ORIGINAL ARTICLE

In vitro antioxidant and anti-inflammatory activities of aqueous extract of an Ayurvedic formulation *Dasamula* and its herbal ingredients: A comparative study

V. Mahadevan[1], V. Vadivel[2], P. Brindha[3]

[1]School of Chemical and Biotechnology, SASTRA University, Thanjavur, Tamil Nadu, India, [2]Chemical Biology Lab, SASTRA University, Thanjavur, Tamil Nadu, India, [3]Deparrtment of CARISM, SASTRA University, Thanjavur, Tamil Nadu, India

Abstract

Aim: *Dasamula* is an Ayurvedic formulation, which is used for the management of pain, arthritis, and inflammatory disorders. Even though this formulation has been widely used in the Ayurvedic system of medicine to cure inflammation-mediated diseases, no deeper research studies have been conducted to prove its antioxidant and anti-inflammatory activities. **Materials and Methods:** In the present study, *Dasamula* was prepared using the roots of available herbal ingredients of such as Bilva (*Aegle marmelos* L. Correa), Syonaka (*Oroxylum indicum* L. Benth. Ex Kurz), Gambhari/Kumil (*Gmelina arborea* Roxb.), Patala/Pathiri (*Stereospermum suaveolens* Roxb.), Agnimantha/Munjai (*Premna serratifolia* L.), Salaparni/Orilai (*Desmodium gangeticum* L. DC), Prisniparni/Sithirapaladai (*Uraria picta* Jacq. Desv), Brhati/Sundai (*Solanum indicum* L.), Kantakari/Vaizhalanthai (*Solanum xanthocarpum* L.) and Goksura/Nerinjil (*Tribulus terrestris* L.) and screened for phytochemical profile, total phenol content, antioxidant activity (1,1-diphenyl-2-picryl-hydrazyl assay), and anti-inflammatory property (red blood cell membrane stabilization assay). **Results and Discussion:** From the results, it was seen that *Dasamula* had an impeccable anti-inflammatory activity. The *Dasamula* preparation contains various phytochemicals such as alkaloids, tannins, saponins, flavonoids and quinones, total phenolic content (567 mg gallic acid equivalent/100 g) and exhibits antioxidant (44.86%) and anti-inflammatory activities (32.56%), which are contributed by its herbal ingredients, especially *A. marmelos* and *G. arborea*. **Conclusion:** Among the roots of 10 different herbal ingredients are used in the preparation of *Dasamula*, only *A. marmelos* and *G. arborea* root extracts are found to be efficient as anti-inflammatory agents, and the remaining ingredients might have some medicinal roles in related therapeutic conditions/alleviating side effects.

Key words: Anti-inflammation, antioxidant, Ayurvedic drug, *Dasamula*, phytochemicals

INTRODUCTION

Dasamula literally means 10 roots in Sanskrit. It is a classically used Ayurvedic multi-ingredient formulation for the management of pain, arthritis, and inflammatory disorders.[1] The roots from five trees and five smaller plants are used as herbal ingredients in this formulation.[2] The herbal ingredients of *Dasamula* are: Bilva (*Aegle marmelos* L. Correa), Syonaka (*Oroxylum indicum* L. Benth. Ex Kurz), Gambhari/Kumil (*Gmelina arborea* Roxb.), Patala/Pathiri (*Stereospermum suaveolens* Roxb.), Agnimantha/Munjai (*Premna serratifolia* L.), Salaparni/Orilai (*Desmodium gangeticum* L. DC), Prisniparni/Sithirapaladai (*Uraria picta* Jacq. Desv), Brhati/Sundai (*Solanum indicum* L.), Kantakari/Vaizhalanthai (*Solanum xanthocarpum* L.), and Goksura/Nerinjil (*Tribulus terrestris* L.). According to the Ayurvedic Pharmacopoeia of India, all the ingredients were pulverized to course powder and mixed in the given proportion and added with four portions of water, kept for 4 h and heat to reduce the volume

Address for correspondence:
V. Vadivel, Chemical Biology Lab, SASTRA University, Thanjavur, Tamil Nadu, India.
E-mail: vadivel@carism.sastra.edu

Received: 07-09-2016
Revised: 04-10-2016
Accepted: 12-10-1016

to one-fourth. Then, the contents were filtered with muslin cloth and the filtrate was used as *Dasamulakvatha* for the treatment of cough, puerperal disorders, burning sensation in palms.[3]

A. marmelos L. Correa is a deciduous thorny tree belonging to the family Rutaceae. Most of the plant parts are of high medicinal value and have been used to treat a variety of diseases and ailments in the indigenous system of Indian medicine. It is used to treat diabetes, high cholesterol, peptic ulcer, inflammation, diarrhea, dysentery, constipation, respiratory infection, cancer, cardio problems, microbial infections, pyretic, analgesic, antioxidant, antiviral, anthelmintic and anti-inflammatory, hepatoprotective, and wound healing properties. The ripe fruit juice is aromatic, has cooling and laxative effects, and arrests secretion or bleeding.[4] Research studies confirm that the plant is rich in aegelin, marmelin, fagarine, marmesin, betulinic acid, auroptene, marmelosin, imperatorin, and limonene.[5]

O. indicum L. Benth. Ex Kurz is a species of flowering plant belonging to the family Bignoniaceae, commonly called midnight horror or Indian trumpet flower. It is a tree which can reach a height of 12 m. The seeds are used in the traditional Indian Ayurvedic medicine. A number of secondary metabolites such as flavonoids, glycosides, alkaloids, tannins, and terpenoids have been reported from various parts of this plant. The roots of the plant have abundant source of oroxylin A, baicalein, chrysin, pterocarpan, rhodioside, p-hydroxyphenylethanols, and cyclohexanols.[6]

G. arborea Roxb. is traditionally used in India for several medicinal purposes such as anthelmintic, diuretic, antibacterial, antioxidant and antidiabetic.[7] Extensive research has been done for the investigations on phytochemicals, anti-hyperglycemic, and *in vivo* toxic effects.[8]

S. suaveolens Roxb. is one among Dashamoola group of herbs. It is used in snake bite, scorpion bite, vomiting, etc. It is also used for neuroprotective and hepatoprotective benefits. The roots of *S. suaveolens* were found to contain p-coumaric acid, triacontanol, 3-acetyl alcohol, oleic, palmitic, stearic acid, lapachol, dehydroalpha-lapachone, and dehydrotectol in root heartwood and β-sitosterol and n-triacontal in root bark.[9]

P. serratifolia L. is a small tree/shrub in the Verbenaceae family. It has cardiotonic, anticoagulant, anti-inflammatory, antihyperglycemic, antiparasitic, antioxidant, and antimicrobial properties. Most of the plant parts of *P. serratifolia* have been used in the traditional system of medicine in India to treat various infectious diseases. The results of the preliminary phytochemical screening of the ethanol extract of *P. serratifolia* revealed the presence of phytoconstituents such as alkaloids, steroids, flavonoids, phenolic compounds, tannins, and glycosides specifically iridoid glycosides.[10]

Shalparni is one of the most important Ayurvedic herb used in Dasamula preparation. Its botanical name is *D. gangeticum* L. DC, belongs to Fabaceae family. Gangetin, a ptero-carpanoid was isolated from the roots of the plant. The roots were reported to have aminoglucosylglycerolipid, desmocarpin, tert-β-phenylethylamines, candicine, Indole-3-alkyl-amines, and β-carbolines.[11]

S. indicum L. belongs to the family Solanaceae, which is one among Dashamoola group of roots having potent anti-inflammatory activity. Apart from its root, fruit is also used in treatment of respiratory disorders. The leaves of *S. indicum* showed the presence of stellate trichome. Ethanolic extract of the leaves of *S. indicum* showed antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus,* and *Escherichia coli*, whereas chloroform extract, acetone extract, and ethanol showed antibacterial against *Pseudomonas*.[12] Antioxidant content of *S. indicum* was high even compared to vegetables and fruits known for high antioxidant contents.

S. xanthocarpum L. belongs to the family Solanaceae. It is also called as yellow-fruit nightshade and Thai green eggplant. Apigenin, arachidic acid, campesterol, coumarin, cholesterol, cycloartanol, and caffeic acid were isolated successfully from the plant parts.[13]

T. terrestris L. is well-known and widely spread member, which has been used in folk medicine throughout history for conditions such as impotence, rheumatism, edema, hypertension, and kidney stones. The chemistry of *T. terrestris* has been extensively studied, and the occurrence of saponins, flavonoids, alkaloids, lignanamides, and cinammic acid amides have been reported.[14-16]

U. picta Jacq. Desv. is commonly known as prishnaparni or pithvan and belongs to the family Leguminosae. The leaves were showed antianxiety activity. The presence of flavonoids and triterpenoids was reported in the plant. The existence of *U. picta* in natural population is highly threatened. It is naturally propagated by seeds. However, the seed set is poor and seed viability and percentage of germination is low. Due to the unavailability of this plant, generally this plant is omitted in the preparation of *Dasamula* formulation. *Dasamula* formulation shows high anti-inflammatory efficacy even without adding *U. picta*.[17]

Even though this formulation has been used widely in Ayurvedic system of medicine to cure all types of edema, renal dysfunction, hydronephrosis, liver dysfunction, and hypothyroidism, there are only a few studies related to its botanical, chemical, and molecular properties.[2,18] However, no deeper research studies have been conducted to prove its antioxidant and anti-inflammatory activities. Hence, the present study was designed to evaluate the antioxidant and anti-inflammatory activities of *Dasamula* decoction to

provide scientific support for the therapeutic efficacy of this drug.

MATERIALS AND METHODS

Preparation of the Drug

Raw drugs *A. marmelos* L. Correa, *O. indicum* L. Benth. Ex Kurz, *G. arborea* Roxb., *S. suaveolens* Roxb., *P. serratifolia* L., *D. gangeticum* L. DC, *S. indicum* L., *S. xanthocarpum* L., and *T. terrestris* L. were collected from Derisanamcope, Kanyakumari District. Only *U. picta* Jacq. Desv. was omitted in the preparation of *Dasamula* drug due to its unavailability. All the ingredients were identified with the help of a Botanist Professor P. Brindha, CARISM, SASTRA University and authenticated using macroscopic and microscopic studies [Figure 1]. All the ingredients were powdered (particle size 1 mm) individually using a lab mill and then mixed according to the formulation given in the Ayurvedic formulary of India.[19] Powders of each individual ingredients and formulation were used for the preparation of decoction by taking 10 g of each drug in 100 ml of distilled water. The contents were heated for 30 min and filtered, and the filtrates were used for the analysis.

Phytochemical Screening

Phytochemical profile of herbal ingredients and formulation extracts were analyzed as per the methodology of Harborne.[20] For steroids test, 1 ml of extract was taken and add few drops of concentrated sulfuric acid, shake well and kept away some time and noted for color change. Terpenoids were analyzed by heating the extract in mild flame with tin and thionylchloride and noted the color change. For alkaloid test, 1 ml of sample was added with 1 ml of diluted acetic acid and few drops of Dragendorff's reagent and noted the precipitate. For the phenol test, 0.5 ml of extract was taken with 1 ml of alcoholic ferric chloride and noted for color change. For flavonoids test, 0.5 ml of extract was taken and 1 mg of magnesium turning and few drops of concentrated hydrochloric acid were added, boiled for 5 min and noted the color change. For the tannins experiment, few drops of ferric chloride solution were added with extract and noted the color change. The presence of saponins was examined by taking the extract with water and shaken hardly and noted for froth. For the quinone, 0.5 ml of extract was added with 1 ml of sodium hydroxide (10%) and observed for color change. For the coumarin assessment, 0.5 ml of extract was taken in a test tube with 1 ml NaOH and shaken well and noted the color change. To check the presence of sugars, extract was treated with Fehling's solution A and B and boiled and noted for precipitation color.

Total Phenol Content

The total phenolic concentration of decoction of all the ingredients and final drug were estimated according to the modified method of Singleton *et al*.[21] Extract (10 µl) was taken in a 96 well microplate, and 25 µl of Folin reagent and 230 µl of 4.4% of Na_2CO_3 were added and incubated for

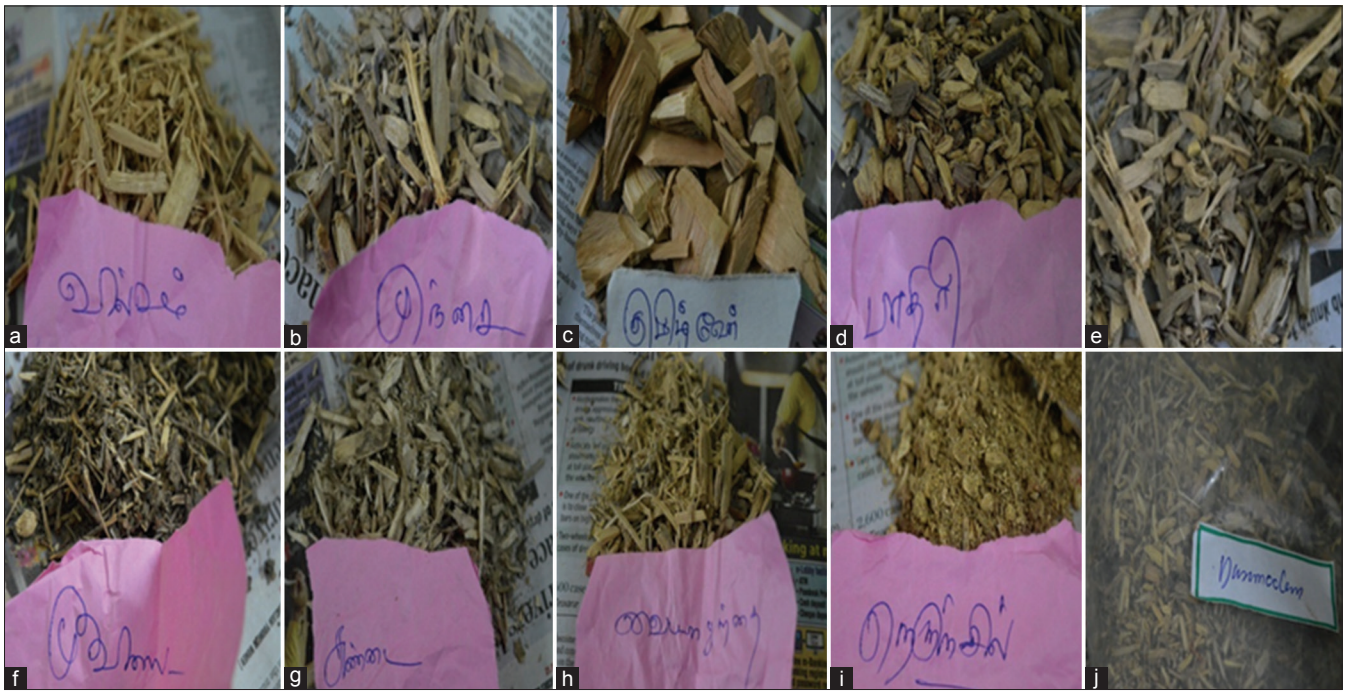

Figure 1: Herbal ingredients of *Dasamula* formulation, (a) *Aegle marmelos*, (b) *Oroxylum indicum*, (c) *Gmelina arborea*, (d) *Stereospermum suaveolens*, (e) *Premna serratifolia*, (f) *Desmodium gangeticum*, (g) *Solanum indicum*, (h) *Solanum xanthocarpum*, (i) *Tribulus terrestris,* (j) *Dasamula* formulation

30 min in dark place. Then, the absorbance was measured at 750 nm in the ELISA plate reader (Make: Biotek, Model: Epoch). A calibration curve was prepared using standard gallic acid (100-1000 mg/L, $R^2 = 0.9978$) and used to express the results as gallic acid equivalents (GAE).

Antioxidant Activity

The antioxidant activity of decoction was analyzed using 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay.[22] Extracts (10 µl) were taken in the 96 well microplate, and 200 µl of DPPH solution (2.5 mg/100 ml) was added and incubated for 30 min in dark place. Then, the absorbance was measured at 515 nm in the ELISA plate reader (Make: Biotek, Model: Epoch). The radical scavenging activity of tested samples was calculated using the formula (Antioxidant activity = Abs control − Abs test/Abs control × 100) and expressed on percentage basis.

Anti-inflammatory Activity

The anti-inflammatory activity of the decoction was evaluated using red blood cell (RBC) membrane stabilization method.[23] Blood sample (2 ml) was collected from a volunteer in a heparinized tube and washed with phosphate buffered saline twice and centrifuged at 3000 rpm for 10 min (Centrifuge Make: Eppendorf, Model 5810-R). Then, RBC was suspended in normal saline and taken in a tube (0.5 ml) with 0.5 ml of extract and 0.5 ml hypotonic solution and incubated for 30 min at room temperature. Then, the contents were centrifuged at 1500 rpm for 10 min and the supernatant was collected and the absorbance was read at 560 nm using Microplate reader (Make: Biotek, Model: Epoch). Based on the absorbance of extract and control, the membrane stabilization effect was calculated and expressed on percentage basis.

RESULTS AND DISCUSSION

Phytochemical Screening

The results of phytochemical screening of herbal ingredients and final formulation of *Dasamula* were given in Table 1. Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Alkaloids are found in *A. marmelos, S. suaveolens, D. gangeticum, S. indicum,* and *Dasamula* preparation [Table 1]. A wide range of biological effects have been reported for alkaloids including emetic, anticholinergic, antitumor, diuretic, sympatho-mimetic, antiviral, antihypertensive, hypnoanalgesic, antidepressant, miorelaxant, antitussigen, antimicrobial, and anti-inflammatory activities.[24] The alkaloids appear to offer the considerable promise for further investigation as anti-inflammatory compounds, and some appears to be remarkably active.[25] For example, colchicine is an alkaloid that reduces pain and swelling in degenerative and immunological inflammatory disease.[26] Tannins are naturally occurring compounds that exist in plants. Extracts of *S. suaveolens, D. gangeticum, T. terrestris, S. indicum, S. xanthocarpum, G. arborea,* and *Dasamula* formulation were found to contain tannins [Table 1]. Tannins are generally helpful in maintaining the antioxidant level in the body, which are useful for fighting against cancer, atherosclerosis, Alzheimer's, Parkinson's, diabetes, and heart diseases. Unlike cytotoxic agents that damage tumor cells, antioxidants act by preventing the onset of cancer during carcinogenesis, and they are generally beneficial to cells.

Saponins are glycosides with a distinctive foaming characteristic and have a bitter taste and consist of polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble)

Table 1: Phytochemical screening herbal ingredients and final formulation of *Dasamula*

Plant	Alkaloids	Tannins	Saponins	Terpenoids	Sterols	Flavonoids	Quinone
A. marmelos	+	−	+	−	+	−	−
O. indicum	+	+	+	−	−	+	+
G. arborea	−	+	+	+	−	+	+
S. suaveolens	+	+	−	+	−	+	−
P. serratifolia	+	+	+	−	−	+	−
D. gangeticum	+	+	+	−	−	+	+
S. indicum	+	+	+	−	−	+	+
S. xanthocarpum	−	+	−	−	−	−	−
T. terrestris	−	+	+	−	−	+	+
Dasamula formulation	+	+	+	−	−	+	+

A. marmelos: Aegle marmelos, O. indicum: Oroxylum indicum, G. arborea: Gmelina arborea, S. suaveolens: Stereospermum suaveolens, P. serratifolia Premna serratifolia, D. gangeticum: Desmodium gangeticum, S. indicum: Solanum indicum, S. xanthocarpum: Solanum xanthocarpum, T. terrestris: Tribulus terrestris

sugar part. Saponins were found in *A. marmelos*, *D. gangeticum, T. terrestris, S. indicum, G. arborea,* and *Dasamula* formulation [Table 1]. Saponins exert a wide range of pharmacological activities including expectorant, anti-inflammatory, vasoprotective, hypocholesterolemic, immunomodulatory, hypoglycemic, molluscicidal, antifungal, antiparasitic, and many others.[27,28] The terpenoids are a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures that differ from one another not only in functional groups but also in their basic carbon skeletons. Terpenoids were found only in *S. suaveolens* and *G. arborea* and absent in other herbal ingredients as well as *Dasamula* formulation [Table 1]. Some terpenoids have been used for therapeutic purposes for centuries as antibacterial, anti-inflammatory, antitumoral agents, and in recent decades, research activity into the clinical potential of this class of compounds has increased continuously as a source of pharmacologically interesting agents. Molecular basis of the anti-inflammatory action of diterpenoids was investigated with special emphasis on their ability to modulate critical cell signaling pathways involved in the inflammatory response of the body such as nuclear transcription factor-kappa B activation.[29]

Sterols, also known as steroid alcohols, are a subgroup of the steroids and an important class of organic molecules. Among the presently studied herbal ingredients, only *A. marmelos* was found to contain sterols [Table 1]. Flavonoids are a group of plant metabolites thought to provide health benefits through cell signaling pathways and antioxidant effects. Flavonoids were found in *S. suaveolens, D. gangeticum, T. terrestris, S. indicum, G. arborea,* and *Dasamula* formulation [Table 1]. Flavonoids have been found to have antimicrobial, antiviral, antiulcerogenic, cytotoxic, antineoplastic, mutagenic, antioxidant, antihepatotoxic, antihypertensive, hypolipidemic, antiplatelet, and anti-inflammatory activities.[30] A quinone is a class of organic compounds that are formally derived from aromatic compounds such as benzene or naphthalene by conversion of an even number of –CH= groups into –C(=O)– groups with any necessary rearrangement of double bonds, resulting in a fully conjugated cyclic dione structure. Quinones were found in *D. gangeticum, T. terrestris, S. indicum, G. arborea,* and *Dasamula* formulation [Table 1].

Total Phenolic Content

The highest level of total phenolic content was found in *G. arborea* (936.00 ± 14.85 mg GAE/100 g), followed by *A. marmelos* (921.00 ± 2.12 mg GAE/100 g) and *Dasamula* formulation (567.75 ± 90.16 mg GAE/100 g) [Table 2]. Phenol is an aromatic organic compound and plays an important role in disease prevention and treatment. Phenolic compounds from medicinal herbs and dietary plants include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects).[31]

Antioxidant Activity

Oxidants such as reactive oxygen and nitrogen species that include the superoxide radical (O2•−), hydroxyl radical (OH•), hydroperoxyl radical (ROO•), peroxynitrite (•ONOO−), and nitric oxide (NO•), damage macromolecules, such as proteins, lipids, enzymes, and DNA. To combat these radicals, living organisms produce enzymes (e.g., catalase, superoxide dismutase, and peroxidase) or rely on nonenzymatic molecules, such as cysteine, ascorbic acid, flavonoids, and vitamin K for protection. An antioxidant is a molecule that inhibits

Table 2: Total phenolic content of herbal ingredients and final formulation of *Dasamula*

Plant drug	Total phenolic concentration (mg GAE/L)	Total phenolic content (mg GAE/100 g)
A. marmelos	1023.33±2.36	921.00±2.12
O. indicum	400.86±12.96	362.75±11.67
S. suaveolens	234.17±12.96	210.75±11.67
D. gangeticum	489.17±1.18	440.25±1.06
P. serratifolia	590.83±288.74	531.75±259.86
T. terrestris	531.67±37.71	478.50±33.94
S. indicum	131.67±16.50	118.50±14.85
S. xanthocarpum	323.33±33.00	291.00±29.70
G. arborea	1040.00±16.50	936.00±14.85
Dasamula formulation	630.83±100.17	567.75±90.16

A. marmelos: Aegle marmelos, O. indicum: Oroxylum indicum, G. arborea: Gmelina arborea, S. suaveolens: Stereospermum suaveolens, P. serratifolia: Premna serratifolia, D. gangeticum: Desmodium gangeticum, S. indicum: Solanum indicum, S. xanthocarpum: Solanum xanthocarpum, T. terrestris: Tribulus terrestris

the oxidation of other molecules. The highest antioxidant activity was shown by *A. marmelos* (65.3 ± 2.16%), which is followed by *S. indicum* (51.04 ± 4.33%) and *Dasamula* preparation (44.86 ± 4.76%) [Table 3]. Five hydroalcoholic extracts of edible plants used in local traditional medicine of Italy for the treatment of inflammatory diseases were found to possess antioxidant property. In vitro antioxidant and antiradical properties of these plants was proven using DPPH radical scavenging assay, and all the extracts showed anti-inflammatory potential in animal model.[32]

Anti-inflammatory Activity

Anti-inflammatory refers to the property of a substance that reduces inflammation or swelling. The inflammatory response is coordinated by a large range of mediators that form complex regulatory networks. They activate specialized sensors, which then elicit the production of specific sets of mediators. The mediators, in turn, alter the functional states of tissues and organs (which are the effectors of inflammation) in a way that allows them to adapt to the conditions indicated by the particular inducer of inflammation.[33] Membrane damage refers to the damage of cell membranes which disturb the state of cell electrolytes, which when constantly increased, induces apoptosis.

RBC is essentially a bag of hemoglobin (Hb). The RBC is unique among eukaryotic cells in that it is a-nuclear, has no cytoplasmic structures and organelles. Structural properties are linked to the membrane. RBCs take up oxygen in the lungs and release it into tissues while squeezing through the body's capillaries. Anesthetics tranquilisers and nonsteroidal anti-inflammatory drugs stabilize erythrocytes against hypotonic hemolysis at low concentration. When RBC is subjected to hypotonic stress the release of Hb from RBC is prevented by anti-inflammatory agents because of membrane stabilization. So, the stabilization of HRBC membrane by drugs against hypotonicity-induced hemolysis serves as a useful *in vitro* method for assessing the anti-inflammatory activity of plant extracts. Maximum level of anti-inflammatory activity was seen in *A. marmelos* (87.49 ± 0.215%), which is followed by *Dasamula* preparation (32.56 ± 0.344%) and *G. arborea* (20.36 ± 0.043%) [Table 4]. Similarly, the membranes stabilizing profiles of various extracts/fractions of *L. camara* on bovine RBC exposed to both heat and hypotonic showed a significant amount of anti-inflammatory activity and exhibited a maximum activity of 27.95%.[34]

Table 3: Antioxidant activity of herbal ingredients and final formulation of *Dasamula*

Plant drug	Antioxidant activity (%)
A. marmelos	65.3±2.16
O. indicum	0
S. suaveolens	0
D. gangeticum	20.32±4.85
P. integrifolia	39.66±16.96
T. terrestris	12.42±14.28
S. indicum	51.04±4.33
S. xanthocarpum	22.96±13.42
G. arborea	13.65±3.20
Dasamula formulation	44.86±4.76

A. marmelos: Aegle marmelos, O. indicum: Oroxylum indicum, G. arborea: Gmelina arborea, S. suaveolens: Stereospermum suaveolens, P. serratifolia: Premna serratifolia, D. gangeticum: Desmodium gangeticum, S. indicum: Solanum indicum, S. xanthocarpum: Solanum xanthocarpum, T. terrestris: Tribulus terrestris

Table 4: Anti-inflammatory activity of herbal ingredients and final formulation of *Dasamula*

Plant drug	Anti-inflammatory activity (%)
A. marmelos	87.49±0.215
O. indicum	0
S. suaveolens	10.86±1.765
D. gangeticum	0
P. integrifolia	0
T. terrestris	0
S. indicum	16.86±1.119
S. xanthocarpum	5.33±1.937
G. arborea	20.36±0.043
Dasamula formulation	32.56±0.344

A. marmelos: Aegle marmelos, O. indicum: Oroxylum indicum, G. arborea: Gmelina arborea, S. suaveolens: Stereospermum suaveolens, P. serratifolia: Premna serratifolia, D. gangeticum: Desmodium gangeticum, S. indicum: Solanum indicum, S. xanthocarpum: Solanum xanthocarpum, T. terrestris: Tribulus terrestris

CONCLUSION

Although *Dasamula* formulation (an Ayurvedic drug) is used routinely to treat inflammation-mediated diseases like edema, there is no scientific validation till date. Hence, in the present study, we have analyzed the phytochemical profile, polyphenolic content, antioxidant and anti-inflammatory properties *Dasamula,* and its herbal ingredients. We have prepared this drug only with nine ingredients similar to commercial preparation because of unavailability of *U. picta*, so the role of this plant is not yet revealed. Among the nine ingredients, *A. marmelos* and *G. arborea* were found to possess higher medicinal effect. Further, actual role of other herbal ingredients such as stabilization of the drug, alleviating the side effects, preventing allergic reactions and also their contribution to the medicinal effect of *Dasamula* could be

revealed scientifically. Since *A. marmelos* and *G. arborea* contributed largely to the antioxidant and anti-inflammatory properties of *Dasamula*, they could be considered for new anti-inflammatory drug formulation in future, and its medicinal property could be proved through *in vitro* and *in vivo* models.

ACKNOWLEDGMENTS

Authors are thankful to the Honorable Vice-Chancellor of SASTRA University for his constant encouragement, support, and provided all the infrastructural facilities to carry out this research work.

REFERENCES

1. Parekar RR, Bolegave SS, Marathe PA, Rege NN. Experimental evaluation of analgesic, anti-inflammatory and anti-platelet potential of Dashamoola. J Ayurveda Integr Med 2015;6:11-8.
2. Rao ML, Savithramma N. Phytochemical screening of dasamoola - An Ayurvedic drug. Int J Pharm Pharm Sci 2011;3:318-20.
3. API. The Ayurvedic Pharmacopoeia of India, Part II. Vol. I. New Delhi: Department of Ayurveda, Yoga, Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Ministry of Health and Family Welfare, Government of India; 2007. p. 65-7.
4. Bharadwaj RL, Nandhal U. Nutritional and therapeutic potential of bael (*A. marmelos* Corr) fruit juice: A review. Nut Food Sci 2015;45:895-919.
5. Baliga MS, Bhat HP, Joseph N, Fazal F. Phytochemistry and medical uses of the bael fruit (*A. marmelos* Correa): A concise review. Food Res Int 2011;44:1768-75.
6. Padgilwara S, Manwara J, Vohrab M, Yogesh S. Traditional uses, phytochemistry and pharmacology of *Oroxylum indicum*: A review. Int J Pharm Phytopharmacol Res 2014;3:483-6.
7. Nayak BS, Ellaiah P, Dinda SC. Antibacterial, antioxidant and antidiabetic activities of *Gmelina arborea* Roxb fruit extracts. Int J Green Pharm 2012;6:3.
8. Attanayake AP, Jayatilaka KA, Pathirana C, Mudduwa LK. Antioxidant activity of *Gmelina arborea* Roxb. (Verbenaceae) bark extract: *In vivo* and *in vitro* study. J Med Nutr Nutraceu 2015;4:32-8.
9. Sreedevi A, Saisruthi K. Pharmacognostic and physiochemical studies of *Stereospermum suaveolens*. Nat Prod Chem Res 2015;3:6.
10. Muthukumaran P, Salomi S, Umamaheshwari R. *In vitro* antioxidant activity of *Premna serratifolia* Linn. Asian Pharma Press 2013;3:15-8.
11. Ganjhu RK, Mudgal PP, Arunkumar G. Pharmacological and phytoconstituent profile of *Desmodium gangeticum* - An update. Int J Pharmacog Phytochem Res 2014;6:643-57.
12. Srividya AR, Arunkumar A, Cherian B, Maheshwari V, Piramanayagam S, Senthoorpandi V. Pharmacognostic, phytochemical and anti-microbial studies of *Solanum indicum* leaves. Ancient Sci Life 2009;29:3-5.
13. Singh OM, Singh TP. Phytochemistry of *Solanum xanthocarpum*: An amazing traditional healer. J Sci Ind Res 2010;69:732-40.
14. Bourke CA, Stevens GR, Carrigan MJ. Locomotor effects in sheep of alkaloids identified in Australian *Tribulus terrestris*. Aust Vet J 1992;69:163-5.
15. Ren YJ, Chen HS, Yang GJ, Zhu H. Isolation and identification of a new derivative of cinnamic amide from *Tribulus terrestris*. Acta Pharm Sin 1994;29:204-6.
16. Li M, Qu W, Wang Y, Wan H, Tian C. Hypoglycemic effect of saponin from *Tribulus terrestris*. Zhong Yao Cai 2002;25(6):420-2.
17. Parmar VR, Jasrai YT. Effect of thidiazuron (TDZ) on *in vitro* propagation of valuable medicinal plant: *Uraria picta* (Jacq.) Desv. Ex DC. J Agric Res 2015;53:513-21.
18. Purushothaman KK, Sarada A, Sasikala E, Gopal H. Studies on *Dasamula* - its chemistry, botany & biology Part - I prisniparni. Anc Sci Life 1986;6:88-96.
19. API. The Ayurvedic Pharmacopoeia of India, Part I. New Delhi: The Ministry of Health and Family Welfare, Department of Indian Systems of Medicine and Homeopathy, Government of India; 2001.
20. Harborne JB. Phytochemical Methods. London: Chapman and Hall; 1973. p. 113.
21. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method Enzymol 1999;299:152-78.
22. Sanchez-Moreno C, Larrauri JA, Saura-Calixto F. Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. Food Res Int 1999;32:407-12.
23. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci 2010;2:146-55.
24. Souto AL, Tavares JF, da Silva MS, Diniz Mde F, de Athayde-Filho PF, Barbosa Filho JM. Anti-inflammatory activity of alkaloids: An update from 2000 to 2010. Molecules 2011;16:8515-34.
25. Filho JM, Piuvezam MR, Moura MD, Silva MR, Lima KV, Ivana LC, *et al*. Anti-inflammatory activity of alkaloids: A twenty-century review. Braz J Pharmacog 2006;16:109-39.
26. Malkinson FD. Colchicine. New uses of an old, old drug. Arch Dermatol 1982;118:453-7.
27. Sparg SG, Light ME, van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol 2004;94:219-43.
28. Sahu NP, Banerjee S, Mondal NB, Mandal D. Steroidal saponins. Fortschr Chem Org Naturst 2008;89:45-141.
29. de las Heras B, Hortelano S. Molecular basis of the

anti-inflammatory effects of terpenoids. Inflamm Allergy Drug Targets 2009;8:28-39.
30. Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. Mechanism of action of flavonoids as anti-inflammatory agents: A review. Inflamm Allergy Drug Targets 2009;8:229-35.
31. Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. Nutr Cancer 2010;62:1-20.
32. Conforti F, Sosa S, Marrelli M, Menichini F, Statti GA, Uzunov D, *et al. In vivo* anti-inflammatory and *in vitro* antioxidant activities of Mediterranean dietary plants. J Ethnopharmacol 2008 28;116:144-51.
33. Medzhitov R. Origin and physiological roles of inflammation. Nature 2008 24;454:428-35.
34. Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. Int J Plant Physiol Biochem 2010;2:46-51.

Source of Support: Nil. **Conflict of Interest:** None declared.