

IMPACT OF PROCESSING ON PROXIMATE COMPOSITION AND MEDICINAL PROPERTIES OF *MUCUNA SANJAPPAE* SEEDS: A FUNCTIONAL FOOD

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ABSTRACT

Mucuna sanjappae seeds are conventionally used as food and medicine in the Western Ghats of India. It possesses good nutritional value with higher level of anti-Parkinson's drug L-DOPA (10.81 g/100g). But, presence of anti-nutritional compounds hardens its preferential use as a food of choice. In the present study, the effect of various commonly used processing methods (soaking, autoclaving, roasting, soaking plus autoclaving and soaking plus roasting) on L-DOPA, nutritional, anti-nutritional factors, antioxidant activity (2,2-diphenyl-1-picrylhydrazyl and ferric reducing/antioxidant power assay) and anti-inflammatory activity was determined. RP-HPLC analysis of processed MS beans showed significant reduction of L-DOPA ($p < 0.001$) due to various treatments among which soaking (2.91 ± 0.09 g/100g) and soaking plus autoclaving (1.10 ± 0.08 g/100g) was most effective treatments. DPPH radical scavenging activity was significantly reduced after the processing treatment ($p < 0.001$) and showed maximum reduction in soaking plus autoclaving process (81.741 ± 0.596 % to 44.311 ± 0.69 %). In contrast, FRAP assay does not showed significant decrease ($p > 0.05$) in activity. Anti-inflammatory activity (BSA anti-denaturation assay and HRBC membrane stabilization activity) was also found to be decreasing after processing of *M. sanjappae* (MS) seeds ($p < 0.001$). This paper has thoroughly given the effect of processing on nutritional value, anti-nutritional compounds, antioxidant potential and has first time reported anti-inflammatory activity of *M. sanjappae* seeds.

Keywords: Anti-inflammatory activity, Anti-nutritional factors, Antioxidants, L-DOPA, Legumes food, *Mucuna sanjappae*

INTRODUCTION

Genus *Mucuna* is well-known for the food, feed, cover crop, medicine and ornamental purpose (Osei-Bonsu *et al.*, 1995; Carsky *et al.*, 1998). This marvelous herb is known by many names including sea beans, buffalo beans, dopa bean, cowitch, kapikachu and atmagupta. It comprises 100 species worldwide and is represented by ten species and four varieties from the Indian subcontinent (Wilmot-Deary 1986, 1991; Patil *et al.*, 2015). These species are rare and mostly found in the specific forest region of the India. The seeds of *Mucuna* contains L-DOPA (L-3, 4-dihydroxyphenylalanine), a promising drug for Parkinson's disease management. These seeds are also widely used against snakebite, uterine stimulant and as an aphrodisiac in traditional system of medicine predominantly in India and West Africa (Patil *et al.*, 2015). Unfortunately, *Mucuna* species other than *M. pruriens* has remained underutilized and neglected for their possible use in food and drug (Patil *et al.*, 2015). It is observed that, regardless of any scientific knowledge, Indian ethnic people are continuously using such species as a staple food and for the cure of different disease including Parkinson's disease and male infertility (Patil *et al.*, 2015; Lampariello *et al.*, 2012). These people have experience based theories about the numerous valuable properties of such plants.

M. sanjappae (MS) is a species of *Mucuna* described from the Junner area of Western Ghats, India (Aitawade and Yadav, 2012). A single pod of *M. sanjappae* contains 5–6 brown and black seeds (Aitawade and Yadav, 2012) having potential nutritional value with gross energy 383 kcal (Patil *et al.*, 2015). But, as found in other legume seeds, it also contains anti-nutritional factors including phenolics, phytic acid, saponin, tannin, etc. Such anti-nutritional factors have adverse physiological effects which harden the use of *M. sanjappae* as a daily food of choice. Though L-DOPA has immense value in the Parkinson's disease treatment, its regular consumption has several anti-metabolic effects like acidity, nausea, and vomiting. Phytic acid and polyphenol decrease digestibility and utilization of legume protein and starch (Nielsen, 1991; Liener, 1994; Bishnoi *et al.*, 1994; Siddhuraju and Becker, 2005; Khattab and Arntfield,

2009). Despite their moderately high amount of protein, calories, vitamins and minerals; their usage in food and feed becomes limited by the presence of antinutritional factors (ANFs) (Khattab *et al.*, 2009). These ANFs have several important functions in the growth and development of plants itself; hence to solve this issue at the genetic level could be extremely disastrous. So, it is important to overcome problem using some adequate, simple and cost effective processing techniques. Such techniques can reduce or completely remove the ANFs from legumes for better consumption by human as well as animals (Khattab and Arntfield, 2009).

In this connection, different pretreatments like soaking, germination, boiling, autoclaving, roasting, fermentation and advanced treatments like gamma radiation are being used for successful reduction of ANFs at a considerable level suitable for the consumption (Chau *et al.*, 1997; Tiansawang *et al.*, 2016). The processing methods being employed also affects the nutritional composition and other properties including antioxidant and anti-inflammatory activity of the processed food. With this background, present research work was attempted to study and compare the effects of various processing methods on the L-DOPA, nutritional-antinutritional composition and antioxidant activity of *M. sanjappae* beans.

MATERIALS AND METHODS

Collection of plant material

MS seeds were collected from Junner area of Maharashtra, India and stored at room temperature in a polyethylene bag ($25 \pm 2^\circ\text{C}$) until use. The herbarium was maintained at Department of Botany, Shivaji University, Kolhapur (Voucher ID-SVG004).

Chemicals and reagents

All the chemicals and solvents used for the experiment were of analytical grade. Folin-ciocalteu reagent, sodium carbonate, aluminium trichloride, ferric chloride, potassium acetate, ascorbic acid, 2,4,6- tripyridyl-s-triazine (TPTZ), 2,2-Diphenyl-1- picrylhydrazyl (DPPH), bovine serum albumin, diclofenac, quercetin, catechin and methanol for HPLC were obtained from Sigma Chemical Co., USA. L-DOPA, phytic acid, tannin monosodium phosphate, di monosodium phosphate was purchased from HiMedia.

Processing methods

The seeds were randomly divided into six groups (30 g/ group). The first group was kept as raw seed without any treatments. The remaining five groups were subjected to various processing methods is shown in fig 1 and briefly described below:

Process A (Soaking): MS seeds were soaked in distilled water (D/W) for 24 hrs.

Process B (Autoclaving): MS seeds were autoclaved at 15 lb pressure (121°C) in D/W for 20 min.

Process C (Roasting): MS beans were roasted on burner using the hot pan for 15 min.

Process D (Soaking plus Autoclaving): MS beans were soaked in D/W for 24 hrs and then autoclaved at 15 lb pressure (121°C) for 20 min.

Process E (Soaking plus Roasting): MS seeds were soaked in D/W for 24 hrs and then roasted on burner using the hot pan for 15 min.

The respective MS seed samples were dried and a fine powder was prepared using mortar pestle for further analysis.

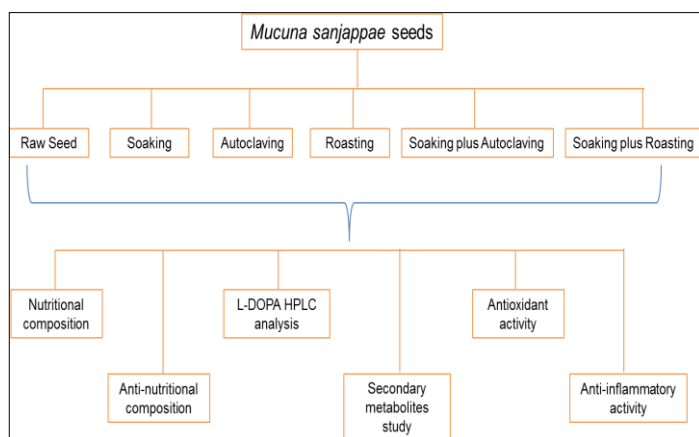


Figure 1 Schematic drawing of different processing methods and further analysis of *M. sanjappae* seeds.

RP-HPLC analysis of L-DOPA

The differentially processed seed samples were analyzed for L-DOPA using reverse phase high performance liquid chromatography (Shimadzu). Sample preparation was carried out as described by Rathod et al. (2014) with slight modifications. One gram of respective MS seed sample was extracted with methanol: 0.1 M HCl (70:30) for 30 min on rotary shaker (120 rpm) and sonicated for 5 min. The samples then evaporated until dry, dissolved in methanol (5mg/10ml) and filtered through 0.45 µm nylon filter (Axiva filters). RP-HPLC analysis was carried out by Shimadzu prominence equipped with degasser DGU-20A 5R, low pressure quaternary pump LC 20 AD and photo diode array detector SPD- M20 A. Chromatographic separation was achieved on a Waters, Nova-Pak C18 column (4 µm, 4.6 × 250 mm). Methanol was used as a mobile phase with a flow rate of 1 ml min⁻¹ for 10 min and UV detector at 280 nm. The commercially available synthetic LDOPA (HiMedia) was used as a standard.

Biochemical analysis sample preparation

200 mg of respective seed powder was added to 10 ml D/W, sonicated for 5 min, crushed using mortar pestle and then centrifuged at 10,000 rpm for 15 min, the supernatant was decanted, and again procedure was repeated for residue. The supernatant was pulled together and diluted to a known volume by D/W. This sample was used for biochemical analysis.

Nutritional factors and secondary metabolites determination

Total ash (AOAC, 2000), total solid (James, 1995) and crude fiber (AOAC, 1990) were determined according to standard methods. The protein content was

determined using Lowry's method (Lowry, 1951). The total carbohydrate content was estimated using the Anthrone reagent method (Trevelyan et al., 1952). The reducing sugar was determined using the method of (Miller, 1959) whereas; crude fat was measured by Mojonnier method (James, 1995). For the starch quantification, the processed seed flour was made sugar free using 80 % ethanol. Then starch was extracted by perchloric acid reagent (McCready, 1950) and quantified colorimetrically at 630 nm. The starch content was determined in mg of glucose X 0.9. The total flavonoids content was determined (Chang et al., 2002), and results were expressed as milligram of quercetin equivalents per gram (mg QUE g⁻¹) of dry weight. The proanthocyanidin content was evaluated (Sun et al., 1998) and expressed as catechin equivalents per gram (mg CAE g⁻¹) of dry weight.

Anti-nutritional factors determination

The spectrophotometric quantification of phytic acid was carried out (Gao, 2007). In brief, 1 ml sample was mixed with 1 ml of Wade reagent and solution was vortexed for 5 seconds and centrifuged for 10 min. The supernatant was used for the measurement of absorbance at 500 nm. The phenolic content of processed MS beans was determined spectrophotometrically (Singleton, 1965). Tannin was determined using the Folin-Denis colorimetric method (Kirk and Sawyer, 1998) while saponin was quantified according to the slandered method (Harborne, 1973).

Antioxidant activity study

DPPH free radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl), free radical scavenging capacity of the differentially processed MS seed samples was determined according to the procedure described by Brand-Williams et al. (1995) with slight modification (Thaipong, 2006). The DPPH radical scavenging capacity was calculated in percent using the following equation:

$$\% \text{ RSC} = \frac{A_{(\text{control})} - A_{(\text{sample})}}{A_{(\text{control})}} \times 100$$

FRAP (ferric reducing/antioxidant power) assay

The FRAP assay was performed to determine the antioxidant capacity of differentially processed MS samples (Benzie et al., 1996). The absorbance of the ferrous tripyridyltriazine complex product was measured at 593 nm using a UV-visible spectrophotometer. A higher absorbance reading revealed a higher reducing power.

In vitro anti-inflammatory activity

Sample was prepared by adding 100mg MS seed powder in 10 ml D/W and extracted in mortar pestle. The extracted sample was then centrifuged at 10000 rpm for 10 min and supernatant was collected. Effective yield was calculated by evaporating water from extract and further diluted to known concentration for experimentation purpose.

Bovine serum albumin (BSA) anti-denaturation assay

BSA protein anti-denaturation test was achieved by method described by Grant et al., (1969) with slight modifications. In brief, different concentrations of extracted MS bean samples and standard anti-denaturation drug Diclofenac were allowed to react with 1ml of BSA solution (1 % BSA in 50mM Tris buffer, pH 6.5). The reaction was accomplished by incubating at 37°C for 20 min and then heated to 64°C in water bath for 5 to 10 minutes till mixture get turbid. After cooling, turbidity was measured at 660 nm by spectrophotometer. Control was D/W instead of test sample for the preparation of control. The denaturation inhibition percentage was calculated as follows:

$$\% \text{ denaturation inhibition} = \frac{A_{(\text{control})} - A_{(\text{sample})}}{A_{(\text{control})}} \times 100$$

Where, A (control) is absorbance of the control and A (sample) is absorbance of samples.

Membrane stabilization test

Fresh whole human blood (15 ml) was centrifuged at 3000 rpm for 10 min and packed RBCs were collected. The packed RBCs were further washed for three times with equal volume of normal saline by centrifugation at 3000 rpm for 10 min. The packed cell volume was measured and diluted as 10% v/v suspension using normal saline (Sadique et al., 1989).

The hypotonicity induced haemolysis assay was carried out as method described by Bhurat et al. (2011). In brief, reaction mixture containing 2 ml hypo saline, 1ml 0.15M phosphate buffer (pH 7.4), 1ml MS extract (200µg-600µg/ml) in isosaline and 0.5ml 10% reconstituted HRBC was incubated at 37 °C for 30 min

and then centrifuged at 3000 rpm for 20 min. Distilled water used instead of hypo saline to prepare control. Hemoglobin content in the supernatant was determined spectrophotometrically at 560 nm. Synthetic diclofenac was used as a standard drug.

Percentage stabilization: 100 – Absorbance of test / Absorbance of control X 100

Statistical analysis

All experimental analysis was carried out in triplicates and results were expressed as a mean ± SEM value. Data were statistically studied using GraphPad Prism 5. The significant difference between means was calculated by one way analysis of variance (ANOVA) followed by Dunnett multiple range test at P<0.05.

RESULTS AND DISCUSSION

Legumes are the low-cost source of protein, complex carbohydrate, dietary fibers and contribute significant amounts of vitamins, minerals with high energy value (Nielsen, 1991; Tharanathan and Mahadevamma, 2003). Number of leguminous species are being used for the daily food requirement, and extensive study has been carried out regarding their nutritional value (Siddhuraju and Becker, 2005; Costa et al., 2006; Mugendi et al., 2010). A well-known species of *Mucuna*, *M. pruriens* has been thoroughly investigated for its L-DOPA content, nutritional, anti-nutritional composition, *in vitro* protein-starch digestibility and possible use in poultry feed after domestic processing (Siddhuraju and Becker, 2001,2005; Nyirenda et al., 2003; Vadivel and Pugalenti 2008; Mugendi et al., 2010).

There is ever increasing demand of food to the growing population of world especially in the developing countries. To fulfill the large demand of food, there is necessity of searching cheaper sources to prevent malnutrition. Numbers of leguminous species are being consumed locally as an optional food but still uninvestigated and unexplored. To this point, our laboratory is comprehensively working on such unexplored species from different leguminous genus for determining their nutritive values. Earlier we had reported nutritional and anti-nutritional composition of *M. sanjappae* (Patil et al., 2015). The present study was undertaken on the new leguminous species *M. sanjappae* for evaluating processing effect on L-DOPA, nutritional-antinutritional factors antioxidant potential and anti-inflammatory activity.

Effect of processing on L-DOPA level

The level of L-DOPA in the raw and processed MS seed sample was determined using RP-HPLC (Fig 2).

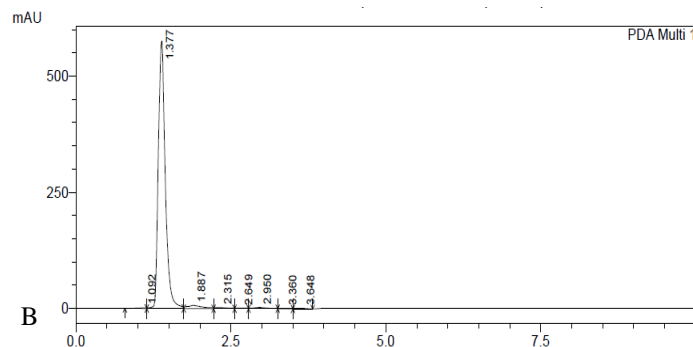
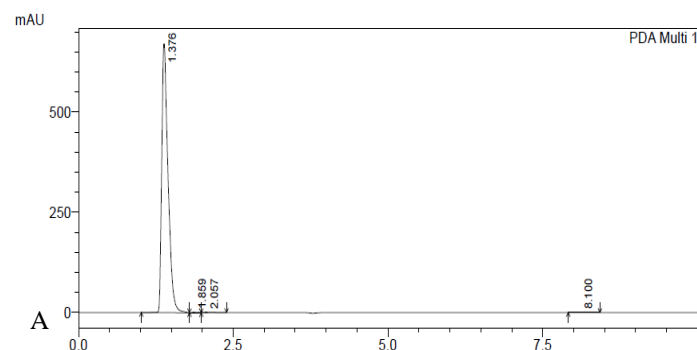


Figure 2 HPLC profile of L-DOPA. a) Standard L-DOPA b) Raw *M. sanjappae* bean.

MS seed showed 10.814±0.242 g/100g (Fig 3 and Table 2) of L-DOPA. Earlier, we had reported 7.3 g/100g of L-DOPA with the simple extraction method using 0.1 N aqueous HCL (Patil et al., 2015). Seasonal variation of collected MS seeds and optimized extraction method might be the reason for the higher quantified level of L-DOPA in the sample. All the cooking processes significantly decreases (p<0.001) the level of DOPA content as compared to raw seed sample.

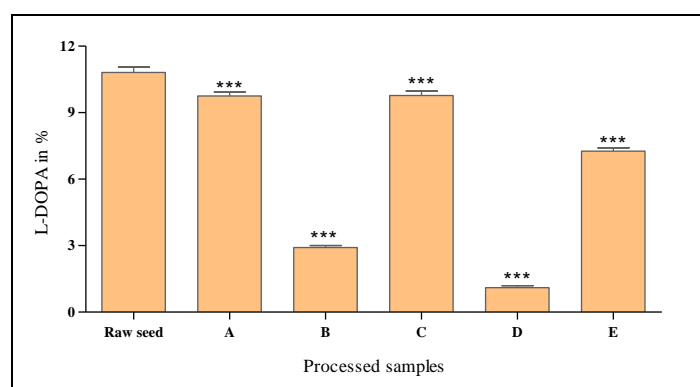


Figure 3 Effect of processing on the L-DOPA content (g/100g) of *M. sanjappae* bean (A-soaking, B-Autoclaving, C-Roasting, D-Soaking plus Autoclaving, E-Soaking plus Roasting). Values are expressed as mean±SEM (n=3) using one-way ANOVA followed by Dunnett’s multiple comparison tests. *** = p<0.001 when compared with Raw seed sample.

As L-DOPA also acts as an anti-nutritional factor, for the consideration of MS beans as a staple food, obliteration of L-DOPA is also important, and we found that soaking plus autoclaving is the excellent treatment for it.

Effect of processing on nutritional value

Results of proximate composition for the variously processed MS seeds are presented in Table 1. The protein level of MS bean is low (6.327±0.2 g/100g) as compared to *M. pruriens* and other cereal pulses (18.5-27.87g/100g) (Siddhuraju and Becker, 2005; Costa et al., 2006; Mugendi et al., 2010). The protein content was significantly reduced due to the different processing (p<0.001). Process D (soaking plus autoclaving) decreases protein content

Table 1 Nutritional composition in raw and processed MS beans (g 100 / g dry matter)^a.

	Raw Seed	A	B	C	D	E
Ash	5.83±0.74	5.45±0.92 (6.51)	4.92±0.672 (15.60)	5.39±0.25 (7.54)	4.28±0.263* (26.58)	4.471±0.41 (23.31)
Total solid [#]	90.24±4.93	88.34±5.52 (2.10)	83.56±6.48 (7.40)	76.36±6.23 (15.38)	85.89±7.91 (4.82)	81.45±4.82 (9.74)
Protein	6.327±0.2 (10.85)	5.64±0.070*** (10.85)	4.99±0.048*** (21.13)	5.591±0.078*** (11.63)	4.13±0.172*** (34.72)	4.731±0.148*** (25.22)
Total Carbohydrates	77.23±0.757	46.232±0.04*** (40.13)	49.036±0.056*** (36.50)	47.22±0.096*** (38.85)	43.88±0.28*** (43.18)	42.48±0.064*** (44.99)
Reducing Sugar	0.1252±0.003 (32.8)	0.0840±0.009 (32.8)	0.080±0.082 (36)	0.106±0.003 (15.2)	0.096±0.005 (23.2)	0.096±0.005 (23.2)
Total Fat	5.78±0.46 (1.03)	5.72±0.61 (1.03)	4.14±0.298* (28.37)	4.35±0.651 (24.74)	3.69±0.816** (36.15)	3.281±0.848** (43.25)
Starch	3.82±0.72 (14.39)	3.27±0.189 (14.39)	2.75±0.478* (28.01)	3.56±0.398 (6.80)	2.68±0.192* (29.84)	2.53±0.223** (33.76)
Total Flavonoids	20.327±0.854	16.916±0.590** (16.78)	6.706±0.598*** (67)	15.7781±0.449*** (22.39)	4.5777±0.370*** (77.51)	5.036±0.359*** (75.22)
Proanthocyanidin	1.89±0.098	1.56±0.15 (17.46)	1.08±0.22* (42.855)	1.67±0.358 (11.64)	0.65±0.075** (65.60)	0.98±0.11* (48.14)

a. Values are expressed as mean ± SEM (n=3) using one-way ANOVA followed by Dunnett’s multiple comparison test. * p<0.05; ** p<0.01; *** p<0.001 when compared with Raw sample. A-soaking, B-Autoclaving, C-Roasting, D-Soaking plus Autoclaving, E-Soaking plus Roasting. # = Total solid was calculated by the formula % total solids = 100 - % moisture. Values in parentheses indicate the percent loss.

(4.13±0.172 g/100g) by 34.72% while process A (soaking) showed the minimal effect on protein level (-10.85%). The decreased level of protein was also found in green gram and black gram due to soaking or cooking (Kakati et al., 2010). The total carbohydrate content of MS beans is high (77.23±0.757 g/100g) than *M. pruriens* (Kakati et al., 2010) which was reduced to 43.88±0.28 and 42.48±0.064 g/100g (-43.88% and -44.99%) through the Process D and E respectively (p<0.001). MS bean has less reducing sugar 0.1252±0.003 g/100g which is lessened to 0.080±0.082 g/100g (-36%) during the autoclaving (Process B) (p>0.05). MS seed contained higher crude fat (5.78±0.46 g/100g) than other legumes except for chickpea (6.7 g/100g) (Mugendi et al., 2010). Process E (soaking plus roasting) found to be decreasing fat content to 3.281±0.848 g/100g (-43.25%) (p<0.001). The starch content of raw MS beans was 3.82±0.72 g/100g which get decreased to 2.53±0.223 g/100g (-33.76%) (p<0.01) during the process E (soaking plus roasting). Process C (roasting) showed minimum effect on starch level (-6.80%) (p>0.05). It was found that the processing methods used in this study resulted in a reduction of the proximate nutritional level of the seeds. Protein and fat content does not decrease at significant level, but total carbohydrate showed an extreme loss due to processing. Flavonoids are the important class of secondary metabolites having role in the treatment of oxidative stress related disorders like diabetes and Parkinsonism (Mu et al., 2009). MS bean showed a high content of flavonoids (20.3279±1.48 g/100g) as compared to *M. pruriens*. A significant decrease in the flavonoid level was observed due to different processing techniques (p<0.001). Soaking and

roasting process (A and C respectively) lessen the amount of flavonoids up to 16.9160±1.02 g/100g (-16.78%) and 15.7781±0.714 g/100g (-22.39%) respectively. Process B, D, and E affected flavonoid content to a greater extent. Proanthocyanidin content of the raw seed sample was 1.89±0.17 g/100g which was decreased to 0.65±0.13 g/100g (-65.60%) (p<0.01) due to the treatment of soaking plus autoclaving while the minimal reduction was found during the roasting treatment that is 1.67±0.62 g/100g (-11.64%). Several workers have reported the similar trend of reduction in nutritional factors and secondary metabolites due to the pretreatment in legumes and pulses (Kakati et al., 2010).

Effect of processing on ANF content

ANF commonly found in legume seeds and possesses the ability to chelates mineral cations and proteins, forming insoluble complexes, which lead to decreased bioavailability of trace minerals, reduced digestibility of proteins and inhibited proteolytic enzyme activity (Duodu and Minnaar, 1999). For the full utilization of legumes as food, deactivation or complete removal of antinutritional factors using economically viable techniques is required (Mugendi et al., 2010). Seed treatments under study are the simple, cheap and routinely used for the consumption of a leguminous plant food. The study showed that level of ANF is decreased during the processing of MS beans (Table 2).

Table 2 Anti-nutritional composition in raw and processed MS beans (g 100 / g dry matter)^a

	Raw Seed	A	B	C	D	E
L-DOPA	10.81±0.24	9.75±0.17*** (9.82)	2.91±0.09*** (73.07)	9.77±0.2*** (9.65)	1.10±0.08*** (89.74)	7.26±0.14*** (32.79)
Total Phenolics	11.11±0.45	7.86±0.24*** (29.23)	6.07±0.254*** (45.33)	9.71±0.29*** (12.62)	3.14±0.26*** (71.68)	5.90±0.381*** (46.88)
Phytic acid	23.67±2.45	18.82±1.56 (20.48)	14.89±3.26** (37.09)	16.76± 2.87* (29.19)	5.23±2.89*** (77.90)	11.42±1.98*** (51.75)
Tannin	0.47±0.08	0.30±0.01** (35.90)	0.25±0.01*** (47.18)	0.22±0.01*** (54.07)	0.10±0.01*** (77.45)	0.13±0.004*** (72.44)
Saponin	2.13±0.67	1.74±0.82 (18.64)	1.42±0.37 (33.08)	1.54±0.18 (27.83)	0.92±0.12* (56.88)	1.16±0.44 (45.64)

a. Values are expressed as mean ± SEM (n=3) using one-way ANOVA followed by Dunnett’s multiple comparison test. * p<0.05; **p<0.01; *** p<0.001 when compared with Raw seed sample. A-soaking, B-Autoclaving, C-Roasting, D-Soaking plus Autoclaving, E-Soaking plus Roasting. Values in parentheses indicate the percent loss.

The phenolics content of the seed was 11.11±0.405 g/100g which gets significantly reduced to 3.147±0.263 (-71.68%) during the process D (Soaking plus autoclaving) (p<0.001). Similar results were found in the treatment of faba beans (Siah et al., 2014). Phenolics reduce the digestion of protein, carbohydrates, and availability of vitamins and minerals (Mugendi et al., 2010). The MS seed showed a high amount of phytic acid (23.67±2.45 g/100g) as compared to other legumes (Embaby, 2011; Patil et al., 2015). Different processing methods reduce phytic acid at a considerable amount. Process D (soaking plus autoclaving) decreases its minimum level 5.23±2.89 g/100g (-77.90%) (p<0.001). We observed process A (soaking) has less effect on the phytic acid level 18.82±1.56 g/100g (p>0.05). The reduction of phytic acid during soaking could be due to the presence of phytic acid in legume seed as a water-soluble salt (probably potassium phytate) (Khatab and Arntfield, 2009). Whereas, a decrease in phytic acid content during heat treatments may be because of the heat sensitive characteristic of phytic acid and its complex formed with other components (Khatab and Arntfield, 2009). Tannin and saponin level in the MS seed was 0.479±0.087 and 2.134±0.67 g/100g respectively. These two factors also showed the same trend of decreasing the concentration by the combinational process soaking and autoclaving (0.1085±0.01403 (p<0.001) and 0.92±0.124 g/100g (p<0.01) respectively). Tannin reduction due to such pretreatments is mainly credited to its water soluble and heat labile nature (Khatab and Arntfield, 2009).

All the processes under investigation affected proximate composition (nutritional and anti-nutritional factors) of the MS seed in varying degrees, but the process soaking plus autoclaving has a considerable effect. Overall, roasting (Process C) has less effect on the nutritional and ANF level, but it was evidenced to be effective in decreasing the amount of biomolecules in combination with autoclaving. Process A and B (soaking and autoclaving respectively) have shown effective processing methods, but the combination of both is more beneficial for reducing the level of ANF.

Antioxidant potential of processed samples

The effect of processing on the antioxidant capacity of MS beans was also determined. However, assessment of antioxidant capacity of plant sample could not be correctly achieved by any single method because of the diverse nature of phytochemicals present in it. Hence, we performed DPPH and FRAP assay for the determination of antioxidant activity of processed MS seeds. We observed that processing techniques reduces antioxidant properties of the MS beans.

DPPH radical scavenging activity

Figure 4 depicts the DPPH radical scavenging activity (DPPH RSA) of processed samples in comparison with raw beans sample. The water extract of raw bean sample showed 81.741±0.596 % activity at the higher dose of 200 µg. DPPH radical scavenging activity was significantly reduced after the processing treatment (p<0.001) of MS seeds. The maximum decrease of 44.311±0.69% was observed during the process D. The order of antioxidant activity was Raw>B>A>C>E>D. The response of DPPH radical scavenging activity for the differentially processed samples was decreased as compared to raw seed sample in which soaking plus autoclaving showed less DPPH radical scavenging capacity. Sweet and bitter *Chenopodium quinoa* seeds also showed decreased DPPH RSA in the due to boiling effect Dini et al., (2010).

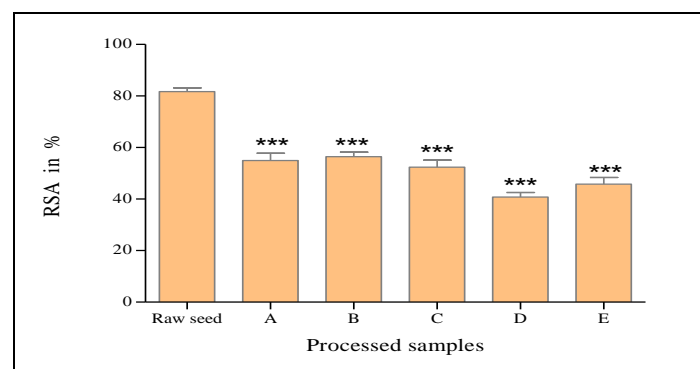


Figure 4 DPPH radical scavenging activity (%) of different processed MS bean water extract (A-soaking, B-Autoclaving, C-Roasting, D-Soaking plus Autoclaving, E-Soaking plus Roasting). Values are expressed as mean±SEM (n=3) using one-way ANOVA followed by Dunnett’s multiple comparison tests. *** = p<0.001 when compared with Raw seed sample.

FRAP radical scavenging activity

The antioxidant potential of the processed bean samples was furthermore assessed using FRAP radical scavenging test (Fig 5). The results were determined in terms of optical density (O.D) at 593 nm. The raw seed sample showed 3.353±0.17 O.D at the higher dose of 200 µg indicating strong FRAP activity. There was no significant reduction in the FRAP activity of MS beans after processing (p>0.05). Highest decrease in activity (2.850±0.18 O.D.) was found for the treatment D (soaking plus autoclaving). The results suggest that

processing of the seeds does not affect drastically on the FRAP radical scavenging activity. The order of activity was Raw>A>B>E>D>C. Dini et al. (2010) likewise found decreased FRAP activity in the sweet and bitter *Chenopodium quinoa* seeds due to boiling effect. Reduction in phenolic content and antioxidant activity of Chickpea (*Cicer arietinum* L.) has also been reported as an effect of soaking and cooking processes (Segev et al., 2011). The decreased level of antioxidant activity after the processing of MS seeds may be attributed to the structural changes or destruction of antioxidant biomolecules present in *M. sanjappae* seeds.

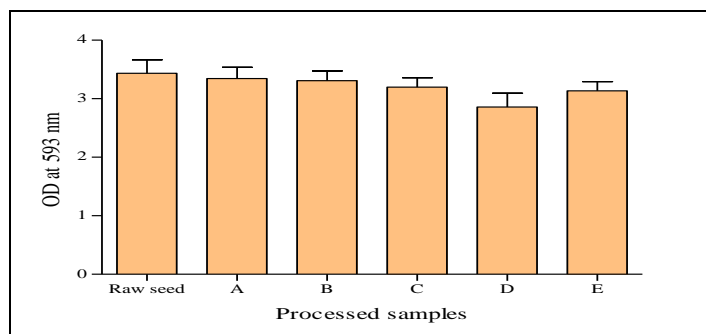


Figure 5. FRAP radical scavenging activity (O.D. at 593 nm) of different processed MS bean water extract (A-Soaking, B-Autoclaving, C-Roasting, D-Soaking plus Autoclaving, E-Soaking plus Roasting). Values are expressed as mean±SEM (n=3) using one-way ANOVA followed by Dunnett’s multiple comparison tests.

Effect of processing on anti-inflammatory activity

Anti-inflammatory activity of plant based drug molecules is mainly depends on secondary metabolites present in it. Hence, it is worthwhile to study the influence of processing methods on the anti-inflammatory activity of MS seeds. This might be the first report showing processing effect on anti-inflammatory potential of leguminous plant.

BSA anti-denaturation activity

Figure 6 depicts the heat induced albumin denaturation activity of differentially processed MS seed. The raw bean sample showed 69.6±0.628 % anti-denaturation activity at the dose of 200 µg. The activity was considerably reduced after the processing treatment (p<0.001) of MS seeds. The maximum decrease of 33.2±0.8% was observed during the soaking plus autoclaving process. The order of BSA anti-denaturation activity was Raw>C>A>B>E>D. The reduction in activity suggests phytochemicals responsible for anti-inflammatory activity gets denatured or completely destroyed due to processing. Standard drug diclofenac showed 72.7±1.25% activity at the same concentration.

Hypotonicity-induced hemolysis inhibition

Study of anti-hemolysis activity is the representative assay of anti-inflammatory potential in drug molecule. Processed MS bean samples were analyzed for their hemolysis inhibition activity and found drastic effect on the activity (Fig 7). Raw sample showed 41.9±0.22% hemolysis inhibition (stabilization) activity for 200 µg extract concentration. The lowest stabilization activity was observed in the soaking plus autoclaving and soaking plus frying process (26.8±0.72 and 26.8±0.2% respectively).

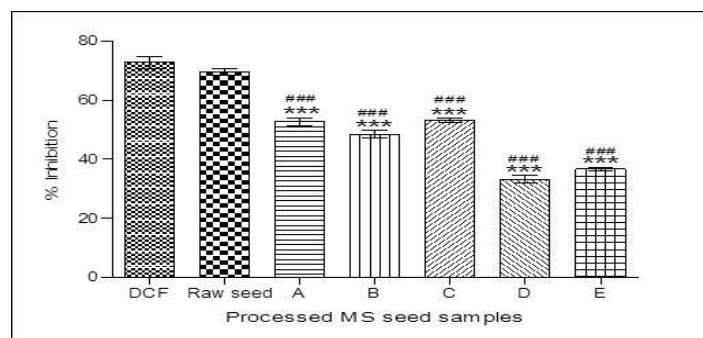


Figure 6 BSA anti-denaturation activity assay of different processed MS bean (A-soaking, B-Autoclaving, C-Roasting, D-Soaking plus Autoclaving, E-Soaking plus Roasting). Values are expressed as mean±SEM (n=3) using one-way ANOVA followed by Dunnett’s multiple comparison tests. *** = p<0.001 when compared with Raw seed sample.

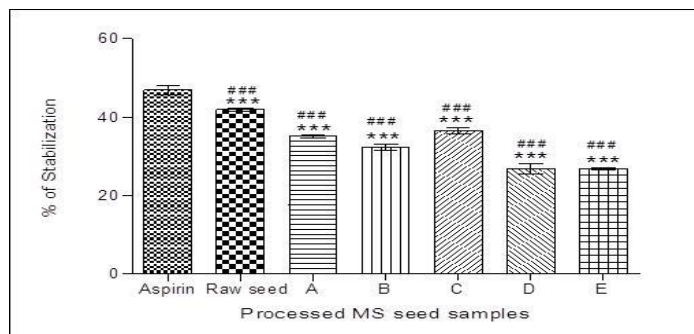


Figure 7 Human Red Blood Cell (HRBC) membrane stabilization activity (%) of different processed MS bean (A-soaking, B-Autoclaving, C-Roasting, D-Soaking plus Autoclaving, E-Soaking plus Roasting). Values are expressed as mean±SEM (n=3) using one-way ANOVA followed by Dunnett’s multiple comparison tests. *** = p<0.001 when compared with Raw seed sample.

Standard drug aspirin showed 46.78±0.7% stabilization activity at the same concentration. Decrease in hemolysis inhibition property in processed MS seed indicates partial denaturation or complete destruction of phytochemicals responsible for it.

CONCLUSIONS

M. sanjappae has tremendous scope for exploring as a functional food due to its anti-Parkinson’s drug L-DOPA and higher level of antioxidants content. From the present study, it can be conclude that, amongst the various processing treatments, soaking plus autoclaving is the most efficient treatment for reducing the L-DOPA and other anti-nutrients. The processing methods also affect nutritional constituents, antioxidants and anti-inflammatory potential. The processing techniques under study are easy and cost effective which can provide an easy way of processing MS bean to utilize its full potential as a functional food. This study will be helpful to explore *M. sanjappae* as a novel source of food to world as demand is continuously increasing due to increasing population.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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