrum inside the tissues of the drug. Imbibition allows the entrapped air to escape, which may resist the flow of menstrum. Soaking of the raw materials soften the raw material through osmosis & diffusion process of solvents into the tissues and cells of raw materials. This process swelled the raw materials and increased diffusion pressure inside the cells which causes bursting of cell wall. Further, applying heat for the extraction, disturb and weakens the bond of phyto-constituents present in the cell and separate hydrophilic & hydrophobic substances. The collapses of cell wall lead to the diffusion and mass transfer of maximum active ingredients into solvent in the form of suspension. The duration of soaking should be decided according to the weather as excess duration may leads to unwanted microbial growth.

Quantum of heat for Kwath preparation

Mild heating with peak temperature 85 ºC along with continuous stirring was applied for proper extraction for reducing the chances of degradation of some of the active constituents, which may decompose due to hydrolysis. As due to the convection current setup by heating at low temperature, does not produce sufficient movement of the liquid. Thus, continuous mechanical stirring must be needed to facilitate the natural circulation evaporation. The quantum of heat applied for the preparation of Kwath should always be mridu (around 80-90 °C), which is needed for Samyak Virya Utkrishtata (proper extraction of the needful principles). Quantum of heat and the duration of heating are of prime concern for Kwatha nirmana. If a drug doesn't contain any of the heat labile substance, then, it could be subjected to repeated boiling for better extraction of the therapeutically active principles from the source drug.

Effect of Agitation in Kwath preparation

All the seers of Ayurveda emphasized on continuous agitation (Darvyavaghattayan) of the contents during the process of boiling. This will hasten the extraction and also avoid the drugs to settle down to the bottom of the container, avoiding possibilities of charring. The rate of transfer of solute from the boundary layer to the surrounding solute will always depend on the concentration gradient between these two regions and on the thickness of the diffusion pathway. Agitation will help to increase a dissolution pathway and by bringing fresh solvent into contact with the boundary layer so producing a high value for concentration gradient. The rate of

dissolution may therefore be markedly affected by agitation or stirring.

Physico-chemical parameters of *Kwath Churna* and Extracts

It was revealed from the result (Table 4 and 6) that *kwath churna* (KC) and both the extracts were found acidic while moisture content was found less in *kwath churna* and aqueous (AQ) extract which prevents the microbial growth while same was found more in hydro-alcoholic (HA) extract but still there is very less chance of microbial growth because of its self-preservative nature. Total ash was found more in KC which indicates presence of more inorganic content. Acid insoluble ash indicates silica content which was in acceptable limit in KC as well in extracts. Water soluble extractive (WSE), alcohol soluble extractive (ASE) and hydro-alcoholic soluble (HA) extractive gives idea about the amount of chemical constituents present in herbal drug. Water soluble extractives was more in comparison to alcohol soluble extractives but hydro-alcoholic extract was more in comparison to WSE and ASE, which indicates synergistic effect of water and alcohol in extraction process. The organoleptic characterization is given in table 5, where both the two extracts were found bitter in taste.

Table 4: Physico-chemical parameters of *Kwath churna* of *Rasna Saptak* formulation

Parameters	*Kwath churna* ± SD
Moisture content (%)	3.26 ± 0.02
pH(5% solution)	5.8 ± 0.057
Total Ash (%)	5.01 ± 0.06
Acid Insoluble Ash (%)	0.54 ± 0.035
Aqueous extract (%)	10. 9 ± 0.35
Alcoholic extract (%)	6.8 ± 0.30
Hydro-alcoholic extract (%)	12.1 ±0.40
Sieve analysis (60# size)	100% pass

Table 5: Organoleptic parameters of extracts of *Rasna Saptak* formulation

Organoleptic parameters	Hydro-alcoholic extract	Aqueous extract
Appearance	Semisolid	Granular
Odour	Aromatic	Aromatic
Taste	Astringent and bitter	Astringent and bitter
Colour	Blackish	Blackish brown
Touch	Sticky and rough	Rough and hard

Table 6: Physico-chemical Parameters of extracts of *Rasna Saptak* for mulation

Physico-chemical Parameters	Hydro-alcoholic extract Mean ± SD	Aqueous extract Mean ± SD
Moisture content (%)	8.6 ± 0.05	4.86 ± 0.2
pH (5%)	5.3 ±0.01	5.1 ± 0.03
Total Ash (%)	0.79 ± 0.03	0.85 ± 0. 03
Acid Insoluble Ash (%)	0.039 ± 0.02	0.046 ± 0.03

Phyto-chemical screening of two extracts

The phyto-constituents were found more in HA extract in comparison to AQ extract (Table 7). The alkaloid and steroid absent in AQ extract while quinine was absent in both the extract. The intensity of color provides idea an about the amount of phyto-constituent present in extract [29]. The intensity of color was found more for phenols, flavonoids and terpenoids while for alkaloid and tannin it was quite less which indicates the former are more in comparison to later ones. Except saponin almost all tested phyto-constituents were more found more in HA extract. Phytoconstituents like phenolic, flavonoids, etc., have been previously found to be useful in the management of arthritis [30]. The presence of the said constituents in both the extracts may be responsible for its clinical usefulness as anti-oxidant agent.

Table 7: Phytochemical screening of extracts *Rasna Saptak* formulation

Phyto-constituent	Reagents used	Observation	Aqueous extract	Hydro-alcoholic extract
Alkaloid	Wagner's reagent	Brown/red precipitate	-	++
Flavonoids	1% potassium hydroxide	Dark yellowcolour	+	+++
Phenols	10% aqueous ferric chloride solution	Blue green colour	+	+++
Tannins	$FeCl_3$	Blue green precipitate	++	++
Saponins	Distilled water heating	Frothing seen	++	+
Terpenoids	Salkowski test	A layer of the reddish brown colouration	+	+++
Quinones	alcoholic potassium hydroxide	Red to blue	-	-
Steroids	Liebermann-Burchard test	Bluish green	-	++

++++ means present in large quantity
+++ means present in moderate quantity
+ +means present in small amount
+ means in trace amount
- means absent

HPTLC fingerprinting of extracts

The HPTLC fingerprinting of phytoconstituents somehow confirm the number and intensity of phytoconstituents in extracts. The figure 1 and 2 revealed the fingerprint of phytoconstituents in HA and AQ extract respectively. The peak areas in HPTLC spectra give indication about the quantity of phyto-constituents [31]. The peaks area are much higher in fig 1 in comparison to fig 2, which indicates the more amount of phyto-constituents in HA extract. In HA extract 8 while in AQ 6 Rf values and having 3 common in both extracts (Table 8), which confirms the presence of three phyto-constituents in both the extract. The Rf value revealed

about the lipophilic and hydrophilic character of phyto-constituents. Compound with the larger R_f value is less polar or non-polar as compare to lower R_f because it does not stick to the stationary phase as long as the polar compound [32]. Both lipophilic as well as hydrophilic components are present in extracts.

Table 8: Rf values for extracts of *Rasna Saptak* formulation

Samples	No. of spots	Rf value
Hydro-alcoholic	8	0.21, 0.26, 0.37, 0.49, 0.63, 0.73, 0.83, 0.96
Aqueous extract	6	0.23, 0.37, 0.45, 0.55,0.73, 0.96

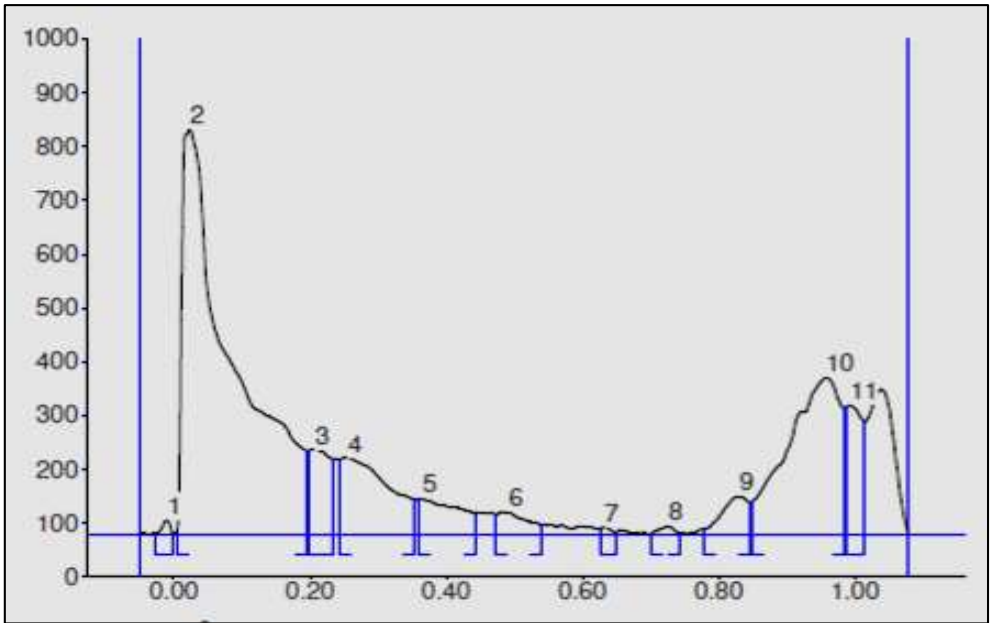

Fig 1: HPTLC fingerprinting of HA extract of *Rasna Saptak* formulation

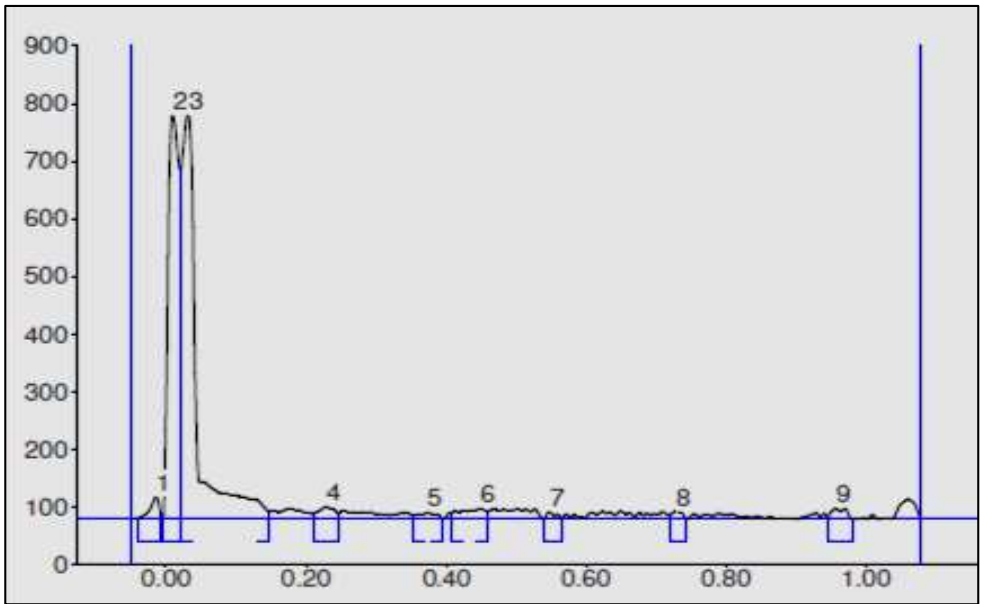

Fig 2: HPTLC fingerprinting of Aqueous extract of *Rasna Saptak* formulation

FTIR screening of both extracts

The FTIR spectrum is used to identify the functional groups of the components present in plant extracts based on the peak values in the region of IR radiation [33]. AQ extract presented the bands showed in figure 3. From the result (Table 9) it can be observed that, bands at 3449 cm^{-1} related to amine group (N-H). Bands at 2929 cm^{-1} represent hydrogen bonding, which could be recognized to alcohol,phenols and acids and tannins. A band was found at 1646 cm^{-1}. This band could be due to stretching vibration of C=C groups, due to aromatic ring deformations, due to flavonoids and amino acids: stretching vibration of C=C, asymmetric bending vibration of N-H.A band at 1413 cm^{-1}, C=C ring stretching or because of N=O bondings which represents nitro compounds. A band at 1104 cm^{-1} was considered to occur due to lipids and alcohol groups (stretching of C-O and bending of C-OH) [34, 35].

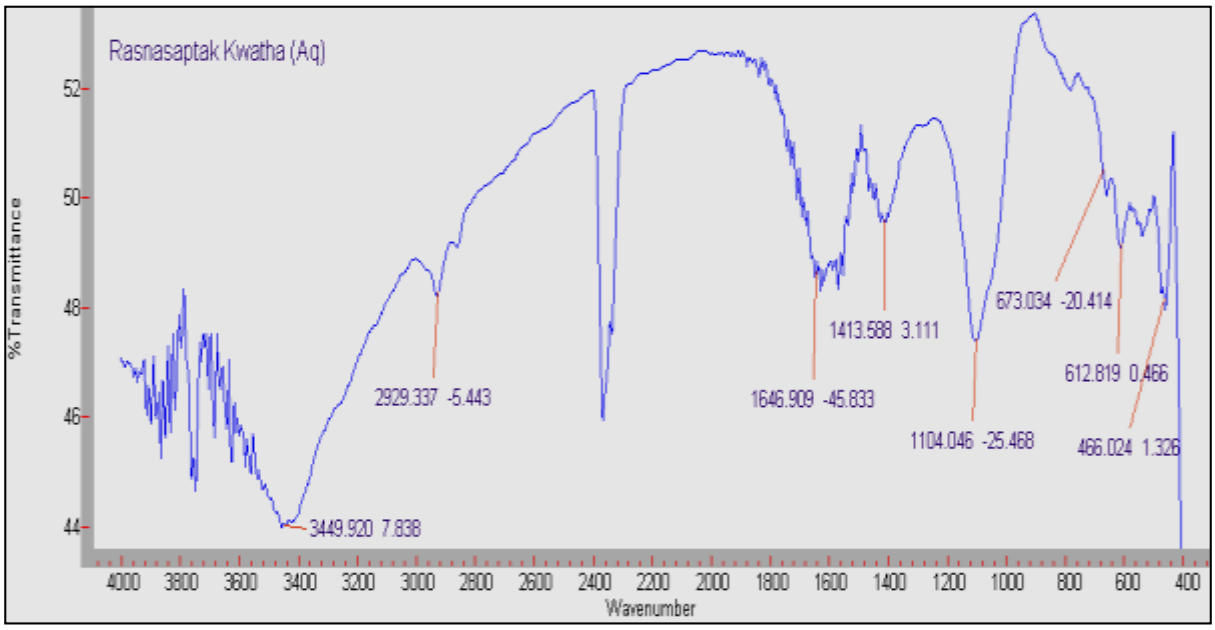

Fig 3: FTIR Spectra of aqueous extract of *Rasna Saptak* formulation

HA extract presented the bands showed in figure 4. It can be observed bands at 3432 cm^{-1}, related to OH wagging (OH of phenolic compounds) therefore it indicates the presence of polyphenols and flavonoids or confirms the presence of N-H i.e. amines or alkaloids. 2928 cm^{-1} and probably related to ethanol, since the samples extracts were alcoholic therefore ethanol bands could be in FTIR spectra. Band at 2664 cm^{-1} denote O-H bond related to acid groups. A band at 1701 cm-1, probably related to: stretching vibration of carboxyl groups, stretching of C = O of flavonoids and lipids. There was a band at 1454 cm^{-1}, probably due to the presence of aromatic -C=C- bond and or to N-O bending vibration which indicates the presence of nitro compounds. Peaks of 1264 correspond to C-N bonding vibration and indicate the presence of polyphenols and phenols. The band at 1067 denotes C-O alcohol and ester. The band at 825 cm^{-1} could be due to aromatic ring vibration or to ethanol and ether [36, 37].

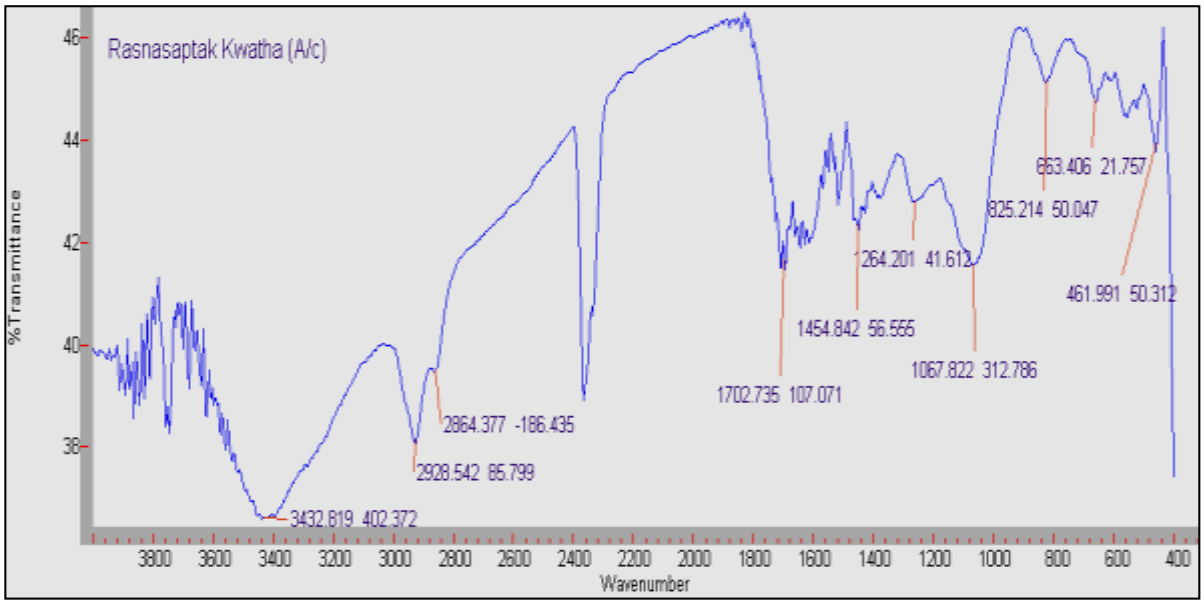

Fig 4: FTIR Spectra of hydro-alcoholic extract of *Rasna Saptak* formulation

The FTIR analysis revealed the presence of alkaloids due to N-H stretching, polyphenols and flavonoids due to O-H stretching, terpens due to C-H group. The FTIR spectrophotometer study predicted the presence of the groups: O-H, N-H, C-H, C-O C=C. in both the extracts. The functional groups present in both the extracts plant are alkaloid, phenols, alcohols, aldehydes, carboxylic group, alkenes, amines, amides, aromatics, ethers.

Table 9: FTIR screening of extracts of *Rasna Saptak* formulation

Aqueous extract	Peak characterization	Hydro-alcoholic extract	Peak characterization
612.819	Characteristic peak of Alkane	663.406	Characteristic peak of Alkane
673.034	Characteristic peak of Alkane	825.214	Characteristic peak of Alkane
1104.046	Alcohol and ether	1067.822	Alcohol and ether

1413.588	Nitro compounds	1264.201	Amines C-N
1646.909	Alkane and Alkene deformation or C-O-H bending or alpha-CH2 bending vibration	1454.842	Nitro compounds
2929.337	Hydrogen bonding in alcohol, Phenol and acid	1702.735	C=O, Aldehuyde, ketone, esters, carboxylic acid
3449.920	amines	2664.377	Hydrogen bonding in alcohol, Phenol and acid
		2928.542	C-H bonding, Hydrogen bonding in alcohol, phenol & Hydrogen bonds in acids
		3432.819	N-H, amines

TLC with marker components

As, it is one of the parameter mentioned in PLIM published by ministry of Ayush. In this study Gallic acid was taken as standard component. The presence of same Rf value in both extracts (Table 10) confirmed the presence of marker components i.e gallic acid in both the extracts. The main objective of our TLC with marker component was to develop unique spots i.e. Rf values (fig.5) in the formulation as an identifier of its ingredient. They also help to identify the presence or absence of the individual herbs in combination herbal formulations

Table 10: Rf value of marker component in extracts of *Rasna Saptak* formulation

Samples	Rf
Gallic acid	0.58
Aqueous extract	0.18, 0.58, 0.69, 0.72
Hydro-alcoholic extract	0.18, 0.34, 0.58, 0.69, 0.72,

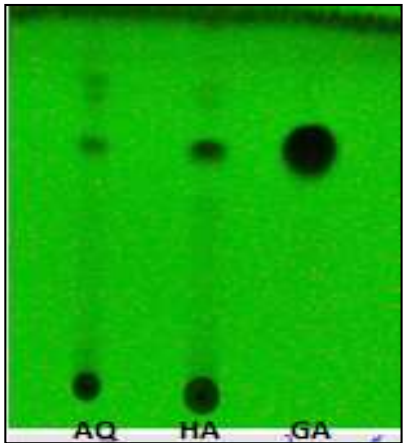

Fig 5: TLC profiling of extracts of *Rasna Saptak* formulation with Marker component

Elemental analysis

Metals are important part of body physiological function. If these metals accumulate in the body, in concentrations sufficient to cause poisoning, then serious damage may occur. Often heavy metals are thought as synonymous of toxins, but lighter metals may also be toxic in certain circumstances [38]. The contaminations of heavy toxic metals in plants could develop serious health problems because there is a narrow concentration range between the deficiency and toxicity levels of the heavy metals in human [39]. API has emphasized on various standard analytical techniques for the analysis of toxic heavy metals in plant products to ascertain their safety. Ayurvedic pharmacopeia of India has given limit for certain heavymetals in plant products to ascertain their safety [40]. Table 11 confirms the presence of heavy metal as well as trace element concentration in limit for cadmium and lead in both the extracts while arsenic and mercury were not detected in extracts. Hence, these two extracts are free from heavy metal contamination and therefore safe to use.

Table 11: Elemental analysis of extracts of *Rasna Saptak* formulation

Element		Wavelength	Aqueous extract(ppm)	Hydro-alcoholic extract(ppm)	Permissible limit(ppm)
Pb	Heavy metals	283.3	1.0	1.0	10
Cd		228.8	0.0114	0.0072	0.3
As		193.69	ND	ND	3
Hg		253.65	ND	ND	1
Zn	Trace metal	213.9	0.9246	0.9758	--

Microbial overload

The presence of microbial contaminants in herbal has the potential to adversely affect patients taking these medicines. Therefore measures should be taken to prepare safe medicines. These herbal extracts either free from microbial contamination or present within the prescribed limit. Table 12 shows that KC and extracts have TBC and TFC in prescribed limits and rest of the pathogens are absent. Therefore these two extracts will not provide any harmful effects to the individuals.

Table 12: Microbial load and specific pathogens in extracts of *Rasna Saptak* formulation

Parameters	Kwath churna	Hydro-alcoholic extract	Aqueous extract	Limits
Total Bacterial Count	1.8×10^{2} CFU/gm	70 CFU/gm	972 CFU/gm	1×10^{5} CFU/gm
Total Fungal Count	0.9×10^{1} CFU/gm	45 CFU/gm	460 CFU/gm	1×10^{3}CFU/gm

E. coli	Absent	Absent	Absent	Absent
Salmonella sp.	Absent	Absent	Absent	Absent
P. aeruginosa	Absent	Absent	Absent	Absent
P. aureus	Absent	Absent	Absent	Absent

Limits: As per Ayurvedic pharmacopeia of India

Free radical scavenging activity

The discoloration of the purple color of the DPPH confirmed the positive antioxidant activity of both the extracts. All of these showed free radical scavenging in a concentration dependent manner. Free radical inhibitory activity of aqueous as well as hydro-alcoholic extract at different concentration have been demonstrated in Figure 6 and 7 respectively. IC_{50} values have been summarized in Table 13. HA exhibited strong antioxidant activity showing lowest IC_{50} at concentration of 33.2 µg/ml while AQ extract showed poor antioxidant activity having highest IC_{50} at concentration of 480 µg/ml. Presence of different phyto-constituents in HA extract responsible for its strong anti-oxidant activity.

Table 13: IC_{50} of different samples of *Rasna Saptak* formulation

Parameter	Hydro-alcoholic extract	Aqueous extract
IC_{50}	33.2 (µg/ml)	480 (µg/ml)

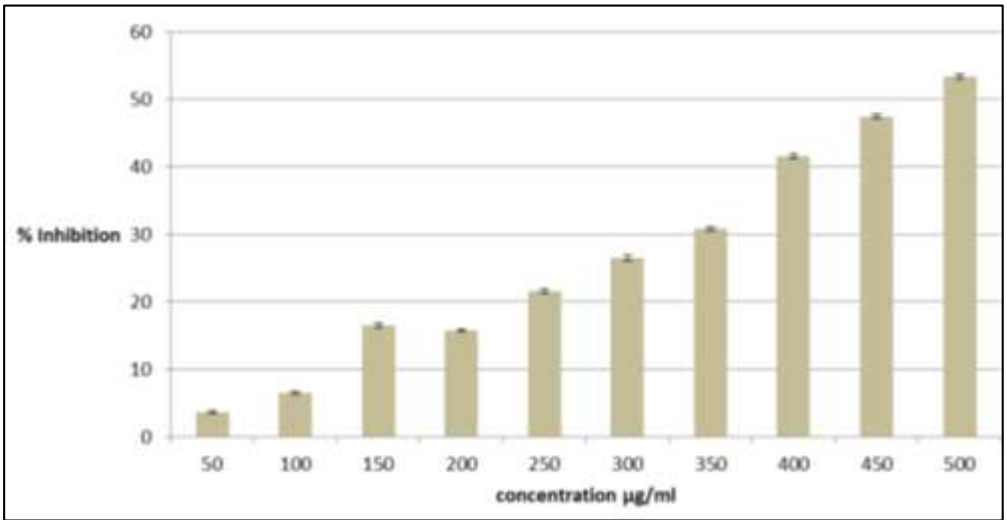

Fig 6: Antioxidant activity of aqueous extract of *Rasna Saptak* formulation

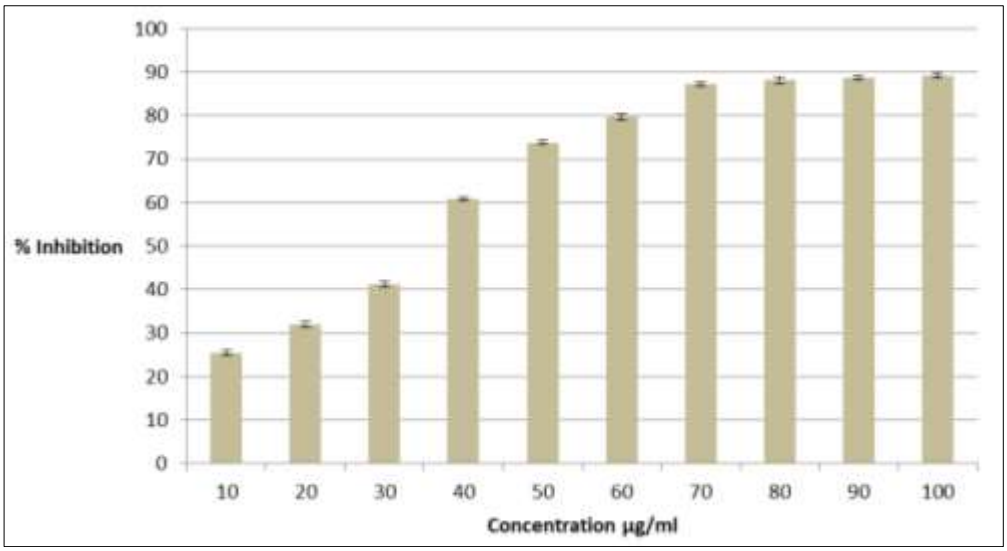

Fig 7: Antioxidant activity of hydro-alcoholic extract of *Rasna Saptak* formulation

The DPPH scavenging activity is correlated to the antioxidant activity of the herbals. DPPH works as a free radical and the herbals ability to scavenge the DPPH radicals shows the herbals' antioxidant activities. The antioxidant activity can be traditionally correlated with the amount of phenolic compounds in plants. Phenolic compounds would be the ones directly correlated with the antioxidant activity, but flavonoids could also be responsible for this effect [41]. Their mechanism of action is related to their ability to donate hydrogen and scavenging free-radicals [42]. The chemical activities of polyphenols in terms of their reducing properties as hydrogenor electron-donating agents predicts their potential for action as free-radical scavengers (ant;oxidants). Flavonoids inhibit the enzymes responsible for superoxide anion production, such as xanthine oxidase and protein kinase C, glutathione *S*-transferase, mitochondrial succinoxidase, and NADH oxidase, all involved in reactive oxygen species generation [43].

It has been proven that free radicals/reactive oxygen species play an important role in inflammation. Recent studies show the evidences for the involvement of free radical or reactive oxygen species in the pathogenesis of rheumatoid arthritis. In rheumatoid arthritis and juvenile idiopathic arthritis, increased oxidative stress and decreased levels of antioxidants have been found. An antioxidant combination to the treatment schedule of the disease revealed that the symptoms of arthritis were better controlled from the first month itself. Number of studies had confirmed that antioxidant intake help to reduce free radical generation and recover antioxidant status in RA patients.

Conclusion

The study reveals that hydroalcohoic (HA) extract, shows the best result in all parameters. The antioxidant revealed that HA extract is much more effective and potent may be because of more number as well as high intensity of phyto-constituents like flavonoids and phenols. Therefore it is suggested to convert this dosage from *kwath* (aqueous extract) to *sandhana kalpana* (hydro-alcoholic extract), to get much better effects in clinical practices to treat RA. Established physicochemical standards give important information for further investigations and facilitate the quality evaluation of herbal formulations in routine industrial production

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Conflict of Interest

There is no conflict of interest between the authors.

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