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Effect of shodhana process on quantity of phytoconstituents of *Semecarpus anacardium* Linn.

Umang Gajjar[1]*, Kapil Khambholja[2] and Rakesh Patel[2]

1, Department of Pharmacognosy, Babaria Institute of Pharmacy, Varnama, Vadodara, (Gujarat) - India

2, Department of Pharmacognosy, S.K.Patel College of Pharmaceutical Education & Research, Ganpat Vidhyanagar, Kherva, Mehsana, (Gujarat) - India

Abstract

Nuts of *Semecarpus anacardium* Linn. are used in the Ayurveda and Siddha systems of medicine, with various therapeutic properties. They are always associated with several side effects, if used unpurified. Many of the traditional books on Ayurveda have described various methods of purification for plants containing toxic materials. Quantitative estimation of active constituents in the herbal drug is an important step in the evaluation of herbal drugs and formulation. There are lots of effects of the Shodhana process on quantity of the phytoconstituent and also on the pharmacological action of the *Semecarpus anacardium* Linn. *Semecarpus anacardium* contains phenolic compounds, flavonoids and carbohydrate as a major phytoconstituents. There is not any significant change in the amount of total flavonoids content & total carbohydrate content in the *Semecarpus anacardium* due to the Shodhana process but there is a drastically change in the concentration of total phenolic content due to the Shodhana process.

Key-Words: *Semecarpus anacardium*, Quantitative estimation, Phenolic, Flavonoid, Carbohydrate.

Introduction

Nuts of *Semecarpus anacardium* Linn. are used in the Ayurveda and Siddha systems of medicine, with various therapeutic properties, such as Anti-helminthes, Antifungal, Anti carcinogenic, Nervous debilities, Arthritis and cardiovascular disease. They are always associated with several side effects, if used unpurified. Many of the traditional books on Ayurveda have described various methods of purification for plants containing toxic materials. These purification methods are known as "Shodhana" process in traditional language. There are different shodhana processes for *Semecarpus anacardium* mentioned in traditional texts[1-4].

Quantitative estimation of active constituents in the herbal drug is an important step in the evaluation of herbal drugs and formulation. Estimation may be done in terms of setting a lower limit on the active constituent level. The marker based standardization may lead to misinterpretation of the quality of herb or formulation when the effects of each and every phytoconstituent are not known.

*** Corresponding Author:**
E-mail: gajjar_umang@yahoo.com
Mob.: 094288 – 06472

The new trend in the standardizations suggests measuring the phytoconstituent in its totality and thus we measure important phytoconstituent viz. total phenolics and total flavonoid.

Material and Methods

Estimation of total phenolic

Preparation of test solution

Before Shodhana Process

Five Gms of *Semecarpus anacardium* fruit were extracted with 100 ml methanol. This methanolic solution was used to estimate the total phenolic.

After Shodhana Process

Five Gms of Shudha *Semecarpus anacardium* fruit were extracted with 100 ml methanol and methanolic extract was concentrated on water bath up to 25 ml.

Materials: Gallic acid (100 – 500 mcg/ml of methanol), Extract or Processing water, Distilled water, Folin – Ciocalteu's Reagents, 7% Na_2CO_3 Solution

Gallic acid was procured from Suyog diagnostics Pvt. Ltd, Mumbai and Folin – Ciocalteu's Reagent was procured from Finar Chemicals Ltd, Ahmedabad. The other entire chemical was analytical grade.

Method

Total phenolics in the methanolic extract of *Semecarpus anacardium* (Before & after Shodhana

process) were determined using the Folin – Ciocalteu's reagent as per the method discussed by Hichami H. and Soulman A. The reaction mixture were prepared by using 50 µl of methanol solution of extract, 3 ml of distilled water, 250 µl of the Folin – Ciocalteu's reagent and 750 µl of 7 % sodium bicarbonate solution. After 2 hour, the absorbance at 765 nm was obtained against blank that had been prepared in a similar manner, by replacing the extract with distilled water. The total phenolic content, expressed as mg Gallic acid equivalents per gm dry weight of sample was determined using calibration curve of Gallic acid standard [5].

Estimation of total flavonoids

Preparation of test solution

Before Shodhana Process

Semecarpus anacardium fruit (5 Gms) were extracted with 100 ml methanol. Methanolic extract was used to estimate the total flavonoids.

After Shodhana Process

Shudha *Semecarpus anacardium* fruit (5 Gms) were extracted with 100 ml methanol and methanolic extract was used to estimate the total flavonoids.

Materials: Quercetin in Methanol, Plant extract in methanol, 10 % Aluminium chloride, 1 M potassium Acetate, Distilled water

Method

Aluminium chloride colorimetric method was used for total flavonoid determination in test extracts. Methanolic solution of plant extract was mixed with 1.5 ml of methanol, 0.1 ml of 10 % Aluminium chloride. The mixture was allowed to incubate at room temperature for 30 minutes. The absorbance of the reaction mixtures was measured at 415 nm against a blank without test drugs. The calibration curve was prepared by treating Quercetin solutions in methanol in similar manner[6].

Estimation of Total Carbohydrate

Material: Plant Extract, 80 % phenol solution, Conc. Sulphuric acid, Sucrose as a standard

Preparation of Test solution

5 Gms of *Semecarpus anacardium* was extracted with 50 ml of water. The water extract was used to estimate the total carbohydrate.

Method

Two milliliters of plant extract was mixed with 0.05 ml of 80 % phenol solution. Then 5 ml of concentrated sulfuric acid is added rapidly, the stream of acid being directed against the liquid surface rather than against the side of the test tube in order to obtain good mixing. The tubes were allowed to stand for 10 minutes, and then they were shaken and placed for 20 minutes in a water bath at 25° C. The absorbance was measured at 490 nm. Blanks were prepared by substituting distilled water for the plant extract. The calibration curve was prepared by preparing sucrose solutions[7].

Results and Conclusion

Table 1 summaries the content of total phenolics, expressed as Gallic acid equivalents (GAE) found in methanolic extract. The Total Phenolic Content (TPC) in *Semecarpus anacardium* before Shodhana process was almost 3 – fold higher than those of TPC in Semecarpus anacardium after Shodhana process. These data indicates significant decrease in the TPC during Shodhana process of Semecarpus anacardium Linn ($p<0.05$). The drastic decrease in the TPC of *Semecarpus anacardium* Linn is due to the removal of phenolics reach toxic oil from nuts and fruits.

Table 2 summaries the flavonoids contents in the methanolic extract of *Semecarpus anacardium* Linn. before Shodhana process (7.513 ± 0.006518 % w/w) and after Shodhana Process (7.435 ± 0.004680 % w/w) which is equivalent to Quercetin.

Table 3 summaries the total carbohydrate contents in the aqueous extracts of Semecarpus anacardium Linn before Shodhana process (0.9077 ± 0.02623 %w/w) and after Shodhana process (0.8531 ± 0.01828 % w/w).

There is not any significant change in the amount of total flavonoids content and total carbohydrate content, while there is a drastically change in the TPC content in the *Semecarpus anacardium* due to the Shodhana process. This may be due to the fact that most of the phenolic substances present in *Semecarpus anacardium* are non-flavonoid type.

We found that the total phenolic concentration in the *Semecarpus anacardium* was drastically decreased due to the Shodhana process, but this change is very much useful to increase the safety margin as phenolic oil present in the *Semecarpus anacardium* is toxic in nature.

Table 1: Total Phenolic as GAE in *Semecarpus anacardium* Linn.

Total Phenolics in Semecarpus anacardium Linn. (% w/w)	
Before Shodhana	**After Shodhana**
28.960 ± 0.4163	8.940 ± 0.08660
Mean of 3 observation	

Table 2: Total Flavonoid in *Semecarpus anacardium* Linn.

Total Flavonoid in Semecarpus anacardium Linn. (% w/w)	
Before Shodhana	**After Shodhana**
7.513 ± 0.006518	7.435 ± 0.004680
Mean of 3 observation	

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Fig. 1: Comparison of TPC as GAE in the sample of *Semecarpus anacardium* Linn Before and After Shodhana process

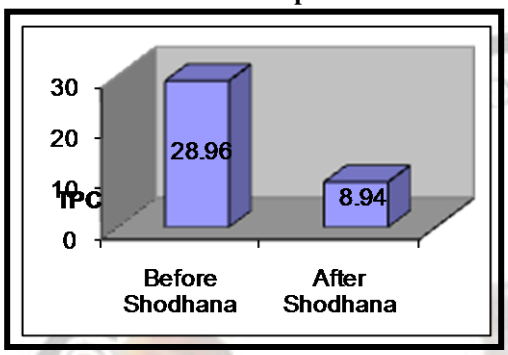


Fig. 2: Comparison of total flavonoids in methanolic extract of *Semecarpus anacardium* before and after Shodhana process

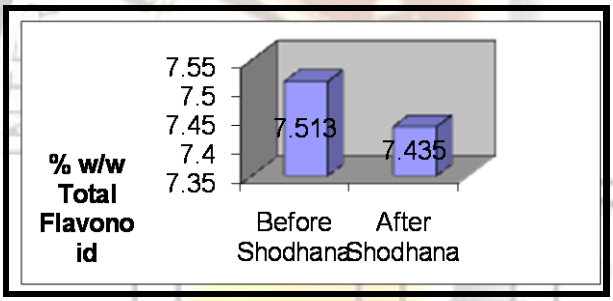
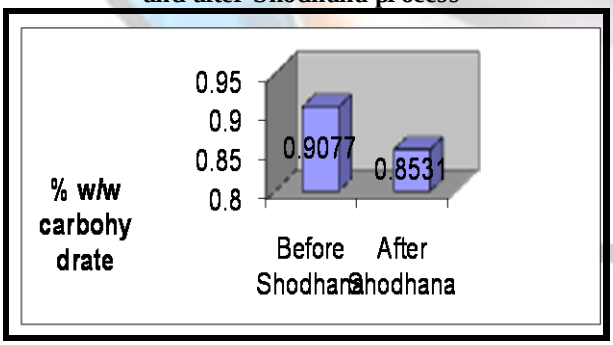


Table 3:Total Carbohydrate in Semecarpus anacardium Linn.

Total Carbohydrate in Semecarpus anacardium Linn. (% w/w)	
Before Shodhana	**After Shodhana**
0.9077 ± 0.02623	0.8531 ± 0.01828
Mean of 3 observation	

Fig. 3: Comparison of total carbohydrate in aqueous extract of *Semecarpus anacardium* before and after Shodhana process



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