



Research Article

Preliminary Phyto-chemical screening of *Abelmoschus esculentus* Linn.

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ABSTRACT

Herbal drugs with little or no adverse effects are becoming a necessity in our daily life. The herbal products & herbal drug extracts are now a day's being used extensively for the preparation of pharmaceutical dosage forms as well as cosmeceuticals. In these work plant parts of *Abelmoschus esculentus* (*Okra*) were authenticated by appropriate authority. The dried pods of *Abelmoschus esculentus* (*Okra*) were extracted with various solvents or combination of solvents by maceration. Several chemical tests were performed on these extracts of the crude drugs. Some tests gave positive results. Some physical tests like loss on drying were conducted. Total phenolic & total flavonoid content were also determined. Different protocols were followed for the screening procedures, references of which were also provided.

Keywords: *Abelmoschus esculentus* (*Okra*), Malvaceae, botanical evaluation, phyto-chemicals, extractive values

Introduction

Materials and Methods:

The fruits (pods) were collected, identified and dried. The extracts of dried pods were used for preliminary phytochemical studies.

1.1 Collection and Identification of plant material:

The fruits of *Abelmoschus esculentus* were collected from Hooghly (West Bengal, India) and identified by the Office of the Scientist-'F' Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah-711103 (West Bengal) and a voucher specimen of plant (No.: CNH/Tech. II/2016/39).

1.2 Sampling of plant material:

The fruits were collected and dried in shade at room temperature, grinded coarsely in mixer grinder, kept in small plastic bags and preserved in air tight containers. The coarsely powdered dried fruits were used for the Phyto-chemical screening and physical evaluation.

20g of the coarsely powdered sample was mixed with 200ml of ethanol in a beaker and kept for 72 h at room temperature. It was then filtered, and

the filtrate was concentrated to dryness in a rotary evaporator and stored in refrigerator at 4°C for further analysis.

1.3 Ash Value:

The ash of any organic material is composed of their non- volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (Metallic salts & silica). This value varies within fairly wide limits & is therefore an important parameter for that purpose of evaluation of crude drugs. In certain drugs, the percentage variation of the weight of ash from sample to sample is very small & any marked difference indicates a change in quality. Unwanted parts of drugs, sometimes possess a character that will raise the ash value; for example, the sclerides in the unwanted pericarp of colocynth & the cork on liquorice, which is not required in the powder of the peeled drug. More direct contamination, such as by sand or earth, is immediately detected by the ash value. The ash value can be determined by three different methods to measure the total ash, the acid insoluble ash & the water soluble ash. [Table-3]

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Procedure:

- 1) Around 0.5 gm of powdered drug was taken.
- 2) Then the drug was transferred in a crucible & it was incinerated at 400⁰ C for hour.
- 3) Then the ash was boiled in water for 10 minutes.
- 4) After that, the ash was dried & weighed.
- 5) After, the water soluble part was dissolved & the rest part was poured in 0.2(M) HCL.
- 6) Then the ash was again dried and weighed.

1.4 Extractive Values:

This method determines the amount of active constituents in a given amount of medicinal plant material when extracted with solvents. It is employed for that material for which no chemical or biological assay method exist. As mentioned in different official books the determination of water soluble & alcohol soluble extractives, is used as a means of evaluating crude drugs which not readily estimated by other means.

The extraction of any crude drugs with a particular solvent yields a solution containing different phyto-constituents. The composition of this phyto-constituent in that particular solvent depends upon the nature of drug & solvent used. The use of a single solvent can be means of providing preliminary information on the quality of particular drug sample; for example, in a drug where the extraction procedure for the constituents commences with water as the solvent, any subsequent aqueous extraction on the re-dried residues will give a very low yield of soluble matter.[Table-4]

Determination of alcohol soluble extractives:

Procedure:

- 1) Weigh about 2 gm of coarsely powdered drug in a weighing bottle and transfer it to a dry 250ml conical flask
- 2) Fill a 100ml graduated flask to a delivery mark with the solvent (90% alcohol).washout the weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask.
- 3) Cork the flask & set aside for 24hrs, shaking frequently
- 4) Filter into a 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of the filtrate

to a weighed, thin porcelain dish, as used for the ash values determinations.

- 5) Evaporate to dryness on a water bath & complete the drying in an oven at 105⁰C for 6 hrs.
- 6) Cool in a desiccator for 30 minutes & weigh immediately.
- 7) Calculate the percentage w/w of extractive with reference to the air dried drug.

Determination of water soluble extractives:

Procedure:

- 1) Weigh about 2 gm of coarsely powdered drug in a weighing bottle and transfer it to a dry 250ml conical flask
- 2) Fill a 100ml graduated flask to a delivery mark with the solvent (Chloroform- water).Washout the weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask.
- 3) Cork the flask & set aside for 24 hrs , shaking frequently
- 4) Filter into a 50ml cylinder. When sufficient filtrate has collected, transfer 25ml of the filtrate to a weighed, thin porcelain dish, as used for the ash values determinations.
- 5) Evaporate to dryness on a water bath & complete the drying in an oven at 105⁰C for 6 hrs.
- 6) Cool in desiccators for 30 minutes & weigh immediately.
- 7) Calculate the percentage w/w of extractive with reference to the air dried drug.

1.5 Loss on drying/ Moisture content:

Procedure:

- 1) Weigh about 1.5 gm of the powdered drug into a weighed flat & thin porcelain dish.
- 2) Dry in the oven at 100⁰ C or 105⁰ C, until two consecutive weighing does not differ by more than 0.5mg.
- 3) Cool in desiccators & weigh. The loss in weight is usually recorded as moisture.

1.6 Extraction of Phyto-Chemical Constituents:

For preliminary phyto-chemical analysis, extract was prepared by weighing 100 gm of the dried powdered fruits were defatted by methanol and were subjected to hot successive continuous extraction in soxhlet apparatus with different solvents with increase in polarity, ethyl acetate, methyl acetate, ethanol and finally with water

(cold/hot). The extracts were filtered in each step, concentrated and the solvent was removed by vacuum distillation. The extracts were dried in the vacuum desiccator and the residues were weighed. The presence or absences of the primary and secondary phytoconstituents were detected by usual prescribed methods.

Qualitative phytochemical Tests for alkaloids

1) Tests for flavonoid

A. Dragendrach's test

Dragendrach's reagent was mixed with solution of extract. Presence of alkaloid was determined by reddish brown precipitate.

B. Mayer's reagent

Mayer's reagent was mixed with solution of extract. Presence of alkaloid was determined by cream colour precipitate.

C. Wagner's reagent

Wagner's reagent was mixed with solution of extract. Presence of alkaloid was determined by reddish brown precipitate.

D. Picric acid test

10% aqueous solution of picric acid was mixed with solution of extract. The presence of alkaloid was determined by formation of yellow colour.

E. Tannic acid test

Tannic acid solution gives buff colour to confirm presence of alkaloid.

2) Tests for flavonoid

A. Shinoda test

Extract solution with concentrated HCl and Mg turning gives pink scarlet/ crimson red/ green/blue colour to confirm flavonoids.

B. Zinc hydrochloride test

Extract solution with Zn dust and concentrated HCl gives red colour to confirm flavonoids.

3) Tests for phenols

A. Ferric chloride test

Solution of extract was heated with 70% alcohol in water bath, and then 5% solution of ferric chloride was added. Phenolic compound was determined by the appearance of blue green to green colour.

B. Lead acetate test

Solution of extract with 10% lead acetate gives bulky white precipitate.

4) Tests for glycoside

A. General test

a) Test A: Extract solution was heated with 10% sulphuric acid by warming on water bath. After

filter acid was neutralized with 5% solution of NaOH, than 0.1 ml of Feling's solution A and B were added until it becomes alkaline, and then heated in water bath for 2 minutes. Appearance of red colour shows presence of glycoside.

b) Test B: Extract solution was heated with water instead of 10% sulphuric acid by warming on water bath. After filter acid was neutralized with 5% solution of NaOH, than 0.1 ml of Feling's solution A and B were added until it becomes alkaline, then heated in water bath for 2 minutes. Appearance of red colour shows presence of glycoside.

1.7 Phytochemical Screening:

The fresh fruits were collected from Hooghly region, dried in shade and reduced to coarse powder passed through mess number 60. The powdered material was extracted with Ethanol, Methanol in Soxhlet apparatus for 4 hrs. The extract was filtered hot and solvent removed by distillation under reduced pressure. The percentage yield was calculated and the extract was further subjected to phytochemical tests for Alkaloids, Glycosides, Flavonoids, Carbohydrates, Tannins.

Quantitative Phytochemical Screening

1) Determination of Total Flavonoid Content:

2.5g of the extract was mixed with 25ml of 80% aqueous methanol. The whole solution was filtered through Whatman filter paper. The filtrate was transferred to a crucible and evaporated into dryness over a water bath and weighed.

Weight of residue

Flavonoid (mg/g) = -----

Weight of sample

2) Determination of Total Saponin Content:

5g of the extract was introduced into a conical flask and 25ml of 20% aqueous ethanol was added. The sample was heated over a water bath for 1 h with continuous stirring at about 55°C. The concentrate was transferred into a 250ml separator funnel and 5ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the ether layer was discarded. 15ml of n-butanol was then added followed by addition of 2.5ml of 5% aqueous NaCl. The remaining solution was heated over a water bath. After evaporation, the sample was dried in the oven to a constant weight.

Weight of residue
 Saponin (mg/gm)= -----
 Weight of sample

1.8 Thin Layer Chromatography:

Thin Layer Chromatographic plates were prepared by spreading silica gel G on glass plate using distilled water as solvent. The plates were activated in oven at 110°C for half hour. All four extracts are applied separately and run in different solvent system of varying polarity. These plates were developed in Iodine chamber for different spot of constituent chemical. Rf value was calculated for different extracts of fruits of *Abelmoschus esculentus* as per Table-5

1.9 Determination of Sulfated ash value:

Procedure:

A porcelain crucible was taken and heated for 10 minutes to red & allowed cooling in a desiccator and weighed accurately. Then 1 gram of air dried fruits of *Abelmoschus esculentus* was taken in a porcelain crucible & ignited gently until it was thoroughly charred. After that the residue was cooled & moistened with 1 ml of concentrated Sulphuric acid and heated gently at 400°C until all black particles disappeared. The crucible was allowed to cool. A few drops of concentrated sulphuric acid was added and heated. The entire operation was repeated until two successive weighing did not differ by more than 0.5 mg.

Result:

Table 1: Qualitative Phytochemical screening of *Abelmoschus esculentus*:

S. No.	Chemical Test	Methanol	Ethyl Acetate	Ethano	Water
1. CARBOHYDRATE					
A	Molish test	+	++	+	-
B	Fehling test	+	++	+	-
C	Pholoroglucinol test	-	-	-	-
D	Tollen's test	+	++	+	-
E	Cobalt chloride	-	+	-	-
F	Iodine test	+	+	+	-
G	Tannic acid test	-	-	-	-
H	Gum test	-	-	+	-
I	Mucilage test	+	+	+	-
2. PROTEIN					
A	Biuret test	-	-	-	-
B	Millon's test	-	-	-	-
C	Sulpher test	-	-	-	-
3. AMINO ACID					
A	Nihydrin test	-	-	-	-
B	Tyrosine test	-	-	-	-

4. FATS AND OILS

A Filter paper test - - - -

5. STEROID

A	Salkowski reaction	-	+	-	-
B	Libermann-Burchard reaction	-	-	-	-
C	Libermann's reaction	-	-	-	-

6. GLYCOSIDES

A	Cardiac glycoside				
A	Legal's test	-	-	-	-
B	Keller-Killani test	-	-	-	-
B	Anthraquinoneglucoside				
A	Borntrager's test	-	-	-	-
B	Modified Borntrager's test	-	-	-	-
C	Saponin glycoside				
A	Foam test	-	+	+	-
D	Flavonoids				
A	Shinoda test	+	++	++	-
B	Lead acetate test	+	+	+++	-

7. ALKALOIDS

A	Dragendorff's test	-	-	-	-
B	Mayer's test	-	-	-	-
C	Wagner's test	-	-	-	-

8. PHENOLIC COMPOUNDS

A	5% FeCl ₃ solution	-	+	-	-
B	Lead acetate test	+	+	-	-
C	Acetic acid solution	-	+	-	-

+ = Present (trace amount), ++ = Moderate amount, +++ = High amount and ND = Not detected

Table 2: Moisture content test by using Carl-Fisher Reagent:

S. No.	Extract	Quantity taken	Moisture content
1	Methyl acetate	1.5 mg	2%
2	Ethyl acetate	1.5 mg	1%
3	Ethanol	1.5 mg	Nil
4	Methanol	1.5 mg	Nil

Table 3: Evaluation of Ash Value of Fruits of *Abelmoschus esculentus*:

Sl. No.	Parameters	Percentage
1	Ash Value	0.37

Weight of empty crucible-19.635 gms

Weight of empty crucible+ Drug- (19.636+ 0.25) gms =19.885 gms

Weight of crucible after incineration=19.110 gms

Weight difference= 0.225 gms

Percentage of ash value=0.37

Table 4: Extractive values of Fruits of *Abelmoschus esculentus*

Sl. No.	Solvent used	Average extractive value in % w/w on dry weight basis
1	Ethanol(Absolute)	0.73
2	Water	95.04

Amount of water soluble ash was also determined

Weight of empty crucible-19.578 gms

Weight of empty crucible+ Drug- (19.578+ 2) gms =21.578 gms

Weight of crucible after incineration=19.636 gms

Weight difference= 0.054 gms

Total ash =0.985 gms

Weight of incinerated filter paper =0.0059 gms

Weight of incinerated filter paper +water= 0.055 gms

Weight difference (water soluble ash) =0.936 gms

Percentage of water soluble ash value= 95.04 %**Amount of acid insoluble ash is also determined**

Weight of empty crucible-19.01 gms

Weight of empty crucible+ Drug- (19.01+ 2) gms =21.01gms

Weight of crucible after incineration=19.02gms

Weight difference= 0.01gms

Total ash =0.97gms

Weight of incinerated filter paper =0.0059 gms

Weight of incinerated filter paper +water= 0.01gms

Weight difference (water soluble ash) =0.007gms

Amount of acid insoluble ash value= 0.73 %**Quantitative Phytochemical Screening of the Dried *Abelmoschus esculentus* L. fruits**

Parameter	Result
Saponins (mg/g)	377 ± 0.02
Flavonoids (mg/g)	235 ± 0.02

Values Are Presented As Mean ± Standard Deviation of Triplicates

Table 5.1: Rf Values of different solvent system of different extract of *Abelmoschus esculentus* fruits**Rf Values for Methyl acetate extract by TLC:**

Sr. No.	Solvent system	Solvent front height(cm)	No. of spots	Spot height(cm)	Rf Value
1	Benzene:Ethanol (9:1)	6	3	3.5,4.2,5	0.58,0.7,0.8
2	Methanol:Benzene (5:5)	6	-	-	0.00
3	Benzene:Acetic acid (9:1)	5.7	2	2.7,3	0.47,0.52
4	Chloroform:Acetone (7:3)	6	3	2.5,3.5,4	0.42,0.5,0.6

Table 5.2: Rf Values for Ethyl acetate extract by TLC:

Sr. No.	Solvent system	Solvent front height(cm)	No. of spots	Spot height(cm)	Rf Value
1	Methanol:Benzene (5:5)	5.8	2	5.2,3.1	0.89,0.53
2	Benzene:Acetic acid (9:1)	6	1	3.8	0.63
3	Chloroform:Acetone (7:3)	5.7	2	4.2, 3.6	0.73,0.63

Table 5.3: Rf Values for Alcoholic extract by TLC:

Sr. No.	Solvent system	Solvent front height (cm)	No. of spots	Spot height (cm)	Rf Value
1	Benzene:Ethanol (9:1)	5.7	1	4.3	0.75
2	Methanol:Benzene (5:5)	6.5	-	-	0.00
3	Chloroform:Acetone (7:3)	6	2	5.2, 4.9	0.86,0.8

Table 5.4: Rf Values for Aqueous extract by TLC:

Sr. No.	Solvent system	Solvent front height (cm)	No. of spots	Spot height (cm)	Rf Value
1	Benzene:Ethanol (9:1)	5.0	-	-	-
2	Chloroform:Acetone (7:3)	5.2	-	-	-

Fourier Transform Infrared (FTIR) Fingerprint Analysis:

Fourier transform infrared (FTIR) spectrophotometer was used to identify the characteristic functional groups in the seed extracts. The AE and ME (5mg), respectively, were thoroughly mixed with potassium bromide (KBr) in a mortar and pressed at pressure of 6 bars within 2min in order to prepare a thin translucent sample discs. The FT-IR spectrum was obtained using Perkin Elmer 2000 spectrophotometer system with a scan range from 400 to 4000 cm^{-1} and analysed using Bruker OPUS software.

Table III: Assignment of FTIR spectra of lignin from okra fibre and stick.

Peak location range (cm^{-1})	Assignment
3412 -3460	O-H stretching
3000 -2842	C-H stretch in methyl and methylene group
1738 -1709	C=O stretch in unconjugated ketone, carbonyl and ester groups
1675 -1655	C=O stretching in conjugated p -subst. Aryl ketones
1593 -1605	Aromatic skeleton vibrations plus C=O stretching; S>G:Gcondensed> G ether ified
1505 -1515	Aromatic skeleton vibrations (G>S)
1460 -1470	C-H deformations (asym in -CH3 and -CH2-)
1422 -1430	Aromatic skeleton vibrations combined with C -H in plane deformations
1365 -1370	Aliphatic C -H stretching in CH3 and phen. OH
1325 -1330	Condensed S and G ring (G ring bound via position 5)
1266 -1270	G ring plus C+O stretching
1221 -1230	C-C + C -O + C=O stretching (Gcondensed>Getherifie d)
1166	Typical for HGS lignins; C=O in ester groups (conj.)
Aromatic C -H in -plane deformation (typical of G unit; Gcondensed> G etherified)	
1125 -1128	Typical of S unit; also secondary alcohol & C=O strt.
1086	C-O deformation in sec. alcohol & aliphatic ether

1030 -1035	Aromatic C-H in - plane deformation (G>S) plus C -O deform.in primary alcohols plus C -H stretching (unconjugated)-HC=CH - out of plane deformation. (trans)
966 -990	C-H out of plane (aromatic ring)
915 -925	C-H out of plane in positions 2, 5 and 6 (G units)
853 -858	C-H out of plane in positions 2 and 6 of S units
834 -835	C-H out of plane in positions 2, 5 and 6 of G units

Table 1: FTIR spectral peak values and functional groups obtained.

Extract	Peak values (cm ⁻¹)	Functional groups
Ethyl acetate extract of <i>Abelmoschus esculentus</i>	917	C-H out of plane in positions 2, 5 and 6 (G units)
	1043	
	1097	
	1233	C-C + C -O + C=O stretching
	1300	
	1372	C-H bending, Aliphatic C -H stretching in CH ₃ and phen. OH
	1446	
	1746	C=O carbonyl group
	2984	
Methyl acetate extract of <i>Abelmoschus esculentus</i>		
	980	C-H out of plane (aromatic ring)
	1044	
	1237	
	1370	C-H bending
	1437	
	1740	C=O carbonyl group
	2956	
	3000	C-H stretch in methyl and methylene group


Fluorescence analysis:

The fluorescence analysis was done to identification when the herbs were fluorescence. The fluorescence character of the were dried leaves powder (40 mesh) was studied both in daylight and UV light (366nm) and after treated with different reagents like Sodium hydroxide 1(N), Hydrochloric acid 1(N), Ferric chloride 1(N), MethanolicNaOH.

Sr. No.	Experiment	Visible light	UV light (366nm)
1	Drug+NaOH	Brown	Dark blue
2	Drug+ HCl	Transparent colour	transparent colour
3	Drug+ FeCl ₃	Dark straw color	transparent colour

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BOTANICAL SURVEY OF INDIA
केंद्रीय राष्ट्रीय पादपालय
CENTRAL NATIONAL HERBARIUM
हावड़ा / HOWRAH - 711 103

No.: CNH/Tech.II/2016/39

Dated: 19-07-2016

To,
Mr. Tamal Maity
Bengal School of Technology
Hoogly -712102
West Bengal

Sub.: Identification of 2 plant specimens – reg.

Dear Mr. Maity,

Please refer to your letter no. BST/16/489 dated 21st June 2016 along with two plant specimens for identification. The specimens have been identified by the concerned expert as:

Sl. No.	Specimen No.	Scientific Name	Family
1.	TM-01	Cajanus cajan (L.) Huth.	Leguminosae: Papilionoideae
2.	TM-02	Abelmoschus esculentus (L.) Moench	Malvaceae

The receipt of ₹ 100/- (Rupees One hundred only) Receipt No. TR-5, C-130457 dated 29-06-2016) is enclosed herewith.

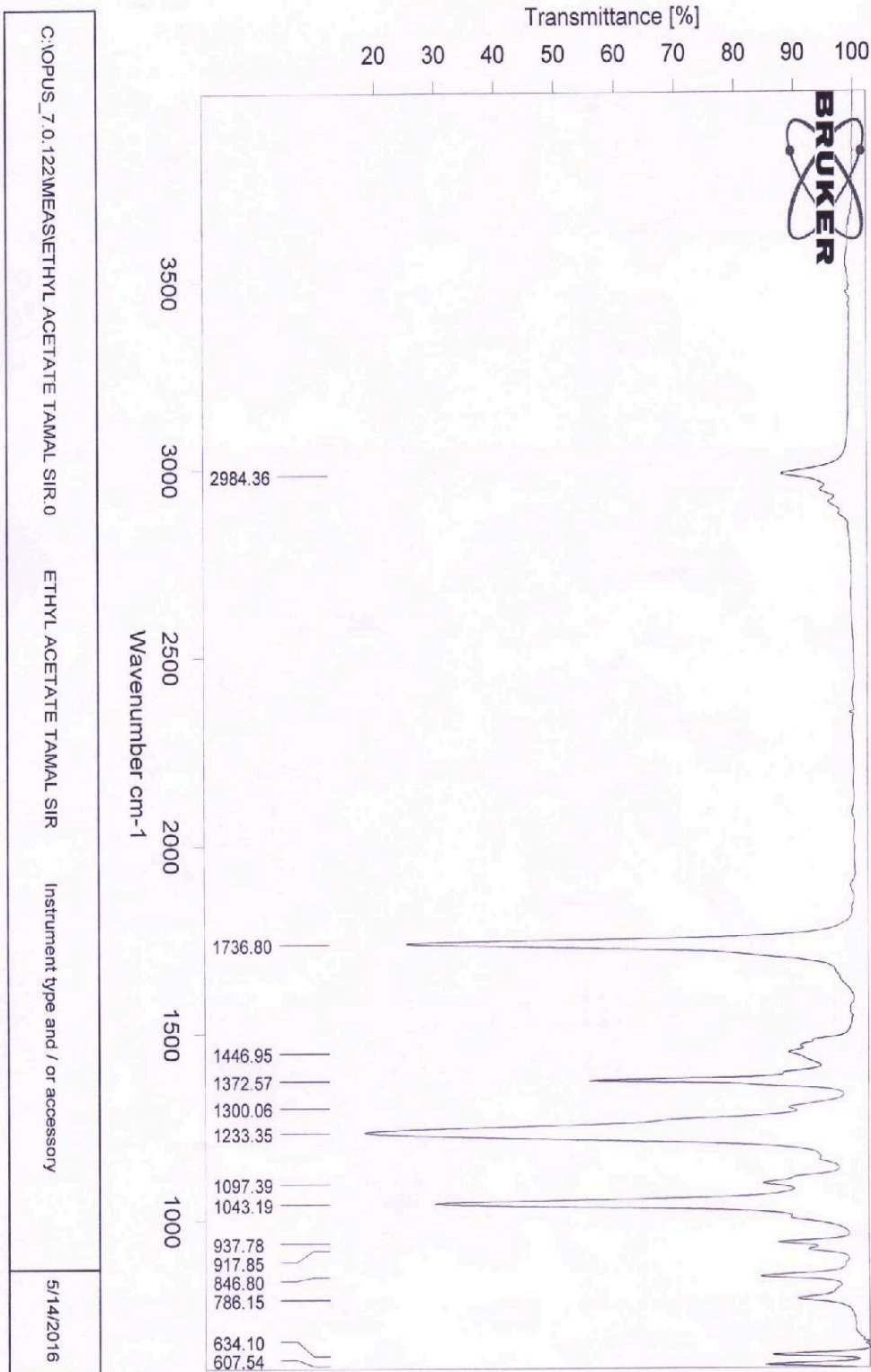
Your specimens are returned herewith.

With best wishes,

Yours sincerely


(R. GOGOI)
Scientist - 'D'

Botanical Survey of India, Central National Herbarium, P.O. – Botanic Garden, Howrah – 711 103, West Bengal, India.



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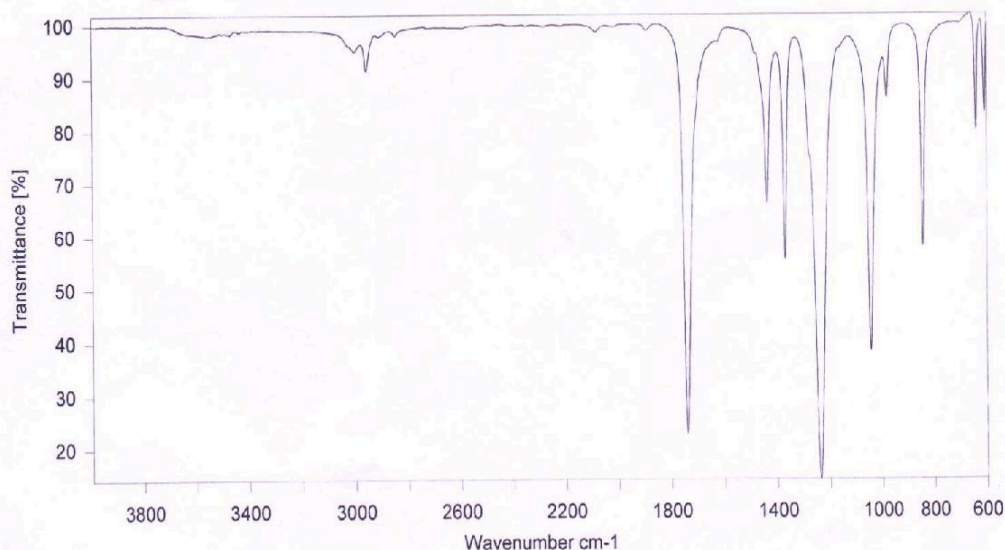
ETHYL ACETATE TAMAL SIR

Instrument type and / or accessory

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METHYL ACETATE TAMAL SIR



Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold <	Shoulder
3000.9093	0.949	0.015	24.5608	1.575459	0
2956.3038	0.913	0.087	26.4796	9.957166	0
1740.2374	0.226	0.770	31.3418	87.378555	0
1437.3812	0.658	0.296	18.2759	32.671722	0
1370.0457	0.552	0.424	14.9772	47.951504	0
1237.2525	0.141	0.869	45.0675	97.986488	0
1044.0957	0.381	0.601	26.2348	67.681328	0
980.7833	0.859	0.087	11.8130	8.531699	0
843.4319	0.575	0.429	15.2442	48.146866	0
639.6845	0.796	0.215	9.2863	23.851643	0
606.9179	0.828	0.118	7.5925	11.048812	0

Experiment ATR_ZNSE.XPM
 Operator Name Administrator
 Instrument Type Alpha
 Resolution 4

Path of File C:\OPUS_7.0.122\MEAS
 Date of Measurement 5/14/2016
 Sample Form Instrument type and / or accessory
 Sample Scans 24

Discussion:

From this work, it is clearly understood that the methyl acetate extract of *Abelmoschus esculentus* (**Okra**), fruits contains some phytochemical compounds. Powder of fruit part of plant was subjected to successive extraction by taking few solvent in increasing order of polarity i.e. ethanol, water, methanol, ethyl acetate & methyl acetate and chemical tests on various extracts and powder material showed the presence of carbohydrate, gums and mucilages, proteins, phytosterols, flavonoids, tannins and phenolic compounds and volatile oil. The methyl acetate extract was found to contain quercetin derivatives & epigallocatechin.

Considering the findings of physicochemical data and phytochemical analysis of different extracts of *Abelmoschus esculentus* (**Okra**), it can be concluded that Methyl Acetate Extract is useful for further studies of Pharmacological parameters.

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