# Anti-oxidant and anti-arthritic potential of Ayurvedic formulations: Maharasnadi quath extract and Stifain tablet

**Nimish Vador[1*], Bhavesh Vador[2], Nirali Rajgor[3]**

[1,2]Dept. of Medical, [3]Dept. of Research and Development, Ayurchem Products, Dombivali, Maharashtra, India

***Corresponding Author: Nimish Vador**
Email: nimishvador@gmail.com

## Abstract
Traditional system of medicines has been in use since ages throughout the world. Ayurvedic granthokt extracts have been in use to treat many disorders which are either non-communicable or lifestyle oriented or due to stress. Rheumatoid arthritis is one of the autoimmune disorder which might arise due to faulty metabolism of body or stress. It is a chronic inflammatory disorder which results in severe pain, discomfort and sometimes disability in the patients. Modern medicines aims to reduce pain but the root cause remains unattended. Hence the main objective was to assess the in vitro Antioxidant and Anti-inflammatory activity of the Ayurvedic formulation Maharasnadi kwath extract and Stifain tablet. The Antioxidant properties of the Maharasnadi kwath and Stifain tablet were evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Anti-inflammatory activity was estimated by inhibition of protein denaturation method. The aqueous extract of Maharasnadi and Stifain tablet showed good anti-inflammatory activities and potent antioxidant capacity.

**Keywords:** Antioxidant, Anti-inflammatory activity, Albumin denaturation, Maharasnadi kwath, Stifain tablet, Nervous system.

## Introduction
Inflammation in any part of the body results either due to physical injury or due to internal faulty metabolism of the body. Faulty metabolism of the body may originate from improper diet, irregular food timings, anger, stress. As per Ayurveda, faulty metabolism of body produces Aam (toxins or Reactive Oxygen Species). These Aam or Reactive Oxygen Speices (ROS) are highly unstable compounds and are key signaling molecules that play an important role in the progression of inflammatory disorders. As per Ayurveda ROS leads to imbalance in the three main pillars of the body that is vata-pitta-kapha. Under normal conditions, ROS levels are controlled by the body's complex antioxidant defense system and there is an equilibrium between ROS formation and degradation. Overproduction of ROS and/or inadequate antioxidant defense disturbs this equilibrium in favor of a ROS upsurge that results in oxidative stress. A deficiency in the body's natural antioxidant defense mechanisms has been implicated as the etiological or pathological factor in several clinical disorders.[1]

Various research studies have indicated the role of ROS in Rheumatoid Arthritis. ROS affects the micro-circulation of blood vessels near the site of inflammation. The microcirculation is the main playground where the process of inflammatory cascade was evaluated and analyzed.[1] Inflammation includes a long chain of molecular reactions and cellular activity, which are designed to restore a tissue from simple skin cut or to cure several burn injuries in inflammatory process, it is important to understand the role of chemical mediators. These mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors vascular permeability, neutrophil, chemotaxins, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or protein denaturation and mediate oxidative damage, causing the protein to lose its molecular conformation and functions or become denatured.[2-5] It is therefore deduced that, compounds which are able to prevent these changes and inhibit thermally or heat induced protein denaturation, have potential therapeutic value as anti-inflammatory agents.[4]

The World Health Organization (WHO) has estimated that 80% of the world inhabitants utilized traditional medicine for their primary health care needs and the majority of this therapy requires the use of herbal extracts and their active components. Various medicinal plant bioactive extracts and their identified/isolated active constituents have shown a variety of medicinal pharmacological properties against various acute and chronic diseases/disorders.[6-8] Currently, the impact of oxidative stress and its associated factors has become an important issue of human health.[9] When the body is under a lot of stress, results in protein denaturation and the production of ROS (e.g., hydroxyl radicals, superoxide anion radicals, and hydrogen peroxide) is amplified.[10]Endogenous enzymatic and non-enzymatic antioxidant substance are not able to handle the overload of ROS and lead to imbalances of the process, cell damage,[11] and health problems.[12]

**Table 1:** Composition of Stifain tablet **(**Each film coated tablet contains)

| S.No. | Ingredient Name. | Part used | Quantity |
|---|---|---|---|
| 1. | Maharasnadi kvatha[13] | Extract | 150mg |
| 2. | Yograj guggul[14] | Powder | 100mg |
| 3. | Mahavatvidhwans ras[15] | Powder | 50mg |

| | | | |
|---|---|---|---|
| 4. | Ekangvir ras[16] | Powder | 50mg |
| 5. | Sameer pannag ras[17] | Powder | 25mg |
| | Processed with (Bhavna Dravya) | | |
| 6. | Brahmi*(Bacopa monneri)*[18] | Whole plant | QS |
| 7. | Eranda *(Ricinus communis)*[19] | Root | QS |
| 8. | Guduchi *(Tinospora cordifolia)*[20] | Stem | QS |
| 9. | Shatavari *(Asparagus racemosus)*[21] | Root | QS |
| 10. | Parsika yavni *(Hyoscymus niger)*[22] | Seed | QS |

Fine powder of all these medicinal ingredients is combined together in Mixer along with other approved Tablet excipients. Granules are prepared from the earlier process is punched in to tablets. Tablets obtained are coated with moisture barrier coat and later with FDA approved color coat to identify the product.

**Table 2: The** Polyherbal Formulation of Maharasnadi kwath[23]

| S.No. | Name of Ingredients | Botnical name | Part used | Proportion |
|---|---|---|---|---|
| 1 | Rasna | Pluchea lanceolata | root | 2 parts |
| 2 | Eranda | Ricinus communisLinn. | Whole | 1 part |
| 3 | Devadaru | Cedrus deodar Roxb. | Root | 1 part |
| 4 | Shati | Hadychium spicatum | Heart wood | 1 part |
| 5 | Vacha | Acorus calamus | Root | 1 part |
| 6 | Vasa | Adhatoda vesica | Whole plant | 1 part |
| 7 | Sunth | Zingiber officinalis | Root | 1 part |
| 8 | Harda | Terminali achebula | Fruit | 1 part |
| 9 | Bala | Sida cordifolia | Root | 1 part |
| 10 | Chavya | Piper chaba | Fruit | 1 part |
| 11 | Musta | Cyperus rotundus | Root | 1 part |
| 12 | Punarnava | Boerhavia diffusaLinn | Root | 1 part |
| 13 | Guduchi | Tinospora cordifolia | Stem | 1 part |
| 14 | Vriddhdaru | Argyreia speciosa | Stem | 1 part |
| 15 | Shatpushpa | Anethum sowa | fruit | 1 part |
| 16 | Gokshura | Tribulus terrestris | Fruit | 1 part |
| 17 | Aswaghandha | Withania somnifera | Root | 1 part |
| 18 | Shatavari | Asparagus recemosus | Root | 1 part |
| 19 | Ativisha | Aconitum heterophyllum | Root | 1 part |
| 20 | Garmala | Cassia fistula | Stem bark | 1 part |
| 21 | Pippali | Piper longum | Fruit | 1 part |
| 22 | Sahachara | Barleria prionitis | Whole plant | 1 part |
| 23 | Dhanyaka | Coriandrum sativum | Fruit | 1 part |
| 24 | Brihati | Solanum indicum | Whole plant | 1 part |
| 25 | Kantakari | Solanum surratance | Whole plant | 1 part |
| 26 | Dhamasa | Fagonia arabica | Whole plant | 1 part |

## Materials and Methods

Chemicals - 2,2-diphenyl-1-picrylhydrazyl Powder (DPPH), Bovine serum albumin Fraction- V(BSA) and Aspirin powder from Hi Media. Solvent Methanol (analytical grade) obtained from Merck.

**Antioxidant activity using determination of DPPH free radical scavenging activity:[25]**

This free radical scavenging Capacity of aqueous Extract of Maharasnadi and Stifain tablet was determined using based on 2, 2-diphenyl-1-picrylhydrazyl.the ability of the antioxidants present in the Maharasnadikwath and Stifaintablet polyherbal formulation to decolorize the DPPH radical. DPPH radicals absorbed maximum at 514 nm, which disappears with reduction by an antioxidant compound.

About 0.5gm of Maharasnadi kwath and Stifain tablet Powder was measured using an Analytical balance (Citizen CY 220) and was added to 5 ml of distilled water separately. The solution was mixed well using a vortex. Boil on water bath for 10 min. Serial dilution from 100 to 1000 µg/ml was performed using methanol for Maharasnadi kwath and Stifain tablet.

**Test solution (3ml)**

2.5ml DPPH solution in methanol (100µM) was mixed with 0.5ml of different Concentration of extracts (mcg\ml).

**Control solution (3ml)**
2.5ml DPPH solution in methanol (100µM) was mixed with 0.5ml of methanol (without extract).

Test solution (0.5 ml) of different concentrations (100 to 1000mcg/ml) and Control solution were mixed with 2.5ml DPPH solution (100µM). Then the samples were incubated at Room temperature in dark for 20min.Absorbance of reaction mixture was measured for each concentration at 514 nm using UV-Visible spectrophotometer (Shimadzu UV-1800) Each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of Radical scavenging activity was determined on a percentage basis with respect to control using the following formula:

**Inhibition of DPPH (%) = (AC - AT/ AC) X 100**
Where,
AC - Absorbance of control solution
AT - Absorbance of Test solution.

**Anti-inflammatory activity using Bovine serum albumin (BSA) denaturation method:**[26]
About 0.2gm of Aspirin, Maharasnadi kwath and Stifain tablet Powder was measured using an Analytical balance (Citizen CY 220) and was added to 20 ml of distilled water separately. The solution was mixed well using a vortex.
Serial dilution from 1000µg/ml to 0.01µg/ml was performed for Maharasnadi kwath, Stifain tablet and for reference Drug (Aspirin).

**Test solution (0.5ml)**
0.45 ml aqueous solution of BSA 0.5%w/v and 0.05ml Test solution of different concentrations were used.

**Test control solution (0.5ml)**
0.45 ml aqueous solution of BSA 0.5%w/v and 0.05ml Distilled water were used.

**Product control (0.5ml)**
0.45ml Distilled water and 0.05ml Test solution of different concentrations were used.

**Standard solution (0.5ml)**
0.45 ml aqueous solution of BSA 0.5%w/v and 0.05ml Aspirin of different concentrations were used.

Test solution (0.05ml) of different concentrations from 0.01 microgram per ml – 1000 microgram per ml and standard drug Aspirin (0.05ml) of different concentrations 0.01, 0.1, 1, 10, 100, 1000 µg/ml) were mixed with 0.5% w/v aqueous solution of BSA (0.45ml). Then the samples were incubated at 37ºC for 20min followed by incubation at 57ºC for 3min. 2.5ml of phosphate buffer (pH 6.4) was added to all the above samples after cooling. Absorbance of reaction mixture was measured for each concentration at 255nm using UV-Visible spectrophotometer (Shimadzu UV-1800) each test was repeated thrice and the mean absorbance was recorded. The percentage of inhibition of protein was determined on a percentage basis with respect to control using the following formula:

**Percentage inhibition (%) = 100- [(ATS - APC)/ ATC] x100**
whereas:
ATS - absorbance of the test solution
APC - absorbance of the Product control
ATC - absorbance of the test control solution

**Statistical analysis**
All data were analyzed statistically using UV spectrophotometer (Shimadzu UV-1800). The descriptive data were expressed as mean ± standard error of mean. Linear regression analysis was performed to find out correlation coefficient. The percentage of inhibition rate between different groups were analyzed by independent sample t-test.

## Results

**Antioxidant activity using DPPH**
Free radical scavenging potential (DPPH) of the polyherbal Formulation Maharasnadi kwath extracts and Stifain tablet at different concentrations is represented below. The free radical scavenging activity increases with increase in the concentration of the sample which was reflected at the decrease in the absorbance. The ability of Maharasnadi kwath extract and Stifain tablet to scavenge DPPH free radical was calculated as percentage inhibition which was 94.609% and 92.602% and respectively at concentration of 1000µg/ml. TheIC50 value of Maharasnadi kwath and Stifain tablet was found to be 395 µg/ml and 426 µg/ml Respectively. (Fig. 1,2)

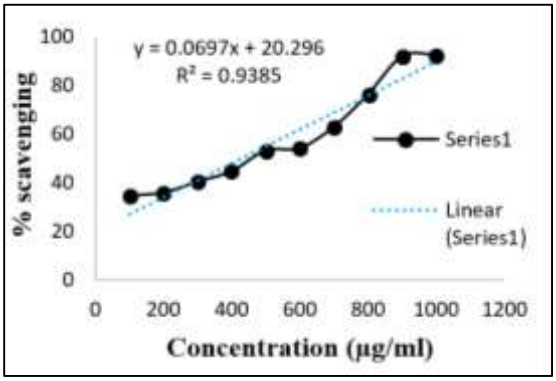


**Fig. 1:** Scavenging effect (%) on DPPH by Maharasnadi Kwath at different concentration

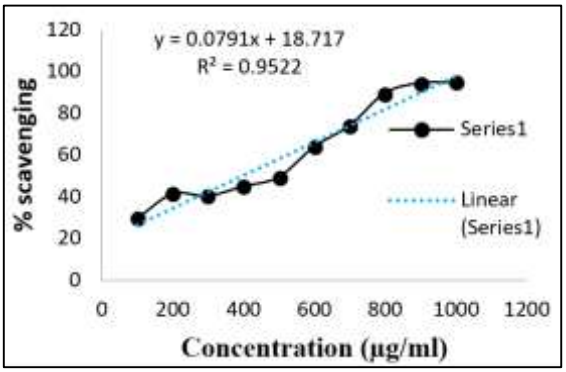


**Fig. 2:** Scavenging effect (%) on DPPH by Stifain tablet at different concentration

**Anti-inflammatory Activity using bovine serum denaturation**

Anti-inflammatory activity of Maharasnadi kwath extract and Stifain tablet was evaluated against BSA denaturation method. The highest inhibition rate was observed at the concentration of 1000 µg/ml shown 10.53% and 14.72%protected Bovine serum albumin (BSA) against heat denaturation at concentrations (Table 3).

This result compared with the reference drug Aspirin, it showed the maximum inhibition 17.43% at the concentration of 1000 µg/ml. (Table 4).

The value of IC 50 of aqueous extract of Maharasnadi kwath and Stifain tablet was 19,758 µg/ml, 11,079 µg/ml, respectively. (Table 3) In addition, the value of IC 50of Aspirin was 8877 µg/ml. (Table 4).

**Table 3:** The percentage of inhibition rate of protein denaturation of Maharasnadi kwath and Stifain table.

| Concentration (µg/ml) | Rate of inhibition (%) | |
|---|---|---|
| | Maharasnadi kwath | Stifain tablet |
| 0.01 | 6.35 ± 0.01 | 9.44 ± 3.16 |
| 0.1 | 8.01 ± 0.40 | 10.57 ± 0.01 |
| 1 | 9.35 ± 0.46 | 10.95 ± 0.81 |
| 10 | 9.53 ± 0.18 | 12.84 ± 0.01 |
| 100 | 9.63 ± 0.01 | 12.86 ± 0.02 |
| 1000 | 10.53 ± 0.20 | 14.72 ± 1.36 |
| **IC 50** | **19758 µg/ml** | **11,079 µg/ml** |

Results are shown as mean ± SEM. SEM: Standard error of the mean

**Table 4:** The percentage of inhibition rate of protein denaturation of reference drug Aspirin.

| Concentration (µg/ml) | Rate of inhibition(%) of Aspirin |
|---|---|
| 0.01 | 12.02 ± 2.80 |
| 0.1 | 12.19 ± 3.00 |
| 1 | 12.50 ± 0.01 |
| 10 | 15.70 ± 1.71 |
| 100 | 16.30 ± 0.93 |
| 1000 | 17.43 ± 0.02 |
| **IC 50** | **8877 µg/ml** |

Results are shown as mean ± SEM. SEM: Standard error of the mean.

## Discussion

Standardized aqueous extract of Maharasnadi quath extract was used to study the anti-oxidant and anti-arthritic property. The extract was standardized to total Phenolics content NLT 5%. The role of Maharasnadi quath extract is mostly observable on the nervous system and musculoskeletal system. Maharasnadi kwath pacifies inflammation and irritation of nerves and removes toxins from the body. Furthermore, it reduces inflammation of organs/parts of the musculoskeletal system. Therefore, it is recommended for paralytic disorders and arthritis. It has antitoxin and AMA Pachak (Detoxifier) actions, which reduces Aam formation, removes aamvisha from the channels and facilitates their quick elimination from the body. Therefore, it helps in diseases in which Aam or aamvisha are responsible or play as an underlying cause of the disease e.g. Rheumatoid Arthritis.

Stifain tablet is the multiherbal combination of Maharasnadi quath extract, Mahavatvidhwans Ras, Ekangveer ras, Yograj guggul processed with Ashwagandha, Shatavari, and other herbs. This tablet combination increases digestion power and opens up all the (shrotas) channels for proper blood circulation towards the site of inflammation. It might also reduce the nerve inflammation there by reducing the pain in rheumatoid arthritis. It possess good antioxidant property which further enhances the activity of the Stifain tablet.

## Conclusion

Standardized extract of Maharasnadi quath extract and Stifain tablets showed very good anti-oxidant and anti-arthritic activity. Stifain tablets may be used as a single drug therapy for arthritis.

## Source of Funding

None.

## Conflict of Interest

None.

## References

1. Schmid-Schonbein GW. Analysis of inflammation. *Annu Rev Biomed Eng*. 2006;8:93-151.
2. Kandikattu K, Bharath RKP, Venu PR, Sunil KK, Singh R. Evaluation of anti-inflammatory activity of Canthium parviflorum by in-vitro method. *Indian J Res Pharm Biotech*. 2013;1(5):729-30.
3. Dharsana JN, Mathew SM. Preliminary screening of anti-inflammatory and antioxidant activity of Morinda umbellata. *Int J Pharm Life Sci*. 2014;5(8):3774-79.
4. Anyasor GN, Funmilayo O, Odutola O, Olugbenga A, Oboutor EM. Evaluation of Costus afer Ker Gawl. In vitro anti-inflammatory activity and its chemical constituents identified using gas chromatography-mass spectrometry analysis. *J Coastal Life Med*. 2015;3(2):132-8.
5. Sridevi G, Sembulingam K, Muhammed I, Srividya S, Prema S. Evaluation of in- vitro anti-inflammatory activity of Pergularia daemia. *World J Pharm Res*. 2015;4(6):1100-8.
6. Chatterjee P, Chandra S, Dev P, Bhattacharya S. Evaluation of anti-inflammatory effects of green tea and black tea: A comparative *in vitro* study. *J Adv Pharm Technol Res*. 2012;3(2):136-8.
7. Tatti PN, Anitha S, Shashidhara S, Deepak M, Bidari S. Evaluation of *in-vitro* anti-denaturation activity of isolated compound of *Butea monosperma* Bark. *Pharma Sci Monit*. 2012;3(4):2314-20.
8. Dar SA, Yousuf AR, Ganai FA, Sharma P, Kumar N, Singh R. Bioassay guided isolation and identification of anti-inflammatory and anti-microbial compounds from *Urta dioica* L. (Urticaceae) leaves. *Afr J Biotechnol*. 2012;11(65):12910-20.
9. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. *Food Bioprod Processing*. 2011;89(3):217-33.

10. Gerber M, Ruault MC, Hercberg S, Riboli E, Scalbert A, Siess MH. Food and cancer: state of the art about the protective effect of fruits and vegetables. *Bull du Cancer*. 2002;89(3):293-312.
11. Bhatia S, Shukla R, Madhu SV, Gambhir JK, Prabhu KM. Antioxidant status, lipid peroxidation and nitricoxide end products in patients of type 2 diabetes mellitus with nephropathy. *Clin Biochem.* 2003;36(7):557-62.
12. Steer P, Millgard J, Sarabi DM. Cardiac and vascular structure and function are related to lipid peroxidation and metabolism. *Lipids*, 2002;37(3):231-6.
13. Sahatrayog (Sanskrit-Hindi translation), Panditrao DV (Anusandhan adhikari) (Ayu.), Nair D. Kendiriya Ayurved Eva Sidhha Anusandhan Parishad Publication: 80-1.
14. Rastantrasar, Published by Krishna Gopal Ayurved Bhavan, Ajmer; 1980;1:642.
15. Rastantrasar, Published by Krishna Gopal Ayurved Bhavan, Ajmer; 1980;1:459.
16. Rastantrasar, Published by Krishna Gopal Ayurved Bhavan, Ajmer; 1980;1:470.
17. Bhavprakash Nighantu – Shri bhavmishra commentary by Chunekar KC edited by Panday GS, Chaukhambha Bharthi Academy Publishers and Distributors of Monumental Treatises of the east. Varanasi. Republished 2004:461.
18. Bhavprakash Nighantu – Shri bhavmishra commentary by Chunekar KC edited by Panday GS, Chaukhambha Bharthi Academy Publishers and Distributors of Monumental Treatises of the east. Varanasi. Republished 2004:299.
19. Bhavprakash Nighantu – Shri bhavmishra commentary by Chunekar KC edited by Panday GS, Chaukhambha Bharthi Academy Publishers and Distributors of Monumental Treatises of the east. Varanasi. Republished 2004:270.
20. Bhavprakash Nighantu – Shri bhavmishra commentary by Chunekar KC edited by Panday GS, Chaukhambha Bharthi Academy Publishers and Distributors of Monumental Treatises of the east. Varanasi. Republished 2004:392.
21. Bhavprakash Nighantu – Shri bhavmishra commentary Chunekar KC edited by Panday GS, Chaukhambha Bharthi Academy Publishers and Distributors of Monumental Treatises of the east. Varanasi. Republished 2004:25.
22. Rasyogsar, Vaidya pandit hariprapannaji, Krishnadas academy Varanasi, Vol.2:489.
23. Shadgdharsahita 1971:226-7.
24. Dar MA, Mubashir H. Masoodi, Kour P, Shapoo NS. In vitro antioxidant activity of methanol aerial extract of mentha arvensis linn from Kashmiri Himalaya. *Am J Pharma Tech.* 2004;4:251-61.
25. Rahman H, Eswaraiah MC, Dutta AM. In-vitro anti-inflammatory and anti-arthritic activity of Oryza sativa var. joha rice (an aromatic indigenous rice of Assam). *Am-Eurasian J Agric Environ Sci.* 2015;15:115-21.

**How to cite this article:** Vador N, Vador B, Rajgor N. Anti-oxidant and anti-arthritic potential of Ayurvedic formulations: Maharasnadi quath extract and Stifain tablet. *Indian J Pharm Pharmacol* 2020;7(1):43-7.