Available online at www.cpronline.in

Current Pharma Research 3 (4), 2013, 1019-1022.

Current Pharma Research

http://www.cpronline.in

Original Article

Comparative study of isolated moiety from Senna leaves, Pods and Powder.

P.A. Shelar[a,*], B.V. Jawlikar[a], M.S. Mohite[a], V.N. Raje[a], M.B.Thorat[a], S.S. Tikole[b]

[a]*Gourishankar Institute of Pharmaceutical Education and Research, Limb, Satara, Maharashtra, India,* [b]*College of Pharmacy, Medha, Satara, Maharashtra, India.*

Received 26 July 2013; received in revised form 19 Aug 2013; accepted 19 Aug 2013
Available online 15 September 2013

Abstract

India is a landmark for traditional system of medicine from past few centuries. Most of the traditional systems of medicine are effective but only one major drawback is lack of standardization. So, there is need to develop standardization technique to mingle this system of medicine in the main stream of health sciences. Senna is reputed drug included in Unani medicine. It is used as laxative & cathartic. This specification covers the standardization & preliminary phytochemical investigation of marketed samples of senna. The marketed samples such as senna leaves, pods & powder were collected from different regions and subjected to phytochemical investigation, fluorescence analysis, thin layer chromatography and infra-red spectroscopy for evaluation of sennosides. It was observed that sample shows variation in chemical analysis, fluorescence analysis, thin layer chromatography and it may be due to raw material collection time, geographical variation, etc. This study helps in proper identification as well as comparision between marketed samples for the amount of sennosides present in leaves, pods and powder.

Keywords: *Standardization, Marketed samples, Chromatography, Spectroscopy.*

1. Introduction

Senna is wonder medicinal plant. It is not a plant of Indian origin. It is native in South Arabia and now under cultivation in India. Senna was known to physicians from very old days and was included in Unani medicine. About 26 species of genus cassia have been reported to contain anthracene derivatives either in free from or glycosides [1]. Senna contain anthraquinone glycoside as sennoside A, sennoside B, sennoside C, sennoside D. Sennoside A and sennoside B are stereoisomer of each other. It contains emmodin, aloe-emmodin, rhein-8-diglycoside, anthrone diglycoside, chrysophanol. It contains two napthalin glycosides that is 6-hydroxy musizin glycoside and tinnevellin glycoside. It also contains phytosterole, mucilage, resin, myricyl alcohol, salicylic acid, yellow flavinol, kaempferol & its glycoside kaempferin. It is used as irritant purgative. Senna leaves are used as laxative & cathartic. Powder senna is mixed with vinegar & applied externally to cure skin disease. The drug is used in acute constipation [1].

2. Experimental Protocol

2.1. Collection of Plant material

The leaves of senna were purchased from satara market, senna powder was purchased from Arkashala Ayurvedic industry (Satara) and senna pods were purchased from Mumbai market.

*Corresponding author.
E-mail address: pshelar82@yahoo.com
(P.A.Shelar)

2.2. Extraction [2]

In the present study, the shade dried leaves, pods and powder of *Cassia aungustifolia* belonging to family Leguminosae were reduced to coarse powder (# 40 size mesh) and around 60gm of each powder was subjected to hot continuous extraction (soxhlet) with methanol. Another batch of powdered samples was macerated with chloroform-water I.P. After the effective extraction, solvent were evaporated to dryness and the extracts obtained were tested for qualitative phytochemical investigation.

2.3. Phytochemical Screening

After extraction of collected leaves, pods and powder with methanol & water, the phytochemical screening of these extracts were carried out according to the standard procedures described by Kokate[3] and Horborne [4].

2.4. Isolation of sennosides from senna [5]

The leaves and pods were dried and powdered while the leaf powder was used as such for isolation. The powdered drug was shaken with benzene on electronic shaker for 2 hrs. Filtered and distilled off the solvent and marc was dried at room temperature and extracted with 70% methanol for 4 to 6 hrs. The extract was filtered under vacuum and it was re-extracted with 70% methanol for 2 hrs, and filter. The methanolic extract was combined and concentrated to 1/8th portion of its original volume. The concentrated solution was acidified with hydrochloric acid to a pH of 3.2. The acidified solution was kept aside for 2 hrs at temperature of 5°C. The solution was filtered and added into anhydrous calcium chloride dissolved in 25ml of

denatured spirit with constant and vigorous stirring. The pH was again adjusted to 8 by ammonia and it was set aside for 2 hrs. The solution was filtered; the precipitation obtained was dried over P_2O_5 in a desiccators.

2.5. Fluorescence Analysis

Many drugs fluorescence when their powder is exposed to ultraviolet radiation. The fluorescence characteristics of powdered drug were studied under U.V. light after treating with different chemical reagents was reported.

2.6. Thin Layer Chromatography

The extracts were subjected to thin layer chromatography for the presence of phytoconstituents. In this technique, the Silica gel-GF_{254} (for TLC) was used as an adsorbent and plates were prepared by spreading technique, then air dried for an over-night and activated for one hour at 110°C and used for analysis.

2.7. Thin Layer Chromatography of Sennosides [6,7]

Sennosides were spotted on Silica gel G plates and developed using ethyl acetate: methanol: water (100:16.5:13.5) as solvent system, red colored spot will appear when the spots were sprayed with 25% nitric acid and turn to yellow when sprayed after drying with alcoholic potassium hydroxide solution.

2.8. Characterization of Isolated Compound

From the separated bands, the substance of interest was scrapped from the plate and it was dissolved in methanol. The mixture was filtered and the filtrate was evaporated to dryness. The isolated compound was then subjected for further studies.

2.9. IR of isolated compound [8,9,10]

IR spectrum was recorded in IR spectrometer for isolated compound.

Result

The results of phytochemical investigation of qualitative analysis of methanolic and aqueous extracts of senna leaves, powder and pods have been discussed here.

Table 1.
Chemical investigation of senna leaves.

Sr. No.	Name of the test	Methanol extract	Aqueous extract
1	Test for sterols	+	+
2	Test for Triterpenoids	+	+
3	Test for glycosides	+	+
4	Test for carbohydrates	+	+
5	Test for alkaloids	-	_
6	Test for flavonoids	+	+
7	Test for tannins	-	-
8	Test for amino acids	+	+
9	Test for fats	-	-
10	Test for Volatile oils	-	-
11	Test for proteins	+	+

Table 2.
Chemical investigation of senna powder.

Sr. No.	Name of the test	Methanol extract	Aqueous extract
1	Test for sterols	-	-
2	Test for Triterpenoids	-	-
3	Test for glycosides	+	+
4	Test for carbohydrates	+	+
5	Test for alkaloids	_	-
6	Test for flavonoids	_	-
7	Test for tannins	_	-
8	Test for amino acids	+	+
9	Test for fats	_	-
10	Test for Volatile oils	-	-
11	Test for proteins	+	+

Table 3.
Chemical investigation of senna pods.

Sr.No.	Name of the test	Methanol extract	Aqueous extract
1	Test for sterols	-	–
2	Test for Triterpenoids	-	–
3	Test for glycosides	+	+
4	Test for carbohydrates	+	+
5	Test for alkaloids	–	–
6	Test for flavonoids	+	+
7	Test for tannins	+	+
8	Test for amino acids	+	+
9	Test for fats	–	–
10	Test for Volatile oils	–	–
11	Test for proteins	+	+

Qualitative phytochemical investigation was showed in Table 1, 2, 3 and chemical investigation was as follows,
Steroid and Triterpenoids were present in leaves while absent in powder and pods. Tannins were present in pods but it was absent in leaves and powder. Flavonoids were absent in powder while present in pods and leaves.

Analysis of extracts
Colour, nature and yield of sennoside extracted from leaves, pods & powder was tabulated in Table 4 and it showed following results.

The pods contained maximum amount of sennoside and powder contain moderate amount of sennoside while leaves contain less amount of sennoside.

Fluorescence analysis
Fluorescence analysis was tabulated in Table 5 and it showed following results.
All the extract showed fluorescence at 254-366 nm.

Chromatography
TLC observations
The observations for TLC are shown in Table 6. By comparing R_f value of isolated compound with R_f value of standard, it was concluded that leaves, powder and pods contain different types of sennosides.

Table 4.
Determination of color, nature & yield of sennoside from leaves, powder and pods.

Sr. No.	Sample name	Nature of Extract	Colour	Weight (g)
1.	Leaves	Semi-solid	Greenish	0.36
2.	Powder	Semi-solid	Greenish black	1.27
3.	Pods	Semi-solid	Reddish brown	2.63

Table 5.
Fluorescence analysis of extracts.

Reagents	Fluorescence observed					
	Leaves		Pods		powder	
	At 254 nm	At 366nm	At 254 nm	At 366 nm	At 254 nm	At 366 nm
Powder + 1N NaOH In Methanol	Green	Green	Green	Light Green	Blue	Green
Powder + 1N NaOH In Water	Green	Green	Dark Green	Light Green	Blue	Green
Powder + 50% Hcl	Blue	Yellow	Yellowish Green	Green	Black	Yellowish Green
Powder + 50% H_2SO_4	Light Green	Light Green	Light Green	Light Green	Green	Green
Powder + 50% HNO_3	Dark Yellow	Greenish Yellow	Dark Yellow	Dark Yellow	Dark Yellow	Dark Yellowish Black
Powder + Petroleum Ether	Faint Yellowish Green	Faint Yellowish Green	Faint Yellow	Green	Faint Yellowish Green	Faint Yellowish Green
Powder +Chloroform	Faint Green	Faint Green	Green	Green	Faint Green	Faint Green
Powder +Picric Acid	Faint Green	Faint Green	Yellow	Yellow	Faint Green	Faint Green
Powder +5% $Fecl_3$	Yellowish Green	Yellowish Green	Faint Green	Faint Green	Yellowish Green	Yellowish Green
Powder +5% Iodine	Green	Faint Green	Green	Faint Yellow	Faint Green	Black Green
Powder +Methanol	Black	Dark Green	Black	Faint Golden	Black	Faint Green
Powder + (HNO_3+NH_3)	Faint Green	Yellowish Green	Dark Green	Light Green	Purple	Yellowish Green

Table 6.
TLC of isolated compound.

Extracts	Observation		R_f values
	No. of spots	Colour of spots	
Leaves	1	Yellowish Orange	0.8543
pods	1	Faint Yellow	0.4384
Powder	1	Yellow	0.5615

Infra Red (IR) Analysis
IR interpretation was mentioned in Fig. 1

From Infra-red interpretation, it was found that isolated compound obtained from extracts of leaves, powder and pods may be of Sennoside.

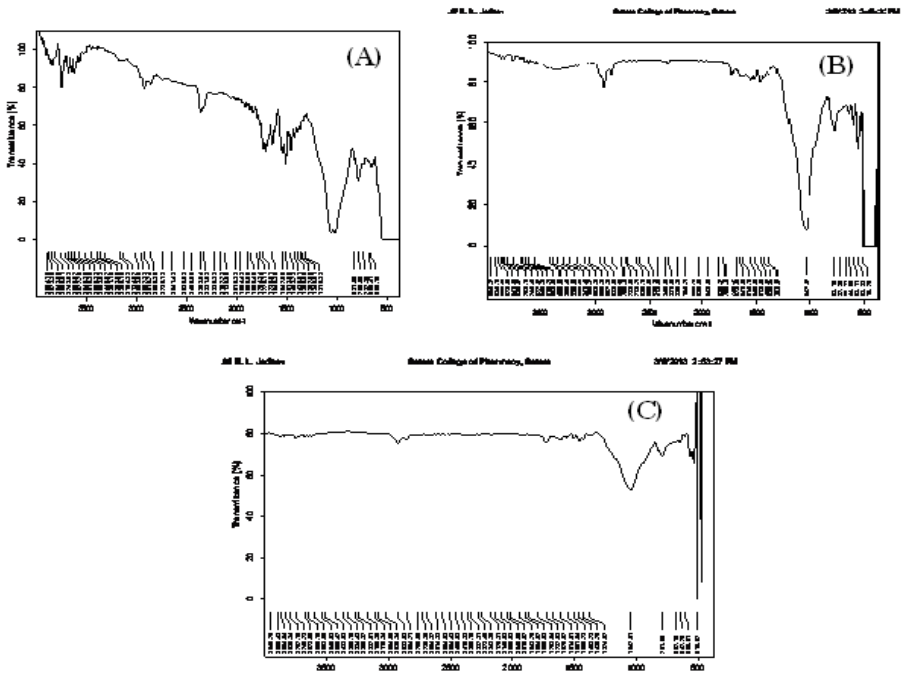

Fig. 1. IR of Senna leaf (A), IR of Senna Powder (B), IR of Senna Pods (C).

Discussion

The quality of herbal marketed samples obtained from different distributors shows fluctuations which can be depicted from experimental data. Qualitative chemical analysis shows variation in the chemical constituents in all the samples. Fluorescent analysis data justifies there is difference in content of fluorescent compound in all the samples. The IR spectroscopy depicts there is uniformity in the content of active constituent that is isolated compound may be of sennoside. TLC chromatogram of isolated compound of all samples shows relative R_f values where all the samples shows presence of different types of sennosides.

Conclusion

The result of present study clearly indicates that there is no uniformity in the marketed samples of senna which may be due to varied geographical locations where these plants grow, coupled with the problems of different vernacular names, these plants are known by great deal of adulteration or substitution. These problems are encountered in the commercial market. It might be useful contribution to the selection of appropriate marketed samples for better effectiveness.

References

[1] C.K. Kokate, A.P. Purohit, S.B. Gokhale, Pharmacognosy, General Introduction, 1990.
[2] J.B. Harborne, Methods of extraction and isolation, London. 1988.
[3] C.K. Kokate, Practical Pharmacognosy, New Delhi 1986.
[4] J.B. Harborne, Methods of extraction and isolation, London 1998.
[5] E. Edwin Jarald, Pharmacognosy and Phytochemistry, New Delhi, 2007.
[6] G.R. Chatwal, S.K. Anand, Instrumental methods of chemical analysis, 1992.
[7] Ergon Stahl, Thin Layer Chromatography, A Laboratory Hand Book, New York, 1990.
[8] William Kemp, Organic Spectroscopy, 1991.
[9] B.K. Sharma, Instrumental method of chemical analysis, Merrut, 2002.