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Investigation of Physicochemical, Preliminary Phytochemical and Quantitative Evaluation of Combination Therapy Using Poly-Herbal Formulation for Diabetes Mellitus

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ABSTRACT

Therapeutic herbal master plan utilise the merging of medicinal herbs to achieve extra therapeutic effectiveness, usually known as poly pharmacy or poly herbalism that may reduce unwanted effects, make the formulation more palatable, and increase its lifespan. Due to synergism, poly herbalism counters some benefits not available in single herbal formulation, Antioxidants can help with diabetes by reducing oxidative stress, which can contribute to the development and progression of diabetes. The excess glucose levels have been found to have a causal link with the development of reactive oxygen species leading to oxidative stress[1]. It is evident that naturally occurring dietary antioxidants such as vitamin E, A and C, plant polyphenols, carotenoids, flavonoids, glutathione, alpha lipoid acid and poly amines all provide significant protection against diabetes. The phytochemical-based formulations containing multiple herbs are liable to produce many metabolites that may act on multiple targets in the body, and hence, poly herbal formulations (which are used in traditional practice) are preferred over mono therapeutic ones[2].

Keywords: Poly herbalism, Antioxidants, Phytochemical-based formulations, Multiple targets, Detailed procedures.

INTRODUCTION

The Indian Traditional System of Medicine is one of the oldest systems of medical practice in the world and played an essential role in providing health care service to human civilisation. India has exclusive distinction of its own recognised traditional medicine; Ayurveda, Yoga, Unani, Siddha and Homoeopathy (AYUSH)[3]. Plants rich in bioactive compounds such as alkaloids, flavonoids, tannins, antioxidants and polyphenols have been used to cure illnesses because of their various pharmacological properties. We aim to discuss in vitro assays for type 2 diabetes (T_2D), which have been utilized extensively by researchers over the last five years, including target-based, non-target based, low- throughput, and high-throughput screening assays[4]. Daily administration of that poly herbal formulation regularly for 6 months resulted in significant reduction of blood glucose and haemoglobin levels. There was also a significant increase in high density lipoprotein cholesterol levels and concomitant decreases in total cholesterol, triglyceride, low density lipoprotein cholesterol and very low density lipoprotein levels. Patients exhibited a significant improvement in the biochemical markers for oxidative stress. It may be a potentially safe and effective therapy for the treatment of type 2 diabetes[5].

PLANT INTRODUCTION

The plant parts which are rich in antioxidant, phenol, flavonoid content and also according to its easy availability and its therapeutic potency are selected.

1) Senna auriculata Flower

2) Eugenia Jambolana Seeds

3) Trigonella foenum-graecum Seeds

4) Gymnema Sylvestre Leaves

MATERIALS AND METHODS

EXTRACTION OF POWDERED PLANT MATERIAL[12]

S.NO	SELECTED HERBS	QUANTITY TAKEN IN GRAMS
1	*Senna auriculata*	50 g
2	*Eugenia jambonala*	75 g
3	*Trigonella foenum-graecum*	50 g
4	*Gymnema sylvestre*	75 g

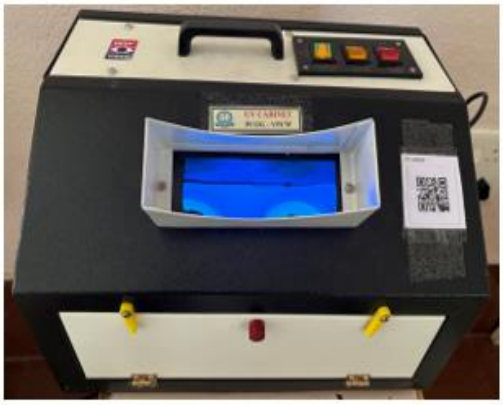
The shade dried leaves, seeds and flowers powdered materials were subjected to sequential soxhlet extraction using the **solvents** like **petroleum ether** (for removing fat and waxy materials) and **Methanol (500 mL)**. The extracts was evaporated to dryness and the yield of the extracts were determined and stored in a refrigerator for further studies.

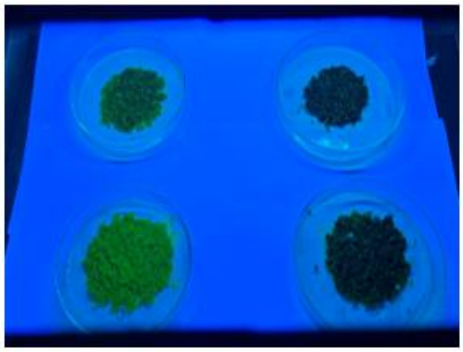
LIST OF HERBS SELECTED FOR EXTRACTION

A) PHYSICOCHEMICAL EVALUATION

1) DETERMINATION OF FLUORESCENCE CHARACTER

Fluorescence characters of powdered plant materials with different chemical reagents were determined under visible, short and long ultraviolet light and reported.





2) MOISTURE CONTENT[15]

Weigh about 1.5 g of powdered drug into a weighed flat and thin porcelain dish. Dry in oven at 100 - 105°C. Cool in a desiccations and watch. The loss in weight in which volatile components are also included was usually recorded as moisture. The observations were tabulated in results.

3) ASH VALUE[16]

The ash value was designed to measure the total amount of material remaining after ignition. This include both the "physiological ash" which was derived from the plant tissue itself, and "non physiological ash", which was the residue of the extraneous matter such as sand and soil adhering to the seed surface.

i**) TOTAL ASH VALUES[6]**

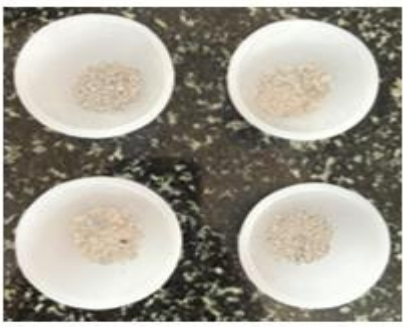
About 3 g of the dried crude powder was accurately weighed in a tarred silica crucible and weighed. The powder was scattered in a fine even layer on the bottom of the crucible. It was ignited by gradually increasing the heat up to 500°C for 3 hour until free from carbon. Cooled in desiccators and weighed. Percentage of ash with reference to the air dried drug was calculated and reported.

ii**) ACID INSOLUBLE ASH[24]**

Acid insoluble ash was the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earths. The total ash obtained was boiled for 5 minutes with 25 mL of dilute hydrochloric acid. The insoluble matter was collected on an ash less filter paper and washed with hot water. Then it was ignited and weighed, after cooling in desiccators. The percentage of acid insoluble ash was calculated and reported with reference to the air dried drug.

iii**) WATER SOLUBLE ASH[18]**

Water soluble ash was the difference in weight between the total ash and the residue after the treatment of the total ash with water. The total ash obtained previously was boiled with 25 mL of water for 5 minutes. The insoluble matter was collected on an ash less filter paper and washed with hot water. Then it was ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. The weight of the residue was reported.



Muffle Furnace Instrument and Crucible with Ash of powdered drug

4) EXTRACTIVE VALUE

Extractive value was useful for the evaluation of a crude drug. Give idea about the nature of the chemical constituents present in a crude drug. Useful for the estimation of specific constituents, soluble in that particular solvent used for extraction.

i**) DETERMINATION OF ALCOHOL SOLUBLE EXTRACTIVES**[12]

Weigh 5 g of powder in a weighing bottle and transfer it into 250 mL conical flask. Fill 100 ml flask to the delivery mark with methanol or 90 % alcohol as solvent. Wash out the weighing bottle and pour the washings together with the reminder of the solvent into the conical flask. Cork the flask and set asides for 24 hr with frequent shaking. Filter into 50 mL cylinder. Collect 25 mL from that filtrate and pour it into porcelain dish, as used for the determination of alcohol soluble extract. Evaporate to dry on water bath and complete the drying in an hot air oven at 100 ℃. Cool in dedication and weigh. Calculate the percentage w/w of extractive with reference to the air dried drug.

ii**) DETERMINATION OF WATER SOLUBLE EXTRACTIVES**[6]

Weigh 5 g of powder in a weighing bottle and transfer it into 250 mL conical flask. Fill a 100 mL flask to the delivery mark with distilled water as solvent. Wash out the weighing bottle and pour the washings together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24 hr with frequent shaking. Filter into 50 mL cylinder. Collect 25 mL from that filtrate and pour it into porcelain dish, as used for the determination of water soluble extracts. Evaporate to dry on water bath and complete the drying in an oven at 100 ℃. Cool in dedication and weigh. Calculate the percentage w/w of extractive with reference to the air dried drugs.

B) PRELIMINARY PHYTOCHEMICAL EVALUATION

The qualitative chemical tests were carried out to find out the various phyto-constituents present in the **methanolic extract** of flowers of *Senna auriculata*, seeds of *Eugenia jambonala* and *Trigonella foenum-graecum* and leaves of *Gymnema sylvestre*. The results were tabulated.

1) TEST FOR ALKALOIDS[29,12]

Mayer's test

About 0.5 g of plant extract was dissolved in 5 mL dilute hydrochloric acid and filtered. The filtrate was treated with 1-2 mL of Mayer's reagent (mercuric chloride and potassium iodide). If yellowish buff colour precipitate forms, alkaloids are present.

Dragendroff's test

Small quantity of plant extract was dissolved in hydrochloric acid and treated with few drops of Dragendroff's reagent (Sodium iodide, basic bismuth carbonate, glacial acetic acid and ethyl acetate). If orange brown precipitate develops, alkaloids are present.

Picric acid test

Small quantity of plant extract was dissolved in distilled water and add few drops of picric acid (picric acid powder was dissolved in ethanol) to it and mix well. If a yellow crystalline precipitate forms, alkaloids are present.

Tannic acid test

Small quantity of plant extract was dissolved in distilled water and add few drops of tannic acid (tannic acid powder was dissolved in ethanol) to it and mix well. If a yellow crystalline precipitate forms, alkaloids are present.

2) TEST FOR FLAVONOIDS[29,15]

Hydrochloric acid test

Add few drops of concentrated hydrochloric acid to a small amount of plant extract. If the extract turns red immediately then flavonoids are present.

Ferric chloride test

Small quantity of plant extract was dissolved in distilled water and filter it. Then add few drops of 10 % ferric chloride solution to 2 mL of the filtrate. If the extract turns green-blue or violet, then flavonoids are present.

Alkaline reagent test

About 2 to 3 drops of sodium hydroxide were added to 2 mL of extract that dissolved in water. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute hydrochloric acid, indicating that flavonoids are present.

3) TEST FOR PHENOLS[29,18]

Ferric chloride test

Small quantity of plant extract was dissolved in distilled water and filter it. Then add few drops of 10 % ferric chloride solution to 2 mL of the filtrate. If the extract turns green-blue or violet, then phenols are present.

Bromine water test

About 1 mg of plant extract was dissolved in 3 mL of glacial acetic acid and add bromine water solution drop wise. If the brown colour of bromine and plant extract mixture disappears, it indicates the presence of phenols.

Litmus test

Phenols are acidic, so they turn blue litmus paper red. This indicates the presence of phenols.

4) TEST FOR STEROIDS[29,21]

About 1 mg of plant extract was dissolved in 5 mL of chloroform, then equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer in the test tube was turns into red and acid layer shows yellow with green fluorescence.

5) TEST FOR CARBOHYDRATES[29,24]

Molish test

To Small quantities of solvent free methanolic extract, few drops of 1 % α-naphthol in ethanol were added. Concentrated sulphuric acid were then added to the sides of the test tube. A brown purple ring formed at the junction of the two liquids indicates the presence of sugars.

Benedict's test

Small quantities of solvent free methanolic extract was separately dissolved in minimum amount of distilled water and filtered. To the filtrate equal volume of Benedict's reagent were mixed in a test tube separately and heated for few minutes. Formation of brick red precipitate confirmed the presence of sugars.

Fehling's test

Small quantities of solvent free methanolic extract was separately dissolved in minimum amount of distilled water and filtered. To the filtrate add equal volume of Fehling's solution A and Fehling's solution B that were mixed in a test tube separately and heated for few minutes. Formation of brick red precipitate confirmed the presence of sugars.

6) TEST FOR PROTEINS AND AMINO ACIDS[29,6]

Ninhydrin test

A small quantity of extract was dissolved in distilled water and boiled with 0.2 % solution of Ninhydrin reagent. Blue colour indicates the presence of free amino acids.

Million's test

Mix 50 mg of plant extract with 2 mL Million's reagent [mercuric nitrate in nitric acid containing traces of nitrous acid]. Appearance of red colour indicates the presence of proteins and free amino acids.

Biuret test

The extract was dissolved in distilled water and that was treated with equal volume of biuret reagent (40 % sodium hydroxide and 1 % copper sulphate solution). Pink or purple colour indicates the presence of proteins.

7) TEST FOR GLYCOSIDES[29,9]

Sodium hydroxide test

About 2 to 3 drops of sodium hydroxide was added to 2 mL of extract that dissolved in water. If deep yellow colour appeared, it indicates presence of glycosides.

Salkowski test

Add 2 mL of concentrated sulphuric acid to the aqueous plant crude extract. A reddish brown colour indicates the presence of steroidal part of the glycoside.

8) TEST FOR CARDIAC GLYCOSIDES[29,21]

Keller-Killiani test

About 1 mg of extract was dissolved in 1 mL distilled water and added with 1.5 mL of glacial acetic acid. Add 1 drop of 5 % $FeCl_3$ and add concentrated H_2SO_4 along the side of the test tube. The presence of purple or brown ring at the interface of two layers indicates the presence of cardiac glycosides in the sample.

9) TEST FOR QUINONES[29,12]

Concentrated HCL test

Plant extract was initially dissolved in distilled water and from that 2 mL was taken and added with few drops of concentrated hydrochloric acid and observe. Yellow precipitate or coloration indicates the presence of quinones.

Diluted NaOH test

Plant extract was initially dissolved in distilled water and from that 1 mL was taken and added with few drops of diluted NaOH. Blue green or red coloration indicates quinones.

10) TEST FOR ANTHRAQUINONES[29,18]

Ammonium hydroxide test

About 10 mg of plant extract was dissolved in isopropyl alcohol that was added with one drop of concentrated ammonium hydroxide. After 2 minutes, formation of red colour indicates the presence of anthraquinone.

orntrager's test

Add 2 mL of dilute sulphuric acid to 2 mL of extract that was previously dissolved in distilled water, boil, filter and cool. Add equal volume of benzene. A rose, pink or red colour ammoniacal layer indicates the presence of anthraquinone glycosides.

11) TEST FOR TANNINS[24]

Lead acetate test

The extract was dissolved in distilled water and filter. To the filtrate add few drops of lead acetate solution. A cream gelatinous precipitate indicates the presence of tannins.

Ferric chloride test

About 0.5 g of plant extract was dissolved in distilled water and filter it. Then add few drops of 10 % ferric chloride solution to 2 mL of the filtrate. If the extract turns green-blue or violet, then tannins are present.

12) TEST FOR SAPONINS[29,15]

Foam test

To the extract, 20 mL of distilled water was added and agitated on a graduated cylinder for 15 minutes. The formation of about 1 cm layer of foam indicates the presence of saponins.

13) TEST FOR COUMARIN[9]

About 2 mg of plant extract was dissolved in 3 mL of 10 % sodium hydroxide solution. If the solution turns yellow the plant extract contains coumarins.

14) TEST FOR TERPENOIDS[29,6]

Salkowski test

About 2 mg of sample was dissolved in chloroform (2 mL) and concentrated sulphuric acid (2 mL) was added carefully along the side of test tube to form a layer. A reddish brown coloration of the interface was formed to show positive result for presence of terpenoids.

15) TEST FOR TRITERPENOIDS[18]

Foam test

If stable form forms above the liquid layer in a test tube indicates the presence of triterpenic glycosides (saponins).

Color test

Add 1 mL of concentrated sulphuric acid to about 2 mL of extract and add a drop of copper sulphate to it. Heat the mixture, a blue-green colour indicates the presence of triterpenoids.

Salkowski's test

About 2 mg of sample was dissolved in 1 mL of chloroform and 1 mL of concentrated sulphuric acid. A yellow colour in the lower layer indicates the presence of triterpenoids.

C) QUANTITATIVE CHEMICAL TESTS

1) ESTIMATION OF TOTAL PHENOL CONTENT[30,31]

Total Phenolic Content of the extracts was measured by Folin - Ciocalteu (F-C) assay. Briefly a volume of 100 µl of sample were added to 2 mL eppendrops tube followed by 86 µl distilled water, 50 µl F-C reagents, mixed and allowed to react for 5 min. Then add 100 µl 20 % Na_2CO_3, 890 µl distilled water, mixed and allowed to stand 60 min at room temperature. Absorbance was measured at 725 nm. The blank was prepared in similar manner without sample or standard, Calibration curve was plotted using Gallic acid as standard (10, 20, 40, 60, 80 and 100 µg/mL). The results were expressed as milligram of Gallic acid equivalents (GAE) per gram of extract.

2) ESTIMATION OF TOTAL FLAVONOID CONTENT[30,33]

Total flavonoid were estimated by Aluminium chloride colorimetric assay. 1 mL aliquot of appropriately diluted sample or standard solution of Quercetin (100, 200, 400, 600, 800 and 1000 µg/mL) was mixed with 50 µl of $NaNO_2$ in 2 mL eppendrop tube. After 6 min, 50 µl of a 10 % $AlCl_3$ solution was added and allowed to stand for 6 min, then 50 µl 1M potassium acetate solution was added to the mixture. Distilled water was added to bring the final volume to 2 mL, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm against prepared blank. Blank was prepared in the same above manner omitting sample or standard. All values were expressed as milligrams of Quercetin equivalent per 1 g of sample.

3) ESTIMATION OF TOTAL ANTIOXIDANT CAPACITY (TAC)[32,33]

Total antioxidant activities of extracts were determined by phosphomolybdenum assay. Briefly 0.2 mL of various concentrations of extract and 2 mL phosphomolybdenum reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulphuric acid) were mixed in a capped test tube and were incubated at 95°C for 90 minutes on a water bath. Test tubes were removed from the water bath, cooled at room temperature and absorbance of reaction mixture was measured at 695 nm. Similarly, series of reaction mixture were made using different concentrations of ascorbic acid (AA).

The blank was prepared in the similar manner without extract. TAC of extract was calculated from the graph and expressed as milligrams of ascorbic acid equivalent (AAE) per gram of dry weight of extract.

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RESULTS AND DISCUSSION

In this study, methanolic extracts of the flowers of *Senna auriculata*, seeds of *Trigonella foenum-graecum*, and *Eugenia jambonala* and leaves of *Gymnema sylvestre* were subjected to pharmacognostical, preliminary phytochemical, anti-bacterial and antihyperglycemic studies. The following datas are evidence of our present studies.

PHARMACOGNOSTICAL STUDY

MORPHOLOGICAL STUDIES

The Pharmacognostical study is useful to find out the external or morphological characters of the respective plant seed. The morphological characters such as colour, odour, taste, size and shape of the flowers of *Senna auriculata*, seeds of the *Trigonella foenum-graecum*, and *Eugenia jambonala* and leaves of *Gymnema sylvestre* were studied.

Character	***Senna auriculata* flower**	***Eugenia jambonala* seeds**	***Trigonella foenum-graecum* seeds**	***Gymnema sylvestre* leaves**
Colour	Yellow	Greenish yellow	Yellowish brown to brown	Dark green
Odour	Characteristic	Slightly sweet and nutty	Bitter and nutty	Aromatic or pungent
Taste	Bitter	Bitter and astringent	Bitter and slightly sweet	Astringent and bitter
Shape	Funnel-shaped	Ovoid or ellipsoidal	Triangular	Elliptic

FLUORESCENCE ANALYSIS

BEHAVIOUR OF POWDERED DRUG WITH REAGENTS

REAGENT	**SHORT-UV 254 nm**	**LONG-UV 365 nm**	**VISIBLE LIGHT**
1) *Senna auriculata* flower			
i) Aqueous potassium hydroxide	Brownish black	Brownish green	Brown
ii) Aqueous ferric chloride (5%)	Green	Brownish black	Green
iii) Picric acid solution	Light green	Green	Dark yellow
iv) Iodine solution	Green	Dark green	Green
2) *Trigonella foenum-graecum* seeds			
i) Aqueous potassium hydroxide	Greenish yellow	Dark green	Orange
ii) Aqueous ferric chloride (5%)	Dark green	Brownish green	Brownish yellow
iii) Picric acid solution	Deep yellow	Yellowish green	Yellow
iv) Iodine solution	Greyish green	Brownish green	Light red
3) *Gymnema sylvestre* leaves			
i) Aqueous potassium hydroxide	Black	Black	Brown
ii) Aqueous ferric chloride (5%)	Dark green	Black	Reddish green
iii) Picric acid solution	Yellowish green	Dark green	Pale yellow
iv) Iodine solution	Black	Black	Brown
4) *Eugenia jambonala* seeds			
i) Aqueous potassium hydroxide	Brownish green	Black	Dark brown
ii) Aqueous ferric chloride (5%)	Black	Greenish black	Black
iii) Picric acid solution	Green	Dark green	Greenish yellow
iv) Iodine solution	Dark green	Brownish black	Blackish brown

PRELIMINARY PHYTOCHEMICAL STUDIES

The Preliminary Phytochemical studies such as moisture content, Ash values and Extractive values were carried out which useful to find out the quality of the sample.

PERCENTAGE LOSS ON DRYING

S.NO	**HERBS**	**LOSS ON DRYING IN %**
1	*Senna auriculata* Flower	11.111
2	*Eugenia jambonala* Seeds	6.382
3	*Trigonella foenum-graecum* Seeds	7.913
4	*Gymnema sylvestre* Leaves	3.448

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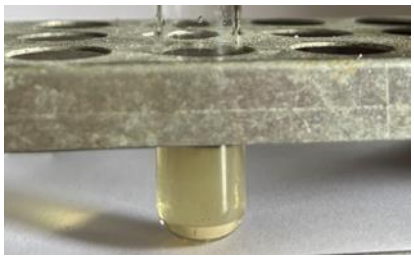
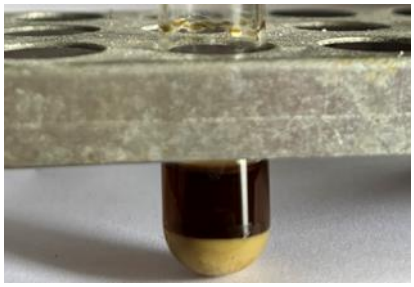
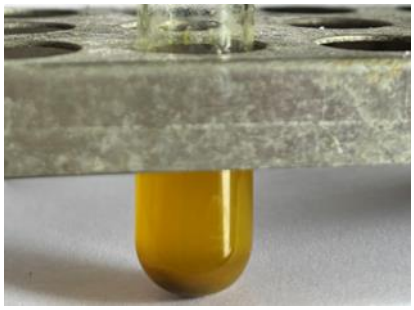
TOTAL, ACID INSOLUBLE AND WATER SOLUBLE ASH VALUES

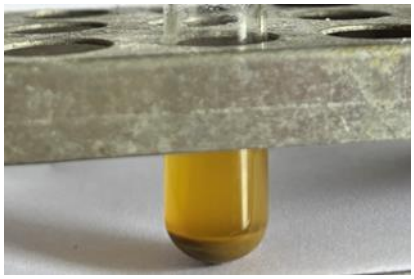
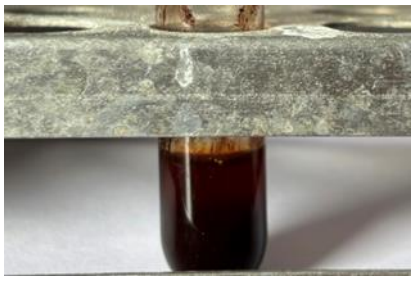
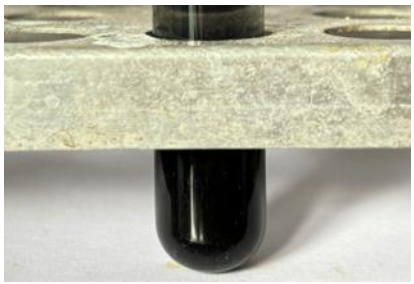
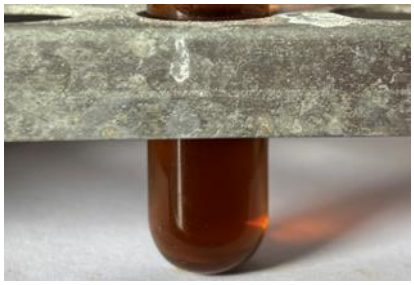
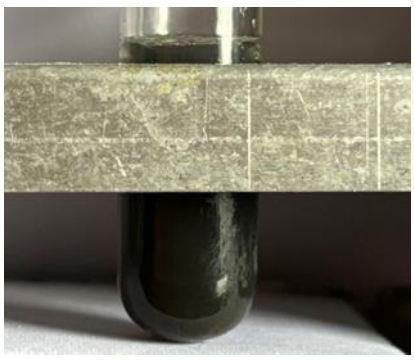
ASH VALUES	*Senna auriculata* Flowers	*Eugenia jambonala* Seeds	*Trigonella foenum-graecum* Seeds	*Gymnema sylvestre* Leaves
Total Ash	5.66%	1.66%	4.67%	11%
Acid Insoluble Ash	13.33%	66.66%	64.28%	57.57%
Water Soluble Ash	94.11%	40%	61.53%	3.12%

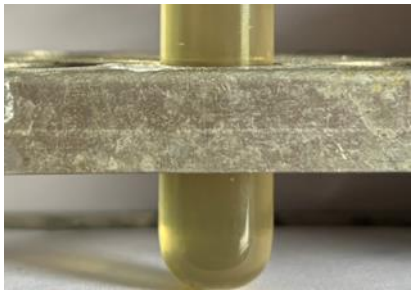
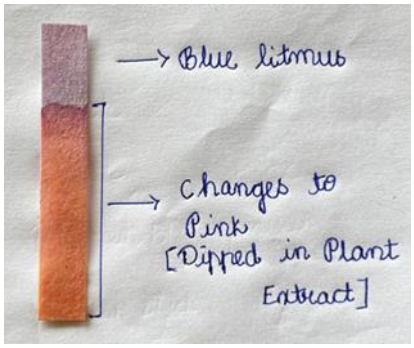
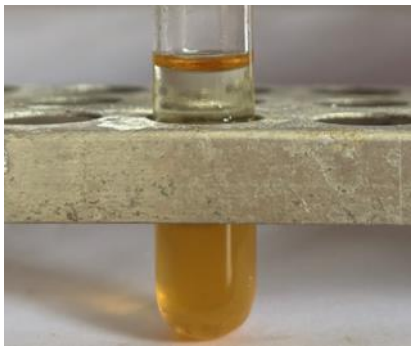
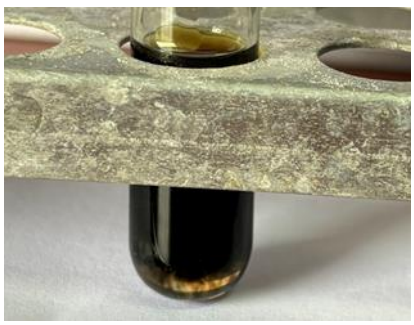
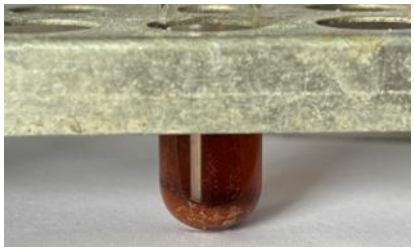
ALCOHOL SOLUBLE AND WATER SOLUBLE EXTRACTIVE VALUES

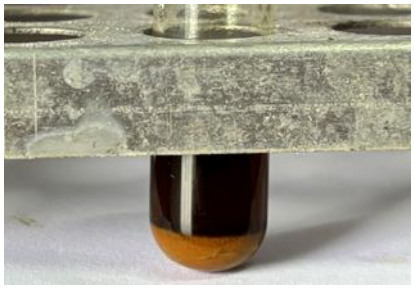
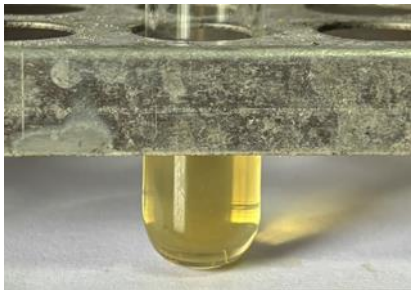
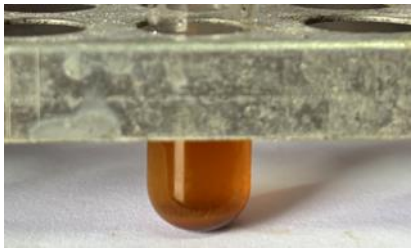
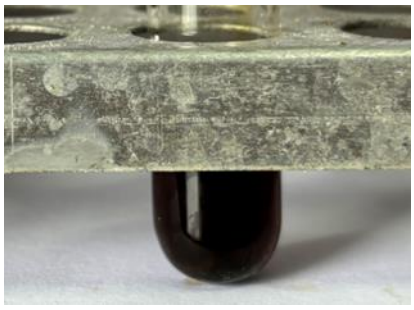
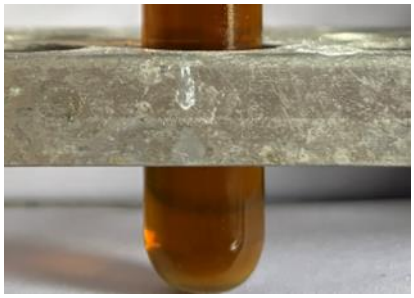
PARAMETERS	PERCENTAGE EXTRACTICE VALUE
Alcohol Soluble Extractive	84.5%
Water Soluble Extractive	27%

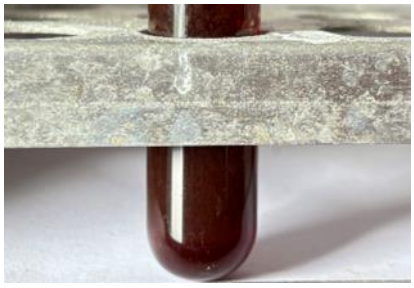
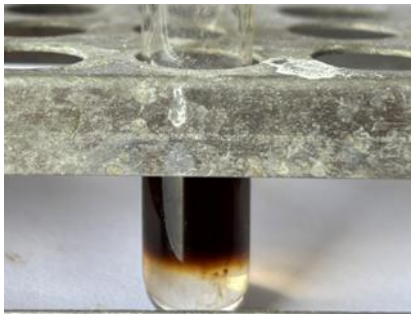
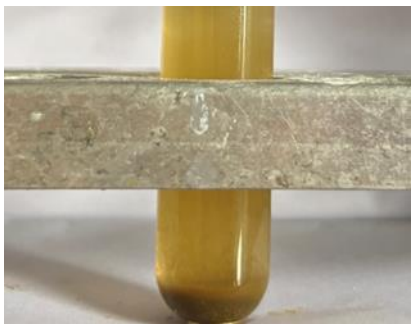
QUALITATIVE CHEMICAL TEST RESULT

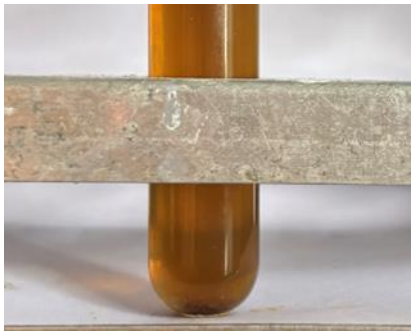
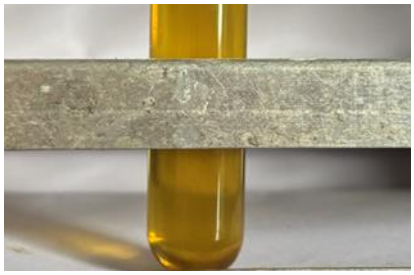
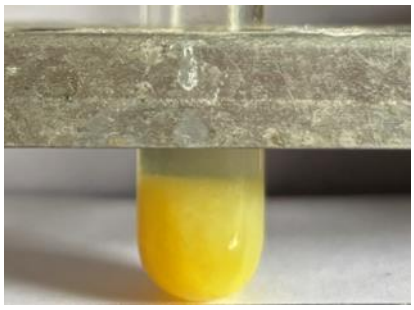
CHEMICAL TESTS	CONSTITUENT IN PLANT EXTRACT	IMAGE OF CHEMICAL TEST
1) Alkaloids	**Presence (+)**	
a. Mayer's test	Fail	
b. Dragendroff's test	Pass	
c. Picric acid test	Pass	

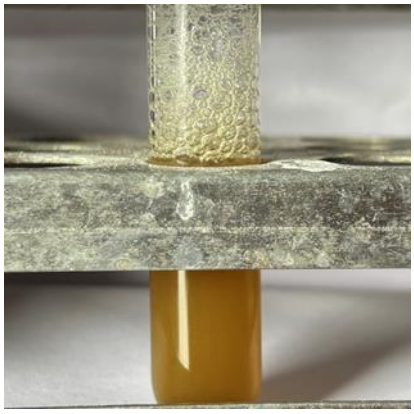
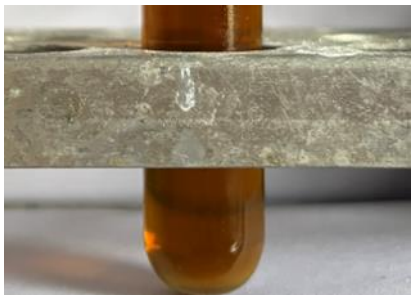
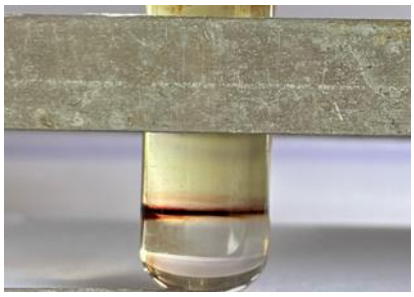
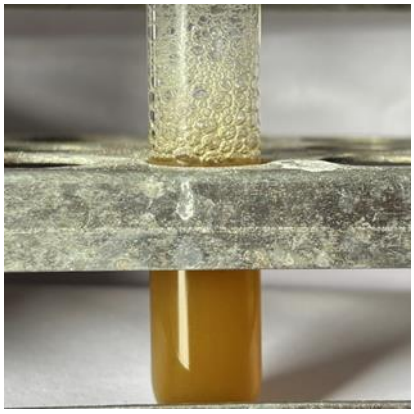
d. Tannic acid test	Pass	
2) Flavonoids	**Presence (+)**	
a. Hydrochloric acid test	Pass	
b. Ferric chloride test	Pass	
c. Alkaline reagent test	Pass	
3) Phenols	**Presence (+)**	
a. Ferric chloride test	Pass	

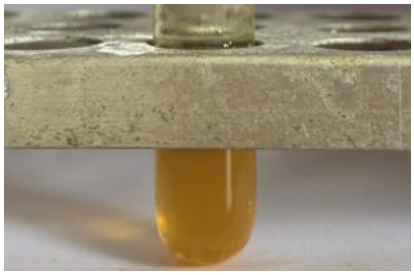
b. Bromine water test	Pass	
c. Litmus test	Pass	
4) Steroids	**Presence (+)**	
a. Steroid test	Pass	
5) Carbohydrates	**Presence (+)**	
a. Molish's test	Pass	
b. Fehling solution	Pass	

c. Benedict's test	Pass	
6) Proteins and Amino acids	**Presence (+)**	
a. Ninhydrin test	Fail	
b. Million's test	Pass	
c. Biuret test	Pass	
7) Glycosides	**Presence (+)**	
a. Sodium hydroxide test	Pass	

b. Salkowski test	Pass	
8) Cardiac glycosides	**Presence (+)**	
a. Keller-Killiani test	Pass	
9) Quinones	**Presence (+)**	
a. Concentrated HCL test	Pass	
b. Diluted NaOH test	Pass	
10) Anthraquinones	**Presence (+)**	

a. Ammonium hydroxide test	Pass	
b. Borntrager's test	Fail	
11) Tannins	**Presence (+)**	
a. Lead acetate test	Pass	
b. Ferric chloride test	Pass	
12) Saponins	**Presence (+)**	

a. Foam test	Presence	
13) Coumarin	**Presence (+)**	
a. Coumarin test	Pass	
14) Terpenoids	**Presence (+)**	
a. Salkowski test	Pass	
15) Triterpenoids	**Presence (+)**	
a. Foam test	Pass	

b. Colour test	Pass	
c. Salkowski's test	Pass	

QUANTITATIVE CHEMICAL TEST RESULT

TOTAL PHENOL CONTENT

ABSORBANCE OF GALLIC ACID STANDARD

S.NO	CONCENTRATION µg/mL	ABSORBANCE
1	10	0.0271
2	20	0.0642
3	40	0.1556
4	60	0.2384
5	80	0.3203
6	100	0.3995

ABSORBANCE OF SAMPLE

SAMPLE	ABSORBANCE
Methanolic extract of the flowers of *Senna auriculata*, seeds of *Trigonella foenum-graecum*, and *Eugenia jambonala* and leaves of *Gymnema sylvestre*	0.6838

Standard curve equation "y" (expressed as y = mx + b) and "R^2" value (closer to 1 indicates a better fit that the regression model accurately predicts the relationship between absorbance and concentration) was determined from the graph. The concentration (x) was found by substituting the absorbance value (y) in the equation.

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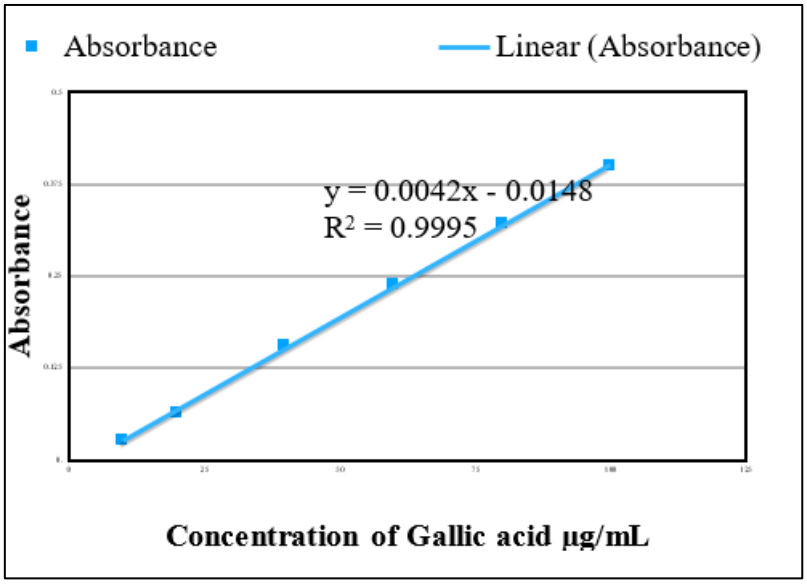


A calibration curve was plotted using Gallic acid as standard (10, 20, 40, 60, 80 and 100 µg/mL) concentrations in x-axis and to its absorbance value in y-axis. A linear graph was drawn by joining the points of best fit. The concentration of sample was determined by plotting the sample's absorbance value on the linear graph to get its appropriate concentration value.

TOTAL PHENOL CONTENT OF SAMPLE

SAMPLE	CONCENTRATION OF SAMPLE FROM STANDARD GRAPH (µg/mL)	mg GAE/g
Methanolic extracts of the flowers of *Senna auriculata*, seeds of *Trigonella foenum-graecum*, and *Eugenia jambonala* and leaves of *Gymnema sylvestre*	171	82.532

The Total Phenol Content was calculated based on the sample volume and weight using the formula, Total Phenol Content (mg GAE/g) = (Concentration from standard curve (mg/mL) × Volume of extract (mL) / Weight of sample (g). The results were expressed as milligram of Gallic acid equivalents (GAE) per gram of extract.

TOTAL FLAVONOID CONTENT

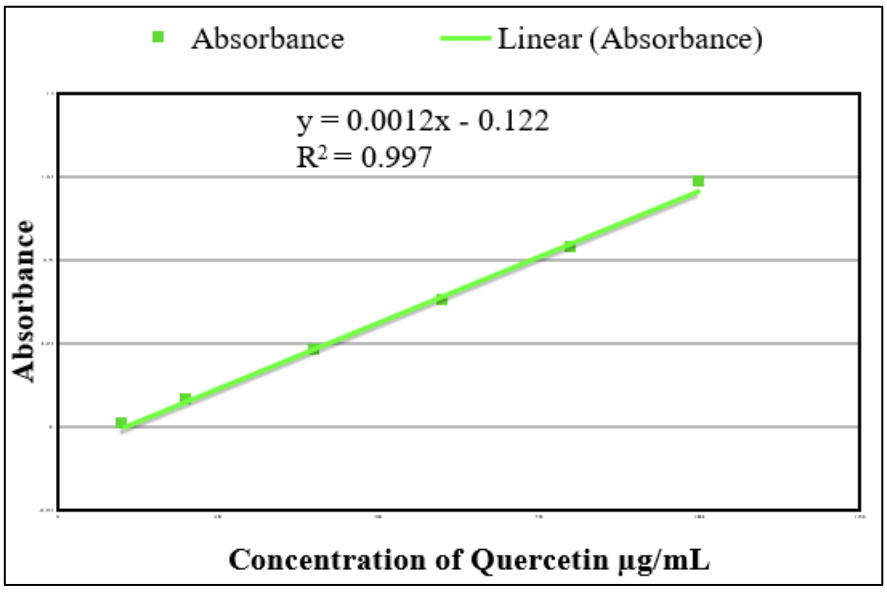
ABSORBANCE OF QUERCETIN STANDARD

S.NO	CONCENTRATION µg/mL	ABSORBANCE
1	100	0.0158
2	200	0.1176
3	400	0.3446
4	600	0.5677
5	800	0.8059
6	1000	1.0987

ABSORBANCE OF SAMPLE

SAMPLE	ABSORBANCE
Methanolic extract of the flowers of *Senna auriculata*, seeds of *Trigonella foenum-graecum*, and *Eugenia jambonala* and leaves of *Gymnema sylvestre*	0.5852

TOTAL FLAVONOID CONTENT OF SAMPLE



SAMPLE	CONCENTRATION OF SAMPLE FROM STANDARD GRAPH (µg/mL)	mg QE/g
Methanolic extracts of the flowers of *Senna auriculata*, seeds of *Trigonella foenum-graecum*, and *Eugenia jambonala* and leaves of *Gymnema sylvestre*	620	299.24

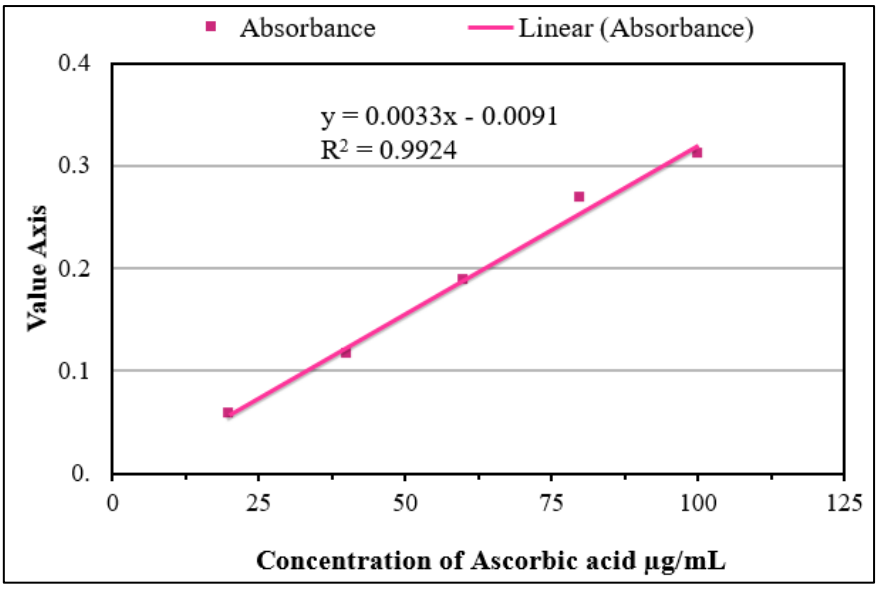
TOTAL ANTI-OXIDENT CAPACITY

ABSORBANCE OF ASCORBIC ACID STANDARD

S.NO	CONCENTRATION µg/mL	ABSORBANCE
1	20	0.0579
2	40	0.1167
3	60	0.1884
4	80	0.2691
5	100	0.3114

ABSORBANCE OF SAMPLE

SAMPLE	ABSORBANCE
Methanolic extract of the flowers of *Senna auriculata*, seeds of *Trigonella foenum-graecum*, and *Eugenia jambonala* and leaves of *Gymnema sylvestre*	1.3080



TOTAL ANTIOXIDANT CAPACITY OF SAMPLE

SAMPLE	CONCENTRATION OF SAMPLE FROM STANDARD GRAPH (µg/mL)	mg AAE/g
Methanolic extracts of the flowers of *Senna auriculata*, seeds of *Trigonella foenum-graecum*, and *Eugenia jambonala* and leaves of *Gymnema sylvestre*	340	164.10

REFERENCES

1) Muhammad Kashif, *et.al.*, (2023) https://search.app/39UmCt76K6ttti96
2) Sunil Mahaian, et.al., (2015) https://www.sciencedirect.com/ science/article/abs/pi S1876382015000086.
3) Indian system of medicine, *et.al.*, (2021) https://www.vbspu.ac.in/e-content/ Dharmendra-Singh/ Traditional%20System%20of%20 Medicine.pdf
4) Jimisha Dharmendrasinh, et.al., (2014) https://search.app/mL3ZCgFL6dP1mf8i8
5) https://www.researchgate.net/publication/269582649_ Exploratioh_of_Preliminary_Phytochemical_Studies_of_Seed_of_syzygium_cumini
6) Jagetia GC, *et.al.*, (2024) https://www.xiahepublishing.com/ m/2572-5505/JERP-2023-00019
7) https://images.app.goo.gl/UZE6xHPZkqSeRfS86
8) https://images.app.goo.gl/wkYvz31y6aGAnqwz6
9) Karunakar Hegde, *et.al.*,(2022) https://globalresearchonline.net/ijpsrr/v74-1/23.pdf
10) https://images.app.goo.gl/Y2MsXR2FZf3xGXVP7
11) https://images.app.goo.gl/2MUJFi6CB5QBkHGt9
12) Oluwole Solomon Oladeii, *et.al.*,(2021) https://www.sciencedirect.com/ science/article/pii/S0254629920311698
13) https://images.app.goo.gl/KR2Mlua2FADng×8r9

14) https://images.app.goo.g!/GfbJkS7HWG6AVZdk7
15) Syed Zameer Hussain, *et.al.,* (2021) https://www.researchgate.net/publication/ 352434487 Anola_Emblica_officinalis_Morphology_Taxonomy-Composition_ and_ Health_ Benefit
16) https://images.app.goo.g!/dwMm3CajiLR6fENPA
17) https://images.app.goo.g!/YG4XmHVgcimreuVu5
18) Ramachandraiah Mallenakuppe, *et.al.,* (2019) https://www.researchgate.net/ publication/333020506_History_Taxonomy_and_Propaga-tion_of_Moringa_oleifera-A Review
19) https://images.app.goo.g!/gsBJdm6HLLcLM4909
20) https://images.app.goo.gl/W4sKdevhe5zmfmtG7
21) Krishnendra Singh Nama, *et.al.*,(2014) https://www.jpab.com/form/ 2014%20Volume%202,%20issue% 206/|JPAB-2014-2-6-318-325.pdf
22) https://images.app.goo.gl/ZyzAk5qdugNworhAA
23) https://images.app.goo.g!/vdEN9BP3wwKbicmy7
24) Krishnapura Srinivasan (CSIR), *et.al.*,(2006) https://www.researchgate.net/ publication/249075462_Fenugreek_Trigonel-la foenum-graecum_A_ Re-view of Health Beneficial Physiological_Effects
25) https://images.app.goo.gl/ 79ZiWYD3UYTtoPfd7
26) https://images.app.goo.g!/ 78Ud1gkH4VQetbbCA
27)https://www.researchgate.net/publication/372194941_Stud-jes_on_Pharmacognosti-cal_Characteristics_and_Phytochemical_Analy-sis_of_Trigonella_foenum-graceum_L_seed
28) https://www.omicsonline.org/open-access-pdfs/pharmacognostic-study-of-trigonella-foenum-graecum-.pdf
29) https://pmc.ncbi.nlm.nih.gov/articles/PMC7035446/
30) https://www.researchgate.net/publication/330131704_Determination_of_To-tal Phenolic_Content_Total Flavonoid Content_and_An-tioxidant_Activity_of_Various_Or-ganic_Crude_Extract-s_of_Licuala_Spinosa_Leaves_from Sabah Malavsia
31) https://iipsr.com/bft-article/determination-of-total-flavonoid-and-phenol-content-in-mimusops-elengi-linn/
32) https://pmc.ncbi.nlm.nih.gov/articles/PMC3480753/
33) https://doi.org/10.1016/j.jff.2015.01.047 7 Get rights and content 7

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Conflict of Interest Statement: All authors have nothing else to disclose.