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COMPARITIVE PHYTOCHEMICAL SCREENING, HEAVY METAL ANALYSIS BY AAS AND IN VITRO ANTI INFLAMMATORY ACTIVITY OF MARKETED AND IN HOUSE FORMULATIONS OF UDARKALP CHURNA

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ABSTRACT

Churna is a fine powder of a mixture of drugs or a single drug prepared by air drying, finely powdering, mixing and sieving the drugs. In any ayurvedic system of medicine, standardization is the need of the hour. The increasing use of herbal drugs by the human is the driving force to evaluate the health claim of these agents and to develop the standards of quality, purity, safety and efficacy. Divya Udarkalp churna is an Ayurvedic formulation made from various medicinal plants and is used for constipation, maintaining good digestive health, as a digestive aid, Dyspepsia, Indigestion, Inflammation of the colon, heartburn, alcohol abuse and ulcers. The main objective of the present study is to perform the comparative evaluation of the marketed and in-house formulations of divya udarkalp churna. The phytochemical screening of different extracts of both the formulations was performed. Both the samples were subjected to atomic absorption spectroscopy for heavy metal analysis to ensure the quality of the churna. The extracts were further screened for In vitro anti-inflammatory activity by protein denaturation assay. The results of the study showed the presence of essential phytochemical constituents, permissible levels of heavy metals and significant anti inflammatory activites in both the marketed and in house formulations. The results obtained may be utilized as tools of assistance to the scientific organizations for developing formulations of potential therapeutic intervention.

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INTRODUCTION

The application of herbs or herbal products for therapeutic intervention of prevailing ailments is the oldest form of healthcare known to humanity.[1] The world health organization (WHO) encourages, recommends and promotes the inclusion of traditional and herbal medicine in Nationwide health care programmes due to their availability at low cost and safety.[2] According to Kochhar, about 88% of the world's inhabitants rely on traditional medicine for their primary healthcare.[3] Divya Udarkalp churna is a product of the Patanjali Ayurveda. The advent of Patanjali Ayurveda dates back to 2007 by a Yoga Guru Baba Ramdev. This Company started with an aim to bring awareness among Indian people towards the materials made in India from the materials that have been produced in India. This company manufactures more than 900 products which include 45 types of cosmetic products and 30 types of food products. [4, 5] Although herbal medicines have been in use for thousands of years, the number of reports of people complaining of negative effects has also been increasing. WHO emphasized the need to ensure the quality of plant products by using modern analytical techniques and by applying suitable standards.[6,7] Several scientific reports have shown that herbal medicines contain toxic heavy metals which lead to toxic effects like cancer, liver dysfunction, lung disease, cerebral hemorrhage and alopecia. Increase in the contamination of general environment has led to the incorporation of toxic heavy metals in medicinal plants. [8] Lead, Cadmium and Arsenic must be controlled in herbal medicines in order to assure their safety.[9] The maximum acceptable limits are lead (10ppm), Arsenic (3ppm) and Cadmium(3ppm).[10] The preparation of Divya Udarkalp churna relies on traditional methods in accordance with the procedures given in classical texts.[11,12] The absence of modern pharmacopeial standards for the preparation of the churna increases the probability of compromised safety, efficacy and batch to batch inconsistency. Inflammation is a complex process involving the reaction of living tissues to injury, infection or irritation. Owning to the presence of liquorice, ginger and fennel in the churna, it claims significant anti-inflammatory properties. A recent animal study indicates that liquorice may be useful in treating lupus. It is now known that glycyrrhizic acid and its aglycone glycyrrhetinic acid present in the root extract are responsible for these biological activities. Non-steroidal anti-inflammatory drugs (NSAID) are the most commonly used agents to tackle acute or chronic inflammation. However these are associated with side effects like gastric irritation and formation of gastric ulcers. Hence, the search for natural sources and phytochemicals with anti-inflammatory activity has greatly increased in recent years. Various scientific studies bolster evidence that natural dietary constituents, such as polyphenols and flavonoids, possess significant anti-inflammatory activity. [13, 14] Phytochemical screening studies have not been reported for this churna. There is no scientific evidence that Udarkalp churna may act as an anti-inflammatory agent. Therefore, the objective of this work was to provide the scientific proof by carrying out preliminary anti-inflammatory activity using in vitro albumin denaturation assay. With this aim, the current project was designed to screen the in-house and marketed formulations of the churna for phytochemicals, screen them in terms of heavy metals (Pd, Cd, As) and evaluate their in vitro anti-inflammatory activities. [9, 15, 17]

MATERIALS AND METHODS:

Divya Udarkalp churna was selected because it had no previous scientific works reported. The composition has been enumerated in Table 1.

Materials

Plant Materials: (Ingredients)

All the crude drugs used in the preparation were procured from the local market and were authenticated by Dr. A. Sabitha Rani, Department of botany, College for women (OUCW), Koti. They were washed, dried and powdered individually following which they were passed through sieve no 50 to obtain a homogenous blend. This churna was stored in a tightly closed container for further study. [12]

Table 1: Composition of the churna.

S. No.	Plant name	Botanical Name	Part used	Quantity(gm)	Uses
1	Liquorice	Glycyrrhiza glabra	Stem	10.55	Anti-Inflammatory agent for stomach ulcers, skin treatment and eczema
2	Rhubarb	Rheum emodii	Root	10.55	Diarrheal agent
3	Fennel	Foeniculum Vulgare	Fruit	5.25	Carminative and anti-inflammatory agent
4	Senna	Cassia Angustifolia	Leaf	26.30	Used for Constipation
5	Ginger	Zingiber officinale	Rhizome	5.25	Used for inflammation and morning sickness
6.	Myrobalan	Terminalia Chebula	Fruit	10.50	Used for constipation and indigestion.
7	Sugar	-	-	31.60	Sweetener and to make the churna palatable

Marketed Formulation of Udarkalp churna:
The marketed formulation was purchased from the Patanjali Arogya Kendra.

Preparation of the drug extract:
The extract was prepared by the hot and cold Maceration processes. 5 gm of the marketed and In-house formulations were taken separately into a glass bottle containing 25 ml of methanol and chloroform solutions respectively and were put aside for 24 hours. These solutions were subjected to filtration by means of whatman filter paper using a Buchner funnel. The extracts were collected and stored in air tight containers.

Preliminary phytochemical screening:
Phytochemical screening was performed on the hot and cold extracts of both the churna formulations to detect the presence of phytochemicals. [17]

Tests for alkaloids:
Small quantities of methanolic and chloroform extracts of the powdered drugs were divided into four portions and the following tests were carried out -
1. Dragendorff's Test: - Absence of the formation of an orange brown precipitate with Dragendorff's reagent (solution of potassium bismuth iodide).
2. Mayer's Test: - Absence of the formation of a creamy white precipitate with Mayer's reagent (potassium mercuric iodide solution).
3. Hager's Test: - Absence of the formation of a yellow precipitate with Hager's reagent (saturated picric acid solution).
4. Wagner's Test: - Absence of the formation of a reddish-brown precipitate with Wagner's reagent (solution of iodine in potassium iodide).

Tests for carbohydrates and reducing sugars:
Small quantities of methanolic and chloroform extracts the powdered drugs were divided into respective portions and the following tests were carried out -
1. Molisch's test: To one portion of the extracts, few drops of α-naphthol solution in alcohol were added and mixed well followed by concentrated sulphuric acid from the sides. Purple ring at the junction of two liquids indicated the presence of carbohydrates.
2. Benedict's test: To one portion of the extracts, add equal volumes of Benedict's reagent and heated in boiling water bath for 5min. The appearance of green, yellow or red color indicated the presence of reducing sugars.
3. Fehling's test: One ml each of Fehling's A and Fehling's B were mixed and heated for one minute and equal volumes of the extracts were added and heated for 5- 10min on a water bath. First yellow, then brick red precipitate indicated the presence of reducing sugars.

Test for saponins:
Foam Test: - Small quantities of the extracts were shaken vigorously with water. Formation of persistent foam indicated the presence of saponin glycosides.

Test for glycosides:

1. Test for Anthraquinone glycosides- Borntrager's test: Marketed and in-house formulations of the churna were boiled with dilute sulphuric acid. Filtered and cooled. The filtrate was extracted with chloroform or benzene and dilute ammonia was added to it. The ammonical turned from pink to red due to the presence of anthraquinones glycosides.
2. Extracts when made alkaline did not show blue or green fluorescence indicating the absence of coumarin glycosides.

Test for Phenols and tannins:

Small quantities of methanolic extracts were treated with the following reagents and the appearance of corresponding color change endpoints indicated the presence of phenolic compounds and tannins.

1. With 5% Ferric chloride solution: Deep blue-black color.
2. With 10% lead acetate solution: White precipitate.
3. With 10% Potassium dichromate solution: Red precipitate.

Tests for flavonoids

Shinoda Test: - Methanolic and chloroform extracts were extracted with 95% ethanol and hydrolysed by concentrated hydrochloric acid. Pink colour appeared after adding the magnesium turnings. Formation of yellow precipitates when lead acetate was added to the residues indicated the presence of flavonoids.

Atomic Absorption Spectroscopy (AAS)

Instrument:

Atomic absorption spectroscopy was performed with SVL Spectronics Atomic absorption spectrophotometer. Hydride generator was used for the quantitative estimation of heavy metals like Pd, Cd and As. As a source of radiation, hollow cathode lamps were employed. Air/Acetylene was used as fuel and nitrogen was used as a carrier gas. Table 2 shows instrumental conditions for analysis

Chemicals: Nitric acid, hydrochloric ac

Nitric acid, hydrochloric acid, sulphuric acid, hydrogen peroxide, sodium borohydride and stannous chloride of analytical grade (E. Merck). The water used in all experiment was double distilled water. The standard solutions were prepared in five different concentrations to obtain calibration curve by diluting stock solutions (E. Merck) of 1000 ppm of each element.

Sample preparation:

Wet digestion method was employed for the digestion of samples. 10 ml of nitric acid was added to 2 g of accurately weighed dried in-house and marketed formulation in a 100 ml beaker and was heated on a hot plate at 95°C for 15 min. The digest was cooled and 5 ml of concentrated nitric acid was added and heated for additional 30 min at 95°C. The last step was repeated and the solution was reduced to about 5 ml without boiling. The sample was cooled again and 2 ml of double distilled water and 3 ml of 30% hydrogen peroxide was added. With the beaker covered, the sample was heated gently to start the peroxide reaction. If effervescence becomes excessively vigorous, sample was removed from the hot plate and 30% hydrogen peroxide was added in 1 ml increments, followed by gentle heating until the effervescence was subsides. 5 ml of concentrated hydrochloric acid and 10 ml of double distilled water was added and the sample was heated for additional 15 min without boiling.[18,19] The sample was cooled and filtered through a Whatman No. 42 filter paper and diluted to 50 ml with double distilled water.

Analysis of samples for heavy metals:

The samples following digestion were analyzed for Pb and Cd using flame atomic absorption spectrophotometer and for As using hydride generation technique.

The 1000 ppm standard solutions of elements were diluted in five different concentrations to obtain calibration curve for quantative analysis. All the measurements were run in triplicate for the samples and standard solutions. The instrumental conditions during the analysis of heavy metals are listed in table-2 giving details about parameters which are defined for respective metals.

Recovery studies:

In order to validate the method use, method of standard addition was used. [20] Hence, a recovery analysis was performed using method of standard addition. Standard solutions containing, Pb, Cd and As were prepared and spiked with digested samples, after dilution of sample to 50 ml.

Table 2: Instrumental Conditions for Analysis.

Parameter	Pb	Cd	As
AAS technique	Flame	Flame	Hydride generator
Wavelength (nm)	217	228.8	193.7
Light source	HCL	HCL	HCL
Flame type	A	A	A
Current	10	3.5	EDL
Slit width(nm)	1.0	0.5	1.0
HCL- Hollow cathode Lamp, Air/C_2H_2- A			

Screening for anti-inflammatory activity by bovine serum albumin protein denaturation assay

The anti-inflammatory activity of the chloroform extracts was studied by using albumin denaturation assay according to the methods described by Gambhire et al [21] and Gunathilake et al [22] with minor modifications. The reaction mixture of 5 ml consisted of 0.2 ml of 1% bovine albumin solution, 2.8 ml of Phosphate buffer saline (pH 6.4) and 2 ml of the extract. The reaction mixtures were incubated at 37ºC for 20 min and then heated to 71 ºC for 5 min. Following this they were cooled to room temperature and turbidity was measured at 660nm (UV Visible Spectrophotometer) The experiment was performed in triplicate.

The Percentage inhibition of protein denaturation was calculated as follows: Percentage inhibition = (Abs Control – Abs Sample) X 100/ Abs control.

RESULTS AND DISCUSSIONS:

The marketed and in-house formulations of the churna were evaluated for their phytochemical constituents, heavy metals and anti-inflammatory property.

Phytochemical Screening

The results of phytochemical screening of alcoholic and chloroform extracts as indicated in the Table 3 revealed the absence of alkaloids and the presence of glycosides, carbohydrates, tannins, flavonoids which correlates with the potent therapeutic activity of the churna. [23]

Atomic Absorption Spectroscopy

The results of Atomic absorption spectroscopic analysis as indicated in table 4 revealed that lead concentrations varied from 0.15 to 0.165 ppm, Cadmium concentrations ranged from 0.05-0.07ppm and Arsenic concentrations were in between 0.25-0.34 ppm. According to WHO guidelines, the permissible limits for lead, Cadmium and Arsenic are 10ppm, 0.3ppm and 10 ppm respectively. [24] The concentrations of heavy metals were found within the permissible limits in both the in-house and marketed formulations. Heavy metals in herbal medicines can cause adverse effects to the consumer. Lead has deleterious effects on the behavioral and physiological activities of humans. It is also known to cause kidney dysfunction and obstructive lung diseases. [24] Cadmium has been associated with carcinogenicity, acute and chronic failures of liver and kidney. [25] Arsenic on the other hand hinders antioxidant enzymes following which there is an increased oxidative stress leading to inactivation of membrane bound enzymes. [26]

Albumin Denaturation Assay

The findings of the albumin denaturation assay as indicated in tables 5, 6, 7 and figures 1 and 2 revealed significant percentage of albumin denaturation. The percentage inhibition of albumin denaturation was between 20-59% for marketed formulation and 28.5-64.2% for in-house formulation. It has been well documented in literature that proteins get denaturated in inflammatory. [27] NSAIDs, owing to their protective action against protein denaturation have antirhumatic activity. [28] Previously, the effect of different plant parts on protein denaturation have been evaluated by many scientists, for example, Semecarpus anacardium bark on bovine albumin [29], an ethanolic extract of Wedelia trilobata on bovine albumin[27], Albucas etosa on egg albumen[30], etc. The ability of studied churna extracts to prevent protein denaturation may be potentially responsible for their anti-inflammatory properties. Although the mechanism of action has to be further evaluated, it has been proposed that the extract might inhibit the release of the lysosomal constituents of neutrophils at the site of inflammation [30].

Table 3: Phytochemical screening results for cold and hot Ethanolic and chloroform extracts of marketed and in-house formulation.

S.no	Main test	Sub test	Observation	Inference
1	Alkaloids	• Dragendroff's test	No orange Brown ppt	-
		• Mayer's test	No creamy ppt	-
		• Hager's test	No yellow ppt	-
		• Wagner's test	No reddish brown ppt	-
2	Carbohydrates	• Molisch test	Purple color at the junction of two liquids	+
		• Benedict's test	Yellow color	+
		• Fehling's test	No brick red ppt	+
3	Glycosides	• Borntrager's Test	Pink color	+
		• Test for Coumarin glycosides	No fluorescence when made alkaline	-
4	Phenols and tannins	• With lead acetate	White ppt	+
		• With potassium Permanganate solution	Red ppt	+
		• With ferric chloride	Deep blue color	+
5	Saponnins	• Foam test	Foam was formed	+
6	Flavanoids	• Shinoda test	Yellow ppt	+

Note: - indicates negative and + indicates positive.

Table 4: Heavy metals concentrations in both the formulations of churna (ppm).

Formulation	Pd	Cd	As
Marketed formulation	0.15±0.002	0.05±0.006	0.25±0.002
In-house formulation	0.165±0.0042	0.07±0.002	0.34±0.008

Table 5: Recovery analysis for heavy metals.

Metal	Base value (ppm)	Quantity added(ppm)	Quantity found[1](ppm)	Recovery [2] (%)
Pd	8.544±2.30	5.00	13.074	91%
Cd	0.758±0.34	1.00	1.67	92%
As	2.962±0.037	0.30	3.225	90%

Mean value (n=3). 2- 100*[(found-base)/added].

Table 6: Anti inflammatory activities of chloroform extracts of the marketed formulation.

S. No.	Sample	Absorbance at 660nm	Percentage inhibition
1.	Control	0.042±0.001	-
2.	100µg/ml	0.033 0±.0014	20.09%
3.	200µg/ml	0.026± 0.0014	38.09%
4.	300µg/ml	0.022 ±0.001	47.6%
5.	400µg/ml	0.017 ±0.001	59.5%

The results are expressed as ± Standard deviation.

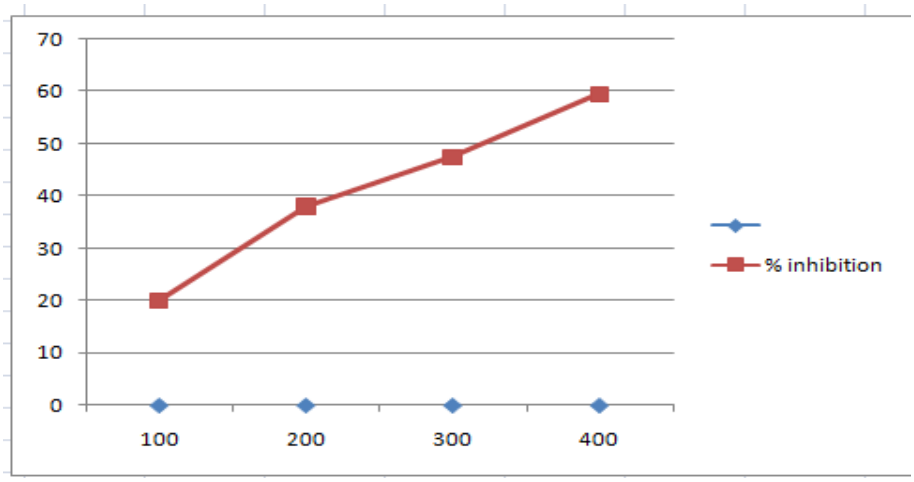

Figure 1: Effect of chloroform extract of marketed formulation of churna on protein denaturation.

Table 7: Anti inflammatory activities of chloroform extracts of the in house formulation.

S. No.	Sample	Absorbance at 660nm	Percentage inhibition
1.	Control	0.042±0.001	-
2.	100µg/ml	0.030±.0014	28.5%
3.	200µg/ml	0.024± 0.0014	42.8%
4.	300µg/ml	0.021 ±0.001	50%
5.	400µg/ml	0.016 ±0.001	64.2%

The results are expressed as ± Standard deviation.

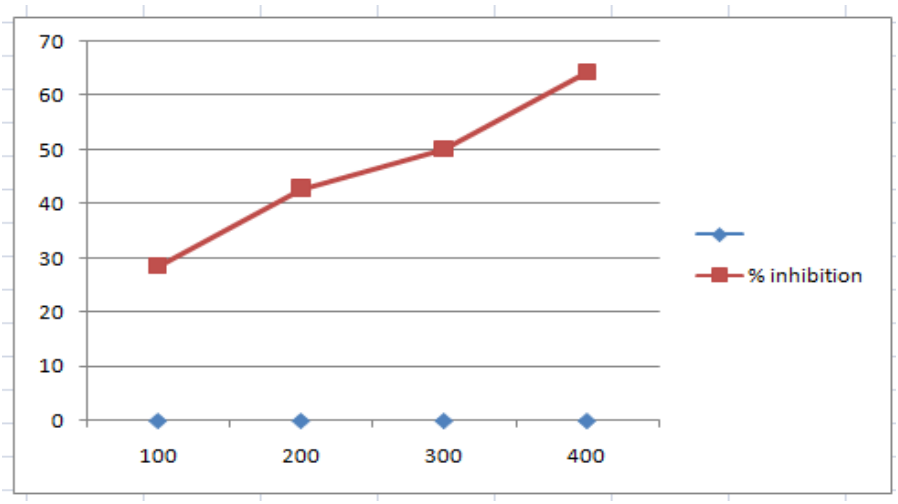

Figure 2: Effect of chloroform extract of in house formulation of churna on protein denaturation.

CONCLUSION

The comparative evaluation of different extracts revealed the presence of phytoconstituents like glycosides, tannins, flavanoids, steroids and carbohydrates. The findings of this study highlight the significance of hygiene and safety practices in terms of toxic heavy metals from the harvest of medicinal plants to their availability for the consumer market. It can be concluded that the marketed and in house formulations of the Udarkalp churna was in accordance with the standards laid down by the WHO in terms of heavy metals concentrations. The in vitro study confirms Udarkalp churna's capacity as a potential anti-inflammatory agent. The results of the present study will serve as a reference in preparation of drug formulations. The specific testing of the quality of the formulations can be further done through various sophisticated instruments like HPLC, Mass Spectroscopy, IR spectroscopy, which will give us a clear idea about the presence and quantity of all the chemical constituents present in the formulation. Based on the results obtained from the in vitro data the anti-inflammatory activity can be further evaluated in animal models and this churna maybe a potential therapy for inflammatory disorders.

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CONFLICT OF INTEREST:

Hereby authors declare that there is no conflict of interest for this publication.

ABBREVIATIONS:

WHO - World Health Organization
AAS - Atomic Absorption Spectroscopy
Pb - Lead
Cd - Cadmium
As - Arsenic
Ppm - Parts per million
Gm - Gram
Nm - Nanometer
mL - Milliliters
µg - Microgram
ºC - degree Celsius.

REFERENCES

1. Firenzuoli F, Gori L, 2007. Herbal medicine today: clinical and research issues. Evid Based Complement Alternat Med. 4(1):37–40.
2. Wani MS, 2007. Herbal medicine and its standardization. Pharmaceutical Reviews. 5(6): 123-125.
3. Kochhar SL. Tropical crops: A textbook of economy botany. London: Macmillan Pub Ltd. 1981. 268–71.
4. Frank, Reema. 2018. Consumer purchase behavior towards patanjali brand in Mangalore. 7(1): 7-9.
5. Jaggi, Ruchi & Ghosh, Munmun. 2007. Consumer Perception of Patanjali Products: An Analytical Study. IUP Journal of Brand Management. 14: 13-23.
6. WHO. 1992. Expert committee on specification for pharmaceuticals preparation. 44–52.
7. Ajasa AMO, Bellob MO, Ibrahimb AO, Ogunwandea IA and Olaworeb NO. 2004. Heavy trace metals and macronutrients status in herbal plants of Nigeria. Food Chem. 85:67–71.
8. Gomez MR, Cerutti S, Somb LL, Silva MF and Martınez LD. 2007. Determination of heavy metals for the quality control in Argentinian herbal medicines by ETAAS and ICP-OES. Food Chem Toxicol. 45: 1060.
9. WHO. 1991. Guidelines for the Assessment of Herbal Medicines, Munich, Geneva. 28:6.
10. Health Canada, Draft guidance on heavy metal impurities in cosmetics, Section 4
11. The Ayurvedic Formulary of India. Part 1, 2nd Edn. Government of India, Ministry of Health and Family Welfare, New Delhi. 2003
12. Lala PK. Lab Manuals of Pharmacognosy 5th Edition. Calcutta: CSI Publishers and Distributors; 1993.
13. Pan M.H, Lai C.S, Ho C.T. 2010. Anti-inflammatory activity of natural dietary flavonoids. Food Funct. 1: 15–31.
14. Pan M.H, Lai C.S, Dushenkov S, Ho C.T. 2009. Modulation of inflammatory genes by natural dietary bioactive compounds. J. Agric. Food Chem. 57: 4467–4477.
15. Rao RN and Kumar Talluri MVN. 2007. An overview of recent applications of inductively coupled plasma-mass spectrometry (ICP-MS) in determination of inorganic impurities in drugs and pharmaceuticals. J Pharm Biomed. 43:1–13.
16. Ang HH. 2008. Lead contamination in Eugenia dyeriana herbal preparations from different commercial sources in Malaysia Food Chem Toxicol. 46: 1969-1975.
17. Choudhury RP and Garg AN. 2007. Variation in essential, trace and toxic elemental contents in Murrayakoenigii-A spice and medicinal herb from different Indian states. Food Chem. 104: 1454.
18. CK Kokate, AP Purohith, SB Gokhale. Pharmacognosy text book, 50th edition. 2015; 22-27.
19. Helaluddin A.B.M, Khalid R.S, Alaama M. and Abbas S.A. 2006. Main Analytical Techniques Used for Elemental Analysis in Various Matrices, Tropical Journal of Pharmaceutical Research. 15 (2): 427-434
20. Hseu Z.Y. 2004. Evaluating heavy metal contents in nine composts using four digestion methods. Bioresource Technol. 5:53–9.
21. Gomez M.R, Cerutti S, Somb L.L, Silva M.F. and MartÄnez L.D. 2007. Determination of heavy metals for the quality control in Argentinian herbal medicines by ETAAS and ICP-OES, Food and Chem. Toxicol. 45: 1060-1064.
22. Gunathilake, K.D.P.P.; Ranaweera, K.K.D.S. Rupasinghe, H.P.V. Influence of boiling, steaming and frying of selected leafy vegetables on the in vitro anti-inflammation associated biological activities. Plants 2018, 7, 22.
23. Gambhire, M.; Juvekar, A.; Wankhede, S. 2009. Evaluation of the anti-inflammatory activity of methanol extract of Barleria cristata leaves by in vivo and in vitro methods. Int. J. Pharmacol. 7: 1–6.
24. Dhan Prakash, Charu Gupta, Girish Sharma. Importance of Phytochemicals in Nutraceuticals. 2012. Journal of Chinese Medicine Research and Development. 1(3): 70-78.

25. Nema S. Shaban, Khaled A. Abdou and Nour EL-Houda Y Hassan. 2016. Impact of toxic heavy metals and pesticide residues in herbal products. Beni-Suef University Journal of Basic and applied Sciences. 5(1): 102-106.
26. Rafati Rahimzadeh M, Rafati Rahimzadeh M, Kazemi S, Moghadamnia AA. 2017. Cadmium toxicity and treatment: An update. Caspian J Intern Med. 8(3):135–145.
27. Figueiredo-Pereira ME, Yakushin S and Cohen G. 1998. Disruption of the intracellular sulphydryl homeostasis by cadmium-induced oxidative stress leads to protein thiolation and ubiquitination in neuronal cells. J Biol Chem. 273: 12703-12709.
28. Umapathy E, Ndebia E.J, Meeme A, Adam B, Menziwa P, Nkeh-Chungag B.N, Iputo J.E. 2010. An experimental evaluation of Albuca setosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J. Med. Plants Res. 4: 789–795.
29. Mizushima Y. 1964. Inhibition of protein denaturation by antirheumatic or antiphlogistic agents. Arch. Int. Pharmacodyn. Ther. 149: 1–7
30. Kumar, A.N.; Bevara, G.B.; Laxmikoteswramma, K.; Malla, R.R. 2013. Antioxidant, cytoprotective and anti-inflammatory activities of stem bark extract of Semecarpus anacardium. Asian J. Pharm. Clin. Res. 6: 213–219.
31. Govindappa, M.; Naga, S.S.; Poojashri, M.N.; Sadananda, T.S.; Chandrappa, C.P. 2011. Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of Wedelia trilobata (L.) Hitchc. J. Pharmacogn. Phytother. 3: 43–51.

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