

A comparison of the antioxidant property of five Ayurvedic formulations commonly used in the management of *vata vyadhis*

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ABSTRACT

Background: The five *kashayams* (*kwaths* - decoctions) *Manjishtadi kashayam* (MK), *Rasna erandadi kashayam* (REK), *Sahacharadhi kashayam* (SK), *Maharasnadi* (or *Rasna dwiguna bhagam*) *kashayam* (MRK) and *Dhanwantharam kashayam* (DK) are widely used in the management of diseases manifested due to vitiation of *vata* and *vatarakta* (mostly diseases of connective tissues, bones, joints and nervous system). Free radicals are generated subsequent to the inflammatory changes in such conditions, and these cytotoxic reactive oxygen species cause oxidative damage to the cells. Phenolic compounds are the most common water soluble antioxidant compounds in plants. **Objective:** The present study aims at evaluating the phenolic content and antioxidant properties of these five *kashayams* and their probable protective role in the management of *vata vyadhis*. **Materials and Method:** The total phenolic contents of these five *Ayurvedic* decoctions were determined using Folin-Ciocalteu method and the antioxidant properties were estimated by DPPH (2,2-diphenyl-1-picrylhydrazine) radical scavenging activity. **Result:** MK exhibited higher property (total phenolic content-15.61 ± 0.006 mg/g wt, EC50-7.2 µg/ml) when compared to other *kashayams*. DK with phenolic content 12.056 ± 0.004 mg/g wt and 22 µg/ml effective concentration for 50% inhibition comes next in the present study. REK, SK and MRK show almost similar phenolic content and antioxidant property. **Conclusion:** From the observations, it is seen that the total phenolic content and the antioxidant property of the products justify the protective and corrective effects produced by the products in *vata* and *vatarakta* disorders.

Key words: Antioxidants, free radicals, *kashayam*, phenols

INTRODUCTION

Kashayam (decoction) is an important primary dosage form in *Ayurveda*. It is effective in attaining various pharmacological actions like *deepana*,^[1] *pachana*, *shamana*, *shodhana*, *tarpana* etc., when prepared according to classical guidelines, used in appropriate concentrations and clinical conditions. *Manjishtadi kashayam* (MK),^[2] *Rasna erandadi*

kashayam (REK),^[2] *Sahacharadhi kashayam* (SK),^[2] *Maharasnadi* (also known as *Rasna dwiguna bhagam*) *kashayam* (MRK),^[2] are quoted in the classical text *Sahasra Yogam* and *Dhanwantharam kashayam* (DK)^[3] is quoted in the classical text *Ashtanga Hrudayam*. These five formulations are extensively used in the treatment of *vata* and *vata-rakta* diseases.

In the management of *vata vikaras*, it is clearly indicated that in conditions where the *vata* alone is vitiated (no association with other doshas: *pitta* and *kapha*), one should initiate the treatment with *snehana karma*. But if other *doshas* are involved in pathogenesis along with *vata*, then *snehana karma* is contraindicated for treatment of the diseases.^[4]

The formulations taken for the study are described in the texts of *Ayurveda* in the *Kashayam* form. The clinical indications of these formulations have the involvement of other *dosas* especially *kapha*. It is observed in clinical practice that in the management of *vata vikaras* with the association of other *doshas* especially *kapha*, the *Kashaya* formulations are very effective and they do the function of *amahara* also.

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These formulations are studied for their total phenolic content and antioxidant properties. An attempt is made to correlate the clinical indications of these *kashayams* with the antioxidant property.

Diseases of connective tissues, bones, joints and nervous system are broadly included in *vatanyadhis* and *vatarakta vikaras*. *Ayurveda* as a system of medicine contributes profoundly to the wellness, curative and preventive aspects of these conditions.

In *vatanyadhis*, there is persistent inflammation in synovial membranes of joints, with migration of activated phagocytes and other leukocytes into synovial and periarticular tissue.^[5] Free radicals are generated in the process of phagocytosis, and these cytotoxic reactive oxygen species cause oxidative damage to the cells^[6] like destruction of membrane lipids, proteins, deoxyribonucleic acid and cartilage.

The enzymatic or free radical damage to proteins like IgG or collagen is also considered as a possibility that causes chronic inflammations in joints, local destruction of cartilage, bone and the systemic manifestations in rheumatoid arthritis.^[7] A molecule capable of inhibiting the oxidation of other molecules is known as an antioxidant; it either prevents the reactive oxygen species from being formed or removes them before it damages vital components of the cell.^[8] Oxidative stress occurs when there is an imbalance between oxidants and antioxidants in favour of oxidants which causes damage.^[9] Oxidative stress has been implicated in a wide variety of chronic inflammatory diseases like rheumatoid arthritis, lupus erythematosus and psoriatic arthritis.

Phenolic compounds are the most common water soluble antioxidant compounds in plants.^[10] The free radical scavenging role of the phenolic compounds is attributed to their unique structure.^[11]

The phenolic compounds in the water extracts thereby take the role of antioxidants and play a protective role in *vatanyadhis* where the damage of bones and cartilage takes place.

MATERIALS AND METHODS

Ayurvedic formulations

All the five formulations included in this study were manufactured by 'The Arya Vaidya Pharmacy' (Coimbatore) Limited.

Determination of total phenolic content

The study materials in the form of a decoction were taken

for the study. The total phenolic content was determined using Folin-Ciocalteu reagent.^[12] Appropriately diluted standard and samples were made up to 3.5 ml with distilled water in a series of test tubes. These tubes were then treated with 0.5 ml 2 N Folin-Ciocalteu reagent and incubated for three minutes at room temperature. The reaction was then neutralized by the addition of 1 ml 20% sodium carbonate. The reaction mixture was then incubated at room temperature for ninety minutes after which the absorbance was read at 760 nm (Shimadzu UV Vis spectrophotometer, 1800). The results were expressed as gallic acid equivalent in milligram per gram of sample, using a standard curve generated with gallic acid.

DPPH radical scavenging activity

The antioxidant activities of the *Ayurvedic* formulations were estimated by DPPH (2,2-diphenyl-1-picrylhydrazine) radical scavenging activity.^[13] Briefly, to various concentrations of the sample, methanolic solution containing DPPH radicals (0.1 mM) was added and shaken vigorously. The reaction mixture was then left to stand for thirty minutes in dark. After the incubation period the absorbance was measured at 517 nm against the corresponding test blanks. The percentage inhibition of DPPH free radical was calculated using the formula, % Inhibition = (Control-sample) / Control × 100.

The sample concentration providing 50% inhibition (EC50) was calculated from the graph of RSA (radical scavenging activity) percentage against sample concentration. Gallic acid was used as standard.

RESULTS

The results from total phenolic content determination and DPPH radical scavenging activity show that the *Ayurvedic* formulation, MK exhibited higher activity when compared to other *kashayams* [Tables 1, 2 and Figure 1]. DK with phenolic content 12.056 ± 0.004 mg/g and $22 \mu\text{g}/\text{ml}$ effective concentration for 50% inhibition comes next in the present study. The other three *kashayams* namely SK, MRK and REK exhibits almost similar activity with total phenolic content 10.61 ± 0.002 mg/g, 10.63 ± 0.006 mg/g, 10.31 ± 0.004 mg/g and percentage inhibition $36 \mu\text{g}/\text{ml}$, $26 \mu\text{g}/\text{ml}$ and $40.48 \mu\text{g}/\text{ml}$ respectively.

DISCUSSION

MK which shows the maximum phenol content and most antioxidant property is a combination of forty five herbs as shown in the Table 1. It is a classical *Ayurvedic* combination detailed in the context of *Vatarakta* in *sahasrayoga*. The combination has a broad spectrum of activity ranging

Table 1: Total phenolic content of kashayams

Sample name	Total phenolic content (mg/g) (*)
Manjishtadi kashayam	15.61 ± 0.006
Dhanwantharam kashayam	12.056 ± 0.004
maharasnadi (or Rasnadwigunabhagam) kashayam	10.63 ± 0.006
Sahacharadhi kashayam	10.61 ± 0.002
Rasna-erandadi kashayam	10.31 ± 0.004

*) Average of five determinations

Table 2: In vitro antioxidant property of kashayams

Sample name	EC50 (*)
Manjishtadi kashayam	7.2 µg/ml
Dhanwantharam kashayam	22 µg/ml
Maharasnadi (or Rasnadwigunabhagam) kashayam	26 µg/ml
Sahacharadhi kashayam	36 µg/ml
Rasna-erandadi kashayam	40.48 µg/ml

*) Effective concentration for 50% inhibition of DPPH radicals

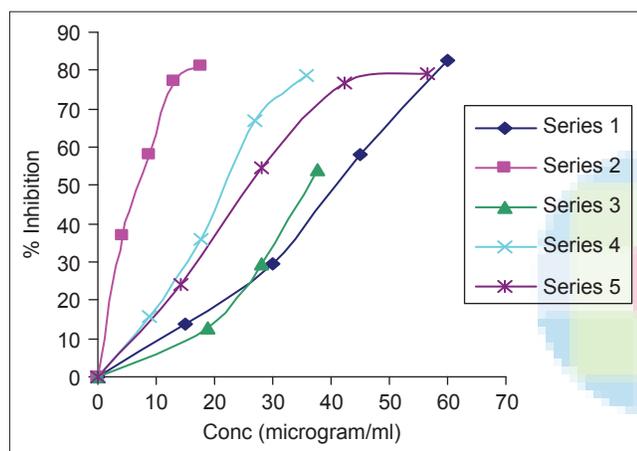


Figure 1: DPPH Radical scavenging activity of various kashayams. Series 1-REK, Series 2-MK, Series 3-SK, Series 4-DK, Series 5-MRK

from *astadasa kusta* (a wide spectrum of skin diseases), *vatarakta* (rheumatoid arthritis), *arditha* (bells palsy) *upadamsa* (venereal diseases), *sleepada* (filariasis), *prasupthi* (numbness), *pakshaghat* (hemi plegia), *medodosha* (diseases related to fatty depositions) *netra roga* (eye disease) etc. In clinical practice it is observed that in conditions like rheumatoid arthritis, skin disorders and degenerative disorders like hemiplegia, Bell's palsy and eye diseases, where oxidative stress causes or worsens the disease process, MK is very effective. The combination of MK has many potent *vatahara* herbs which are also antioxidants like *Rubia cordifolia*,^[14] *Cedrus deodar*,^[15] *Zingiber officinale*,^[16] *Tinospora cordifolia*,^[17] *Sida rhombifolia*,^[18] *Triphala*,^[19] *Hemidesmus indicus*^[20] etc. The antioxidant effect of the combination possibly makes it a useful combination in the treatment of *vatayadhis*.

DK stands next in the phenol content and antioxidant property. It is a versatile product with forty ingredients.

Even though it is indicated in the context of *garbha vyapad* (anomalies of pregnancy), it is effective in *vatarogas* (diseases affecting many connective tissues like bone, joints, ligaments, muscles and nervous system). It is also effective in traumatic conditions of bone and vital points. It is effectively used in *bala rogas* (pediatric diseases) and *soothika vikaras* (post partum care). It is also indicated in *javara* (pyrexia), *Gulma* (flatulence), *grabam unmada* (mental disorders) *Moothraghata* (urinary obstructions) and *andra vridhhi* (hernia).^[21] It is clinically seen that in conditions subsequent to trauma and also in arthritis or degenerative conditions subsequent to trauma, this formulation is exceptionally advantageous. In traumatic cartilage and joint injury there is an increased production of reactive oxidant species and reduced antioxidant defence. As a result of this imbalance, cell death and degradation of extracellular matrix occur.^[22] There is significant improvement in chondrocyte viability and protection against extracellular matrix damage, following joint injury when there is brief exposure to free radical scavengers.^[23] OS (oxygen species) play a role in multiple physiological processes from oocyte maturation to fertilization and embryo development. Oxidative stress occurs due to excessive production of free radicals and/or impaired antioxidant defence mechanism. Studies point that excessive free radicals precipitate in female reproductive tract pathologies.^[24] The antioxidant property of DK possibly takes care of the gynaecological anomalies.

MRK is the next product with highest antioxidant property. It is combination of twenty five herbs indicated in the context of *vatayadhis* in *sahasrayoga*. It is effective in *vatayadhis*, *sarvangavata*, *pakshagata*, *apabhabyugam*, *gridrasi*, *filariasis*, *andravridhi*, *sukla roga*, *linga roga*, *yoni roga*, *infertility*. It is anabolic to body and helps in the management of *infertility*. In clinical practice it is observed that the *brihmana* (nourishing) nature of this formulation helps in the correction and redeeming painful conditions of *vatayadhis* and also in correcting *infertility*. In both these conditions antioxidants play a vital role. The ingredients *Asparagus racemosus*,^[25] *Withania somnifera*,^[26] *Kaempferia galangal*,^[27] are all nourishing herbs which are *vatahara* and also anti oxidant.

SK stands fourth in the antioxidant property of the *Kashayams*. It is indicated in *vatayadhis prakarana* of *sahasrayoga* and is a combination of three herbs as shown in the table. It is particularly effective in the afflictions of lower limbs.

REK shows the least antioxidant property of the five *kashayams* indicated in *vatarakta* chapter of *sahasrayoga*. It is a combination of thirteen herbs as shown in the table. It is also indicated in *ekanga vatam* (hemiplegia) *swasa kasa*

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(respiratory disorders) *kanda rogas* (disease of throat) *soothika roga* (diseases in post partum stage), *jibwa sthambam* (paralysis of lingual muscles), (filariasis).

The phenol content of the formulations MRK, SK and REK is comparable.

From the observations, it is seen that the antioxidant property of the products substantiate the clinical indications of the products, where oxidative stress affects the system and the products provide corrective or protective functions in the conditions. The comparison of the antioxidant property is done to provide an idea on the sequence of the formulations which could be effectively used in an oxidative stress induced condition associated with the *vata* and *vatarakta* disorders.

CONCLUSION

The sequence of the kashayams in the descending order of the antioxidant property is as follows: MK, DK, MRK, SK and REK. The phenolic content is highest in MK, followed by DK. The phenolic content of MRK, SK and REK are comparable. The antioxidant properties of the *kashayams* justify the protective and corrective effects produced by the products.

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