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A STUDY OF VARIOUS MARKET SAMPLES OF SWEET FLAG (ACORUS CALAMUS LINN) WITH SPECIAL REFERENCE TO ITS PHARMACOGNOSTIC & PHYTOCHEMICAL ASPECTS.

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ABSTRACT

Sweet flag is one of the extensively prescribed herbs in Ayurvedic formulations. It has quoted in *Brihatrayi* i.e, *Charak Samhita, Sushruta Samhita* and *Astanga Hridaya* several times for many diseased conditions especially in speech problems. Rhizome is medicinally useful part and botanical resource is *Acorus calamus Linn*, belongs to Family *Araceae*. The rhizome is acrid, bitter, aromatic , intellect promoting , emetic , carminative , stomachic , expectorant , antispasmodic, anticonvulsant, anti-inflammatory , anti-pyretic , voice promoting and useful in mental disorders. Its market demand increases day by day comparing to supply. This leads to premature harvesting of rhizome and intentional practice of adulteration with so many other species. Sometimes such types of raw materials have less aromatic smell and essential chemical components. Ultimately it hampers the therapeutic efficacy. Therefore a sincere effort has made by procuring market samples from different markets and to analyze on basis of their different Pharmacognostic & Phytochemical parameters .The reported data also compared with standard parameters of Ayurvedic Pharmacopoeia.

Key Words: *Sweet flag, Acorus calamus, Market Sample, Pharmacognostical study & Phytochemical study.*

INTRODUCTION: In various Ayurvedic formulations sweet flag has referred several times. It has quoted in *Brihatrayi* i.e, *Charak Samhita, Sushruta Samhita* and *Astanga Hridaya* frequently for speech problems. Rhizome is medicinally useful part and botanically known as *Acorus calamus Linn*, Family *Araceae*. Rhizomes contains volatile oils like asaryladehyde , asarone, acorin,eugenol, crystalline; alkaloids like calamine ; mucilage ; starch, less amount of tannin and resinous bitter principle acoretin etc. It is aromatic, emetic ,intellect promoting, voice promoting , stomachic , carminative , expectorant , antispasmodic, anticonvulsant, anti-inflammatory , anti-pyretic , sialagogue, tranquilizing ,nervine tonic , sedative, odontalgia, amentia and mental disorders. This drug is indigenous to Eastern Europe and central Asia. It is a semi aquatic perennial herb usually cultivated in damp marshy places in India & Burma. This is common in Manipur, Assam, Kashmir, Sirmoor , Sikkim & Nagaland especially Tumkur district of Karnataka . There are so many adulterants sold in the name of *Vachā* & *Ghor Vachā* in local markets apart from genuine rhizomes of *Sweet flag*.

Therefore a sincere effort has made by procuring genuine market samples from

four different markets of Vadodara, Delhi, Belgaum & Kolkata. The reported comparative Pharmacognostic and Phytochemical data has given in this article.[1, 2 & 3.]

Morphology of Sweet flag: Rhizome is woody, branched, light brown, cylindrical to flatten and 10-15 mm in diameter with distinct nodes and internodes. Nodal regions are broad with leaf scars and hair like fibers. Internodes are 8-10mm in length, ridged and furrowed. Undersurface is provided with zigzag line of circular root scars. Transversely cut surface is cream in color with pinkish tinge and differentiated into scars. Freshly exposed surface is granular and porous with soothing aromatic odor. [3]

Adulteration & Substitution: Sweet flag is adulterated with several other drugs like- *Alpinia Officinarum Hance, Alpinia galanga Willd, Iris germanica Linn, Acorus gramineus Soland* and *Alpinia calcarata* etc. In local markets these sold in the name of *Vachā*, *Ghor Vachā* and *Śweta-Vachā* .

Alpinia galanga Willd- A branched rhizome, about 12mm thick, in pieces about 5 or 10 cm long. Frequently cylindrical but sometimes tapering or enlarged , dull reddish-brown, longitudinally striated at intervals of about 5mm , pale encircling remains of leaves. Root remains attached to the rhizome. Interior of the drug is reddish brown in color, strongly pungent taste and agreeable spicy odor.

Iris germanica Linn- Rhizomes occurs usually in pieces from 5-10 cm, long up to 4 cm, wide about 2cm, thick, pale cream color, often dorsy ventrally flattened and constricted at intervals or bearing 1 or 2 short lateral branches at the apex. Under surface small dark circular scars of roots and on upper surface traces of leaf marks present. It is slightly bitter and aromatic in smell.

Althaea offincinalis L. - Its tap root is grayish yellow, corky externally and white fibrous within, slightly scented, sweetish and astringent.

The powdered drugs are reported to be adulterated with Siliceous earth, Ground root of *Althaea offincinalis L.* (marsh mallow) & Cereal flours.[1, 2, 4-10.]

MATERIALS & METHODS: Four different market samples of sweet flag were purchased from the markets of Vadodara, Delhi, Belgaum & Kolkata. These four samples were named as Sample –A, Sample –B, Sample –C & Sample –D respectively. All the Pharmacognostic & Phyto-chemical studies were carried out in Department of Pharmacognosy , Goa College of Pharmacy, Panaji, Goa as per the standard procedures.[10-14]

Organoleptic characteristics: The organoleptic characters of Sweet flag were analyzed on basis of criteria i.e, Physical appearances, Color, Smell &Taste etc.

Table-1:ORGANOLEPTIC EXAMINATION:

Character	Sample-A	Sample-B	Sample-C	Sample-D
Physical appearances	Rhizomes are sub cylindrical, tortuous & shrunken. Transverse leaf scars encircle the rhizome on upper surface, lower surface spots of	Rhizomes are cylindrical flattened & slightly tortuous. Leaf scars on upper surface encircle the rhizome & lower surface have Pinkish root scars.	Rhizomes are cylindrical, tortuous, having deep longitudinal wrinkles. Leaf scars cover the upper surface of the rhizome, Lower surface having Reddish tinge root scars.	Rhizomes are cylindrical, slightly flattened, tortuous, shrunken, deep longitudinal wrinkles. Leaf scars seen alternately arranged on upper surface and encircled the

	reddish root scars.			rhizome, lower side elevated light brown spots of root scars are present.
Color	Brown	Light brown	Yellowish brown	Light brown
Smell	Aromatic	Characteristic and Aromatic.	Less aromatic	Characteristic and Aromatic.
Taste	Pungent, Bitter.	Less pungent, Bitter.	Pungent, Bitter.	Bitter, Pungent , Disagreeable.

Common identification tests for Sweet flag:

Dried rhizomes are kept in oven at 60 ^{0}c for 4 to 6 hrs to make moisture free and grounded in the grinder.

1. Powder is pale brown, fibrous and grainy.
2. When mixed the powder with water, powder settles at the bottom giving a straw yellow color solution which on heating gives a sweet aromatic smell.
3. When powder added with 5% KOH solution, powder settles at the bottom giving a clear orange brown solution.
4. On mixing powder with 5% H_2SO_4 solution, most of the powder settles at the bottom, small quantity floats on the surface giving faint cream color turbid solution.
5. On addition of $FeCl_3$ solution, some powder settles and some floats giving a thick dark brown solution.
6. On addition of KI and iodine solution powder settles at the bottom giving a dark brown solution.

Table-2:- COMMON IDENTIFICATION TESTS OF ALL SAMPLES:

Test	Sample-A	Sample-B	Sample-C	Sample-D
When mix the powder with water, powder settles at the bottom.	Yellow colored solution.	Straw yellow colored solution.	Pale yellow colored solution.	Straw yellow colored solution.
On heating	Aromatic smell	Sweet aromatic smell	Less aromatic smell	Sweet aromatic smell.
On addition of 5% KOH solution on powder , it settles at the bottom and gives	Hazy , orange brown solution	Clear ,orange brown solution	Light orange dark brown solution	Clear ,orange brown solution
On mixing the powder with 5% H_2 SO_4 solution , most of the powder settles at the bottom, small quantity floats	Cream colored turbid solution	Faint yellowish colored turbid solution	Cream colored turbid solution	Faint, cream colored turbid solution.

on the surface				
On addition of $FeCl_3$ solution, some quantity of powder settles and some floats on surface.	Thick light brown solution	Thick dark brown solution	Thin light brown solution	Thick dark brown solution.
By adding KI and iodine solution on powder it settles at the bottom.	Light brown solution	Dark brown solution	Light brown solution	Dark brown solution.

In Physical constant values, quantitative standards like-foreign matter, total percent of moisture , total ash, acid insoluble ash, water soluble ash , water soluble extractive, alcohol soluble extractive, total percentage of volatile oil, specific gravity of aqueous extractive value, pH of aqueous extractive value, total percentage of resin, sulphated ash, acid value , ester value, saponification value etc. were determined.

Qualitative chemical tests were done for aqueous extracts to analyze & identification of the phyto-constituents present in it. Study for carbohydrate, starch, proteins, steroids, alkaloids, tannins & phenolic compounds, fat, glycosides, flavonoids, saponin, gum , mucilage & volatile oil etc. were done. Chemical tests for detection of inorganic matters were conducted for iron, chloride, sulphate, calcium, magnesium, aluminium, sodium & potassium.

Table-3:- PHYSICO- CHEMICAL ANALYSIS:

Name of the Test	***Sample-A***	***Sample-B***	***Sample-C***	***Sample-D***	***Standard Pharmacopoeia Value***
Foreign matter	2.3	1.6	2	1.3	NMT 1%
Total % of moisture	11.4	9.8	12.35	6.6	NMT 1%
Total ash value	10.8	5.7	9.2	8.2	NMT 7%
Acid insoluble ash value	2.3	1.1	1.8	1.1	NMT 1%
Water soluble ash value	4.2	1.9	5.2	4.6	-
Water soluble extract value	28.2	24.4	26.1	29.70	NLT 16%
Alcohol soluble extractive value	24.3	12.6	25.6	35.6	NLT 9%
Total % of volatile oil w/v	2.8	3.1	1.7	3.8	NLT 2%
Specific gravity of aqueous extractive value	0.650	0.755	0.580	0.975	-
pH of aqueous extractive value	5.5	4.8	6.3	6.8	-
Total % of resin	1.8	2.8	2.6	3.5	-
Sulphated ash	3.3	4.2	3.6	2.9	-
Acid value	2.6	1.8	1.5	1.3	-
Ester value	2.5	2.3	1.7	1.6	-
Saponifiction value	2.9	4.1	3.2	5.1	-

Table-4:- PRELIMINARY PHYTOCHEMICAL STUDY:

Test For	***Sample-A***	***Sample-B***	***Sample-C***	***Sample-D***
Carbohydrates (Molish's test)	Positive	Positive	Positive	Positive
Reducing test (Fehling's test)	Positive	Positive	Negative	Positive
Reducing test (Benedict's test)	Positive	Positive	Positive	Positive
Tannic test for starch	Positive	Positive	Positive	Positive
Proteins (Biuret test)	Positive	Positive	Positive	Positive
Proteins (Million's test)	Positive	Positive	Positive	Positive
Steroids (Liebermann-Burchard's reaction)	Negative	Negative	Negative	Positive
Steroids (Salkowski) reaction	Negative	Negative	Negative	Positive
Alkaloids (Dragendorff's test)	Positive	Positive	Negative	Positive
Alkaloids (Mayer's test)	Positive	Positive	Negative	Positive
Tannin and phenolic compounds($FeCl_3$)	Positive	Positive	Positive	Positive
Tannin & phenolic compounds(lead acetate)	Positive	Positive	Positive	Positive
Fat	Negative	Negative	Negative	Negative
Glycosides(Legal's test)	Negative	Positive	Positive	Positive
Glycosides(Liebermann-Burchard' test)	Negative	Positive	Positive	Positive
Flavonoids(Shinoda test)	Negative	Negative	Negative	Positive
Flavonoids(Lead acetate solution)	Negative	Negative	Negative	Positive
Saponin **(**Foam test)	Positive	Positive	Positive	Positive
Saponin(Haemolytic test)	Positive	Positive	Positive	Positive
Gum	Negative	Negative	Negative	Negative
Mucilage(Ruthenium red solution)	Positive	Positive	Negative	Positive
Mucilage(KOH)	Positive	Positive	Negative	Positive
Volatile oil (Sudan III solution)	Positive	Positive	Negative	Positive
Volatile oil (Tincture alkana)	Positive	Positive	Negative	Positive

Table-5:CHEMICAL TEST FOR DETECTION OF IN-ORGANIC CONSTITUENTS:

Test For	***Sample-A***	***Sample-B***	***Sample-C***	***Sample-D***
Iron	Negative	Positive	Positive	Positive
Chloride	Positive	Negative	Negative	Negative
Sulphate	Negative	Negative	Negative	Negative
Calcium	Positive	Negative	Negative	Positive
Magnesium	Positive	Positive	Positive	Positive
Aluminium	Negative	Negative	Negative	Negative
Sodium	Positive	Positive	Positive	Positive
Potassium	Negative	Positive	Positive	Positive

RESULT & DISCUSSION:

Raw material samples of Sweet flag were purchased from four different places i.e Vadodara, Delhi, Belgaum & Kolkata have a variation in physical appearances. The rates of samples were also different. It has been observed that Kolkata having low rate & Belgaum, Delhi, Vadodara having gradually higher in rates. The samples purchased from Kolkata (sample –D) satisfy all the physical constant values/standards of Ayurvedic pharmacopoeia of India.[12]

Macroscopic and Organoleptic observations:

Physical appearances: Rhizomes were sub cylindrical, tortuous in Sample A & C while Sample B & D were cylindrical and slightly flattened. Sample C & D were having deep longitudinal wrinkles. Samples A, B & C were having transverse leaves scars encircle the rhizome on upper side and spots of root scars were present in lower side. The root spots were having reddish, pinkish and reddish tinge color respectively. The Sample D was having alternately arranged leaf scars on upper surface encircle the rhizome and elevated spots of root scars were present in lower side.

Color: Sample –B & D were having light brown color where as Sample A & Sample C brown and yellowish brown respectively.

Smell: Sample –B & D were having characteristic and aromatic smell, while Sample A was aromatic and Sample C was comparatively less aromatic.

Taste: Sample –A & C were pungent & bitter where as Sample B was less pungent and bitter. Sample D was bitter, pungent and disagreeable.

These suggest that Sample-D possess through all macroscopic & organoleptic characteristic as mentioned in Ayurvedic pharmacopoeia of India. It indicates that Sample –D was not adulterated and more genuine.

Common identification test:

Presence of straw yellow colored solution on mixing the powder with water, sweet aromatic smell on heating, clear orange brown solution on addition of 5% KOH solution, faint-cream colored turbid solution on mixing the powder with 5% H_2SO_4 solution, thick dark brown solution on addition of $FeCl_3$ solution and dark brown solution by adding Iodine solution possess the all test of genuinity in Sample –D.

On Physico-chemical analysis less percentage of foreign matter & moisture content i.e, 1.3 % & 6.6 % shows the maximum purity in sample D than others. Total ash value and acid insoluble ash value were observed within standard limits in the same sample i.e Sample D. Water soluble ash value were 4.2%, 1.9%, 4.6 % and 5.2% respectively.

The water / alcohol soluble extract value, total % of volatile oil, specific gravity of aqueous extractive value, pH of aqueous extractive value & Total % of resin were obtained as higher in Sample-D.

Less percentage of Sulphated ash in Sample-D indicates its more genuinity.

Higher saponifiction value of Sample-D justifies the presence of more water soluble constituents.

Less percentage of ester value and acid value in Sample-D in comparison to others proves its less chances of rancidity.

These show the maximum genuinity of Sample-D which was procured from Kolkata.

On Preliminary Phytochemical screening Positive result obtained for the test of carbohydrate, test for reducing sugar (Benedict test), tannic test for starch, Biuret and Millions test for protein, test for Tannin and Phenolic compound and test for Saponin (Foam test & Haemolytic test) in all four Samples.

Test for Steroids (Liebermann-Burchard's) / (Salkowski reaction) and test for flavonoids (Shinoda test)/ (Lead acetate solution) shows positive result in Sample-D where as negative in other Samples.

Test for fat & gum shows negative result in all Samples.

Except Sample C positive result obtained in all other three Samples under reducing sugar test (Fehling's test) , test for alkaloids (Dragendorff's test)/ (Mayer's test) , test for mucilage(Ruthenium red solution) / (with KOH solution) and test for volatile oil (Sudan III solution) /(Tincture alkana).

Except Sample A Positive result obtained in all other three Samples in tests for

Glycosides i. e, both by Legal's test and Liebermann-Burchard' test.

The presence of carbohydrates, starch, protein, Tannin and Phenolic compound, Saponin, Steroids, flavonoids, alkaloids, mucilage, Glycosides and volatile oil etc. suggest better chemical constituents in sample-D as per textual references.

On Chemical Test For Detection Of In-Organic Constituents: - Positive result obtained in test for iron & Potassium in all three samples except Sample A. This was opposite in test for chloride which was negative in all three samples except Sample A. Calcium was present in both the samples of A & D , where as absent in sample B & C. Sulphate & Aluminium was absent irrespective of all four Samples where as Magnesium & Sodium was present in all four samples.

It suggests presence of more organic constituents in Sample-D.

CONCLUSION:

This study gives clear evidence about the practices of adulteration in available market samples of Sweet flag. When such raw material used in medicinal preparations it will have less efficacy to combat the pathology. It may produce several health hazards. Further studies for adulteration can be confirmed by TLC, HPTLC techniques.

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