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Pharmacognostic Standardization and Phytochemical Profiling of *Syzygium hemisphericum*: Implications for Antioxidant Activity

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ABSTRACT
India has a rich heritage of traditional medicine constituting different components such as Ayurveda, Siddha, and Unani. The development of these traditional systems of medicines with the perspectives of safety, efficacy, and quality will help in the preservation of tradition in healthcare. The Plant constitute an effective source of traditional and modern medicines and play an important role in health care programs. Comprehensive pharmacognostic studies provide critical information on the morphological, microscopic, and physicochemical characteristics of plant materials. The present study aims to establish pharmacognostic standardization parameters of *Syzygium hemisphericum* through organoleptic, macroscopic, and microscopic analyses, along with the determination of ash values, moisture content, extractive values, foreign matter, and fluorescence characteristics in accordance with World Health Organization guidelines. Additionally, phytochemical screening of different extracts, quantification of phytoconstituents, and evaluation of antioxidant activity were carried out for the leaf and stem extracts of *Syzygium hemisphericum*. The findings of this study provide essential data for the identification, quality control, and standardization of this medicinal plant.

INTRODUCTION:

Plants have been always used as medicine by mankind to treat health-threatening diseases and still popular to obtain new drug candidates as it is the oldest medical practice for humans. The use of botanical natural health products is on the increase all over the world. It is known that almost 80% of the populations in developing countries rely on the traditional medicine, mainly composing herbal prescriptions.[1]

Today, with the current surge of interest in phototherapeutics, the availability of genuine plant material is becoming scarce. Since crude plant drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity becomes an essential part of its study. It is extremely important to make an effort toward standardization of plant material as medicine. The process of standardization can be achieved by stepwise Pharmacognostic studies These studies help in identification and authentication of the plant material.[2]

Syzygium hemisphericum wt. astlon is a flowering plant species in the family Myrtaceae, commonly called the hemispheric rose-apple.[3] Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. Phytochemicals could exhibit bioactivities such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties.[4] Different species of Syzygium have been previously investigated in

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several pharmacological activities such as antibacterial, antifungal, analgesic, anti-inflammatory, antihyperglycemic, antioxidant, anticarcinogenic, and antihypertensive properties. This plant derived phytochemicals with therapeutic properties could be used as single therapeutic agent or as combined formulations in drug development. So, there is need to explore the traditional use of plant for therapeutic effectiveness.

Fig 1. Syzygium hemisphericum wt. astlon plant

MATERIAL AND METHOD:

a). Collection and Authentication of plant material:

The leaves and stem of Syzygium hemisphericum wt. astlon were collected from western ghat region. The material was identified and authenticated by Botanical Survey of India Pune, Maharashtra. Collected samples of leaves and stem were dried under shade and powdered separately. The powder drug was stored in air tight and light-resistant container for experimental work.

b). Organoleptic evaluation:

It refers to the evaluation of plant material by color, shape, odour, taste, size, fracture, and other surface characteristics etc. Different dried parts of Syzygium hemisphericum wt. astlon were considered for macroscopical evaluation [5]

1. Macroscopic evaluation

Fresh and healthy plants of Syzygium hemisphericum wt. astlon were assessed for their external characteristics.

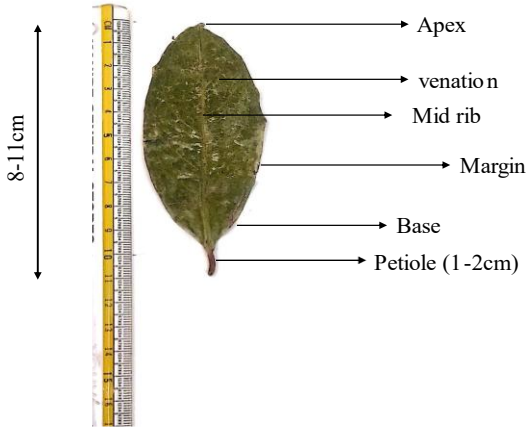

Fig 2. Syzygium hemisphericum wt. astlon plant Leaf macroscopy

2. Microscopic Evaluation

a) Anatomy

Microscopic Evaluation: - The microscopic study is the anatomical study which is done

by taking appropriate section of the plant parts under study.

Transverse sections of fresh materials of different parts of Syzygium hemisphericum were cut with the help of sharp blades. Peels were obtained from fresh leaves and stems by forceps. Different sections/peels were stained with phloroglucinol and HCL and observed under microscope and photographed by using a digital micro imagining adaptor(SGL-11A).

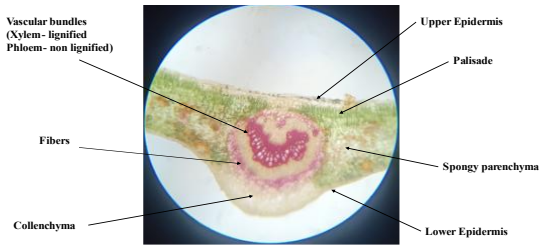

Fig.3. T. S. of Syzygium Hemisphericum Leaves

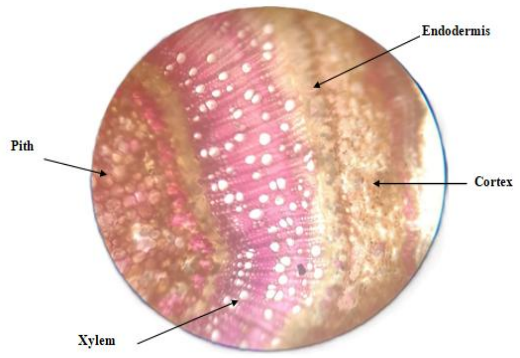

Fig 4. T. S. Of Syzygium Hemisphericum Stem

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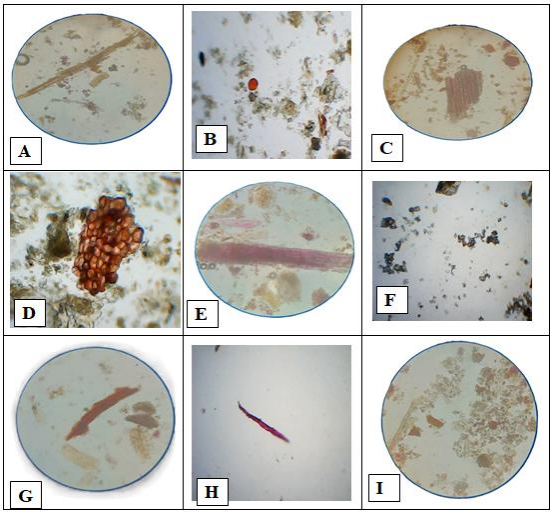
b) Powder Microscopy: Cell structure and behaviour of the leaf and stem powders were observed using standard procedures. The powder form of leaf and stem sieved. For the analysis of plant powder, pinch of fine powder is taken in a test tube and boiled in chloral hydrate solution for few minutes. A few drops of powder were smeared on a slide mounted with phloroglucinol followed by few drops of concentrated HCl [6]. The prepared slides were then observed under a microscope and photographed by using a digital micro imagining adaptor (SGL-11A).



A-Epidermal cell, **B**- Oil globules, **C**-Xylem vesicles, **D**-Clustered Sclerides, **E**- Lignified fibers, **F**-Calcium oxalate crystals, **G**-cork cells, **H**-Cuticle**, I**- Stone cells

C) Fluorescence analysis:
Fluorescence study of leaf and stem powder was performed as per procedure.22 A small quantity of the powder was placed on a grease free clean microscopic slide, and 1-2 drops of the freshly prepared reagent solution were added, mixed by gentle tilting the slide and waited for 1-2 min. Then the slide was kept inside the ultraviolet (UV) cabinet and observed in visible light, short (254 nm), and long (365 nm) ultraviolet radiations.[7]

D) Physicochemical parameters:
Various physicochemical parameters studied are moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values, petroleum ether soluble extractive value, ethyl acetate soluble extractive value, n-hexane soluble extractive value, etc. were investigated on the powdered sample of both leaves and Stem of Syzygium hemisphericum wt. astlon.[8]

Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The wate soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not.[9]

E) Phytochemical characterization:
The collected leaves and stem of Syzygium hemisphericum wt. astlon were separately shade dried and powdered. Successive Soxhlet extraction method was used to extract leaves and stem of the plant sample and were performed using different solvents including Pet ether, n-hexane ethyl acetate ethanol and distilled water. After the extraction the solvent was evaporated using rotary evaporator Dried extract were stored in refrigerator for further studies. The crude extract in different solvents is tested for various phytoconstituent present in them by standard procedure. [10,11,12] They are generally tested for presence of Alkaloids Flavanoids, tannins Glycosides, Phenols, Triterpins, Steroids, and Saponis.

F) Quantification of phytoconstituents: -
a). Total Flavonoid content: - The total flavonoid content was estimated on the methodology of (Experimental pharmacognosy by Dr. S.S. Khadabadi) The extract 0.5 ml was mixed with 0.1 ml aluminium chloride (10%), 0.1 ml sodium acetate (1M), 1.5 ml ethanol and 2.8ml distilled water. Similarly, 0.5 ml of standard quercetin (40 ppm) was also mixed with above reagent. This mixture allowed to stand for 30 minute and absorbance was measured at 415nm using SHIMADZU (1900-i) UV-VIS spectrophotometer. [13,14] The total flavonoid content was expressed as percentage and calculated using the formula TFC(g extract/mg QE)=Aliquots/(g)×1000(mg/µg) X(mL/µg)×V final(mL)

b) Total Phenolic content: -The total phenolic content was determined by Folin ciocalteu reagent. (diluted with water) The extract 1ml was mixed with 5 ml of Folin ciocalteu reagent and 4 ml of sodium carbonate. The tubes are vortexed and allowed to stand for 30 min in the dark at room temperature. [15] Absorbance was recorded against reagent blank at 765 nm using SHIMADZU (1900-i) UV-VIS spectrophotometer. The total phenolic

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concentration was expressed in terms of gallic acid by using formula TPC (mg GAE/g extract) =MC×V

G) DPPH radical Scavenging Assay: -

DPPH radical scavenging activity of the pet ether extract of both stem and leaves was done using reported method the reaction mixture containing 1 ml of DPPH solution (0.1mm/L in methanol V/V) with different concentration of the extract was shaken and incubated for 30 min in dark and the absorbance was measured at 517 nm against blank. [16,17] The radical scavenging activity was measured as decrease in the absorbance of DPPH and calculated using the equation

% Inhibition =Control -sample / Control x 100

Result and discussion

1. **Macroscopical/organoleptic:** Syzygium hemisphericum wt. astlon leaves were 18-11cm in length 4-5 cm in width. The leaves were simple alternate thick coriaceous base obtuse -sub cordate. Margin was centerate, -serrate, apex obtuse or subacute, petiole was 1-2cm long.

Table 1. Organoleptic characteristics of plant

Sr. no	Organoleptic characteristics	Nature
1.	Colour	Pale green
2.	Sand silica	Absent
3.	Odor	pungent
4.	Taste	bitter
5.	Insect infestation	Absent
6.	Rodent contamination	Absent

2. Microscopic Evaluation

a. Leaf Microscopy:

Transverse section of leaf passing through midrib showed a single layer of the epidermis on both surface and was covered with cuticle. Upper epidermis with thin walled tangentially elongated cells with one layer with distinct cuticle. **Stomata were absent**. Followed by palisade double layer with compactly arranged elongated and thin walled cells. In midrib region several layers thick-walled collenchyma and thin-walled parenchymatous tissue surrounded the central vascular bundle present. Xylem was lignified while phloem was non-lignified.

b. Stem Microscopy:

The transverse section of stem showed the presence of epidermis covered with cutical ,cortex beneath the epidermis, endodermis separating cortex and vascular bundle with ploem and xylem.Medullary rays between vascular bundeles. Pith central zone with large, loosely packed parenchymatous cells.

3. Physicochemical analysis

Leaves-:

Table 2. Physicochemical analysis of Leaves

Sr. No.	Test	Results
1.	Loss on drying	5.1%
2.	Total ash	2.5%
3.	Acid insoluble ash	0.75%
4.	Water soluble ash	3.5%
5.	Alcohol soluble extractive value	18.4%
6.	Water soluble extractive value	15.2%
7.	Pet Ether extractive soluble extract	2.4%
8.	n-Hexane soluble extractive value	3.2%
9.	Ethyl acetate extractive value	5.6%
10.	Foreign matter	Nil

Stem: -

Table 3. Physicochemical analysis of Stem

Sr. No.	Test	Results
1.	Loss on drying	6.2%
2.	Total ash	2%
3.	Acid insoluble ash	1%
4.	Water soluble ash	1.5%
5.	Alcohol soluble extractive value	28.8%
6.	Water soluble extractive value	19.2%
7.	Pet Ether extractive soluble extract	6.4%
8.	n-Hexane soluble extractive value	4.8%
9.	Ethyl acetate extractive value	8%
10.	Foreign matter	Nil

4. Fluorescence analysis

Table 4. Fluorescence behaviour of powdered leaf and Stem treated with different reagents

Sr. no.	Testing	Visible Light		Short UV (254nm)		Long UV (365nm)	
		Leaves	Stem	Leaves	Stem	Leaves	Stem
1.	**Powder (p)**	Green	brown	Green	Gray	brown	reddish
2.	**P + 1 N NaOH in methanol**	Green	brown	Greenish	Brownish	black	Greenish black
3.	**P + conc. HCL**	Blackish black	black	black	black	black	black
4.	**P +NH_3**	Greenish black	Green	Gray	Greenish black	Gray	Greenish black
5.	**P+ conc H_2SO_4(1:1)**	Bluish black	Brownish	black	Brownish black	black	Brownish black
6.	**P + 50% H_2SO_4**	Blackish	Redish black	Redish	Redish black	Redish	Redish

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7.	**P + 50% HNO_3**	Yellowish green	Orange	Yellowish	Redish	Yellowish	Brown
8.	**P+ conc HNO_3**	Yellowish	Yellowish orange	Yellowish orange	Redish	Yellowish	Black
9.	**P + acetic acid**	Green	brown	Greenish yellow	Brownish yellow	Brown	Black

5. Phytochemical Evaluation: -

Table 5. Preliminary Phytochemical analysis of various extract of leaves and stem of Syzygium hemisphericum.

Extracts	**Pet ether**		**N-hexane**		**Ethyl acetate**		**Ethanol**		**Aqueous**	
Phytoconstituents	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem
A. Carbohydrate	+	+	+	+	+	+	+	+	+	+
B. Test for Steroids										
1. Salkowaski Test	+	+	+	-	+	+	-	-	-	-
2. Libermamann Test	+	+	+	-	+	+	-	-	-	-
3. Libermann burchard test	+	+	+	-	+	+	-	-	-	-
C. Test for Glycosides										
1. Baljet test	+	-	+	-	+	+	+	+	+	+
2. Killer kilani	+	-	+	-	+	+	+	+	+	+
3. Legals test	+	-	+	-	+	+	+	+	+	+
C. Test for Flavonoids										
1. Shinoda test	+	+	+	-	+	+	+	-	+	-
2. $H_2So_{4\ test}$	+	+	+	-	+	+	+	-	-	-
E. Test for Alkaloids										
1. Dragan droff reagent	+	+	+	-	+	+	+	+	-	-
2. Hagers test	+	+	+	+	-	+	-	+	-	-
3. Mayers test	+	+	+	+	-	+	+	+	-	-
4. Wagnor reagent	+	-	+	-	+	+	+	+	-	-
F. Terpenoids	+	-	+	+	-	+	-	-	-	-
I.Fixed oils and fats	+	+	+	+	+	+	-	-	-	-
J. Test for Phenolic and Tannins										
1. $FeCl_3$	-	-	-	-	-	-	+	+	+	+
2. Lead acetate	-	-	-	-	-	-	+	+	+	+
3. Iodine	-	-	-	-	-	-	+	+	+	+
4. Pot. Dichromate	-	-	-	-	-	-	+	+	+	+
K. Saponin	-	-	-	-	-	-	+	-	-	-

6. Total Phenolic content estimation:

Table 6. Absorbance for standard Gallic acid

Sr. No	**Concentration Gallic acid (µg/mL)**	**Absorbance (Mean ± SD)**
1	100	0.150±0.001
2	200	0.298±0.000
3	300	0.440±0.002
4	400	0.590±0.001
5	500	0.730±0.002

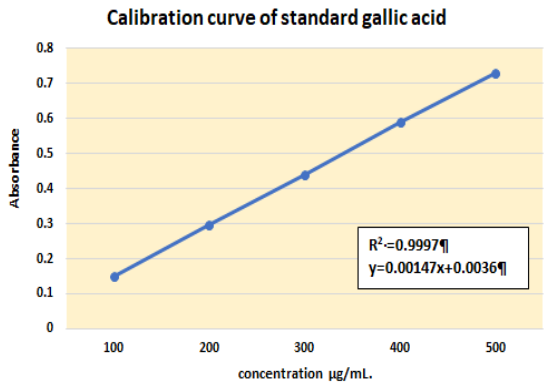

Figure 6. Calibration curve of Gallic acid

Table 7. Absorbance for Leaves extracts of SH

Extract	**Observed Abs. (Mean ± SD)**	**Dilution**	**Corrected Abs.**
PE	0.360±0.001	1	0.360
N-hexane	0.207±0.002	1	0.207
Ethyl acetate	0.665±0.001	2	1.330
Ethanol	0.896±0.000	5	4.480
Aqueous	0.678±0.001	2	1.356

Table 8. Absorbance for Stem extracts of SH

Extract	**Observed Abs. (Mean ± SD)**	**Dilution**	**Corrected Abs.**
PE	0.310±0.001	1	0.310
N-hexane	0.039±0.000	1	0.039
Ethyl acetate	0.789±0.001	1	0.789
Ethanol	0.692±0.001	5	3.460
Aqueous	1.079±0.002	2	2.158

Table 9. Total Phenolic content in extract of leaves and stem of SH

Extract	**Leaves (mg GAE/g)**	**Stem (mg GAE/g)**
Pet Ether	0.246	0.21
N-hexane	0.139	0.023
Ethyl acetate	0.914	0.541

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Ethanol	3.086	2.383
Aqueous	0.932	1.484

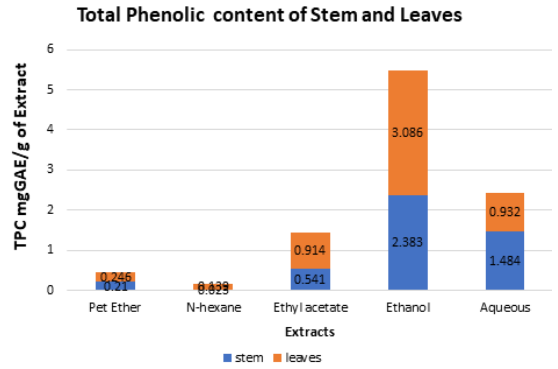

FIG 7: -Graph of Total Phenolic content in extract of leaves and stem of SH

Ethanol extracts of leaves and stems showed the highest total phenolic content, confirming the suitability of polar solvents for efficient extraction of phenolic compounds. The study demonstrated that solvent polarity plays a crucial role in the extraction of phenolic compounds. Ethanol extract of leaves showed the highest total phenolic content, suggesting its potential application as a natural antioxidant source.

7. Total Flavonoid content estimation:

Table no 11. Absorbance for standard Quercetin

Sr. No	Concentration Quercetin(µg/mL)	Absorbance (Mean ± SD)
1	150	0.158±0.001
2	300	0.294±0.002
3	450	0.402±0.000
4	600	0.501±0.001
5	750	0.595±0.001

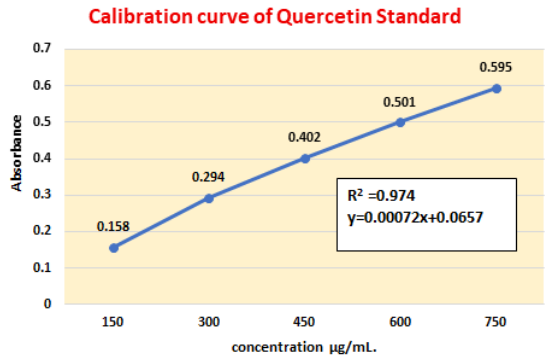

Fig 8. Calibration curve of standard Quercetin

Table 12. Absorbance for Leaves extracts of SH

Extract	Absorbance (Mean ± SD)	Total Flavonoid Content (mg/L QE)
Pet Ether	0.646±0.000	805
Ethyl acetate	0.541±0.002	662
N-hexane	0.468±0.001	561
Ethanol	0.047±0.002	0
Aqueous	0.043±0.001	0

Table 13. Absorbance for Stem extracts of SH

Extract	Absorbance	Total Flavonoid Content (mg/L QE)
Pet Ether	0.151±0.001	129
Ethyl acetate	0.249±0.002	263
N-hexane	0.098±0.001	57
Ethanol	0.165±0.001	149
Aqueous	0.042±0.000	0

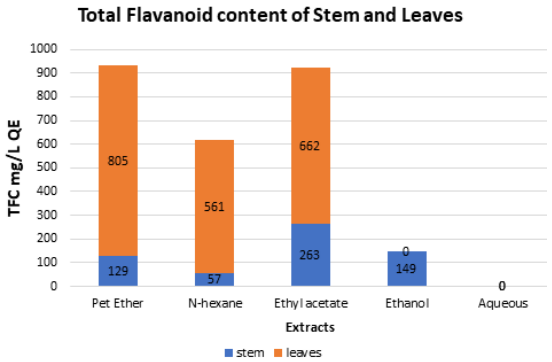

Fig 9: -Graph of Total Flavonoid content in extract of leaves and stem of SH

The total flavonoid content of leaf and stem extracts was expressed as milligrams of quercetin equivalents per liter (mg/L QE). Among the leaf extracts, petroleum ether extract showed the highest flavonoid content (805 mg/L QE), followed by ethyl acetate (662 mg/L QE) and n-hexane (561 mg/L QE), indicating efficient extraction of non-polar to moderately polar flavonoids. Ethanol and aqueous leaf extracts exhibited flavonoid content below the detection limit. In stem extracts, ethyl acetate extract demonstrated the maximum flavonoid content (263 mg/L QE), followed by ethanol (149 mg/L QE), petroleum ether (129 mg/L QE), and n-hexane (57 mg/L QE), while the aqueous extract showed negligible flavonoid content. Overall, leaves contained significantly higher flavonoid levels than stems, and ethyl acetate emerged as the most effective solvent for flavonoid extraction from stem material. This variation highlights the influence of both plant part and solvent polarity on flavonoid extraction efficiency.

DPPH radical Scavenging Assay:

Table 14. DPPH free radical scavenging activity of ascorbic acid

Concentration (µg/mL)	Absorbance (Mean ± SD)	% Inhibition
100	0.031 ± 0.000	95.44
200	0.035 ± 0.002	94.88
300	0.061 ± 0.001	91.09
400	0.063 ± 0.001	90.76
500	0.086 ± 0.001	87.35
	Equation: $y = -0.0206x + 97.38$	**R^2 = 0.935, IC_{50} = 2299 µg/mL**

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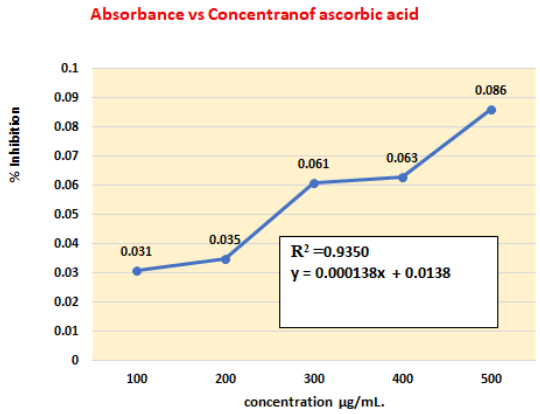

Fig 10: -Graph of absorbance vs concentration of Ascorbic Acid

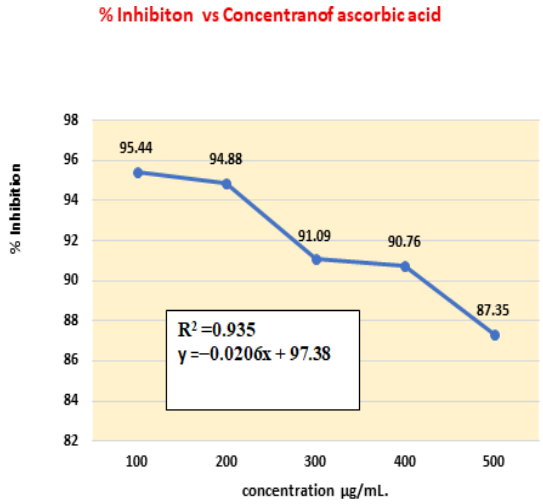

Fig 11: -Graph of % Inhibition vs concentration of Ascorbic Acid

Ethyl acetate Leaves extract of SH: -

Table 15. DPPH free radical scavenging activity of Ethyl acetate Leaves extract

Concentration (µg/mL)	Absorbance (Mean ± SD)	% Inhibition
100	0.087 ± 0.000	87.21
200	0.079 ± 0.001	88.38
300	0.047 ± 0.001	93.09
400	0.046 ± 0.001	93.24
500	0.035 ± 0.000	94.85

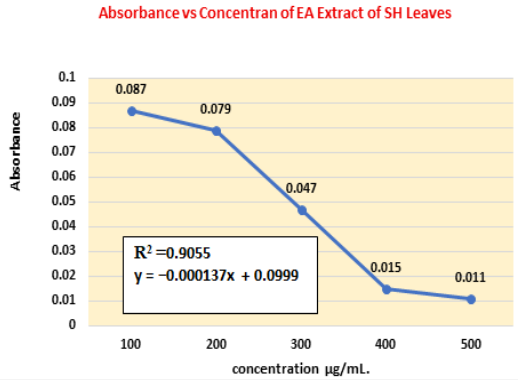

Fig 12: -Graph of absorbance vs concentration of Ethyl acetate Leaves

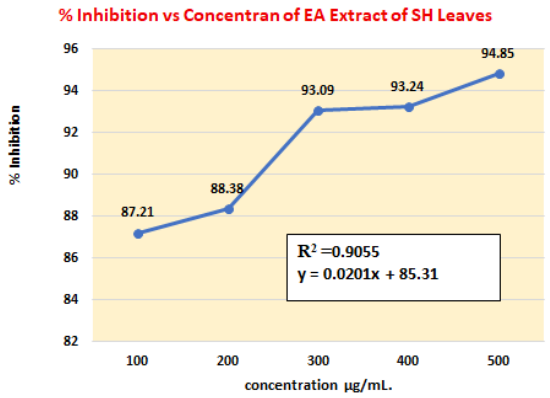

Fig 13: -Graph of % Inhibition vs concentration of Ethyl acetate Leave

Ethyl acetate Stem extract of SH: -

Table 16. DPPH free radical scavenging activity of Ethyl acetate Stem extract

Concentration (µg/mL)	Absorbance (Mean ± SD)	% Inhibition
100	0.067± 0.001	90.15
200	0.057 ± 0.001	91.62
300	0.046± 0.000	93.24
400	0.044 ± 0.001	93.53
500	0.043 ± 0.002	93.68

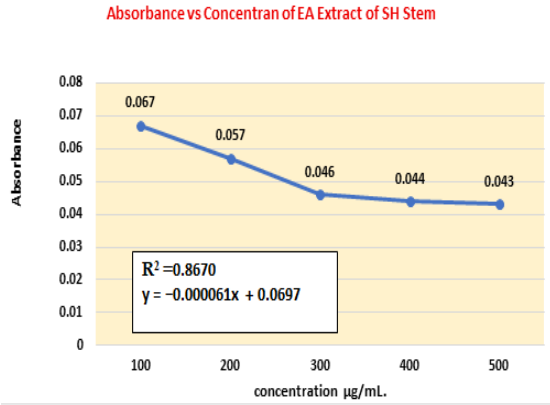

Fig 14: -Graph of absorbance vs concentration of Ethyl acetate Stem

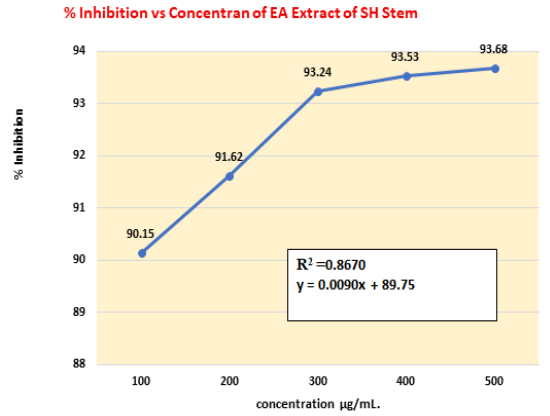

Fig 15: -Graph of % Inhibition vs concentration of Ethyl acetate stem

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Ethanol Leaves extract of SH: -

Table 17. DPPH free radical scavenging activity of Ethanol Leaves extract

Concentration (µg/mL)	Absorbance (Mean ± SD)	% Inhibition
100	0.082± 0.002	87.94
200	0.070 ± 0.000	89.71
300	0.052± 0.001	92.35
400	0.046 ± 0.001	93.24
500	0.022 ± 0.000	96.76

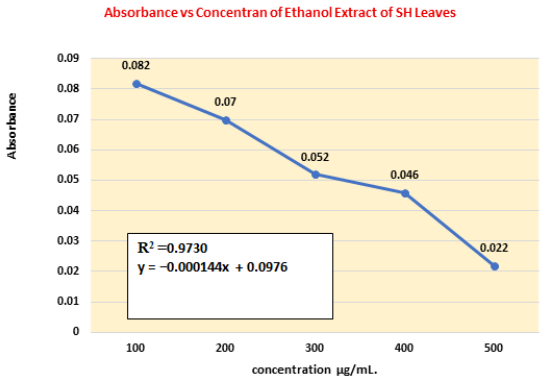

Fig 16: -Graph of absorbance vs concentration of Ethanol Leaves extract

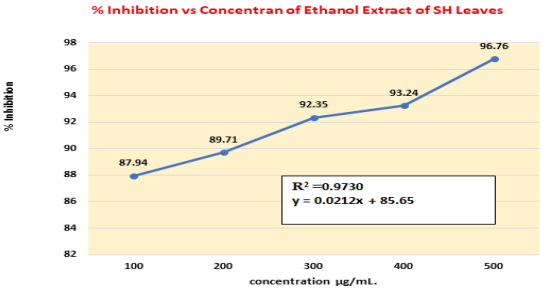

Fig 17: -Graph of % Inhibition vs concentration of Ethanol Leaves extract

Ethanol Stem extract SH:

Table 18. DPPH free radical scavenging activity of Ethanol Stem extract

Concentration (µg/mL)	Absorbance (Mean ± SD)	% Inhibition
100	0.079± 0.001	88.38
200	0.053 ± 0.002	92.21
300	0.047± 0.000	93.09
400	0.030 ± 0.001	95.59
500	0.020± 0.000	97.06

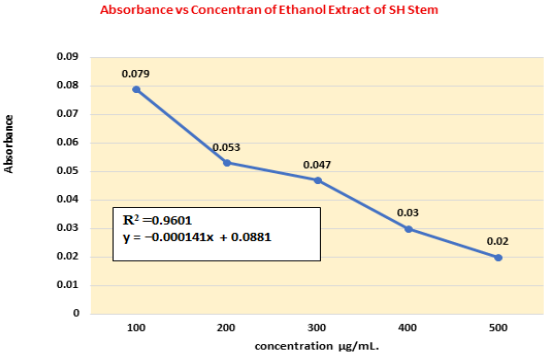

Fig 18: -Graph of absorbance vs concentration of Ethanol stem extract

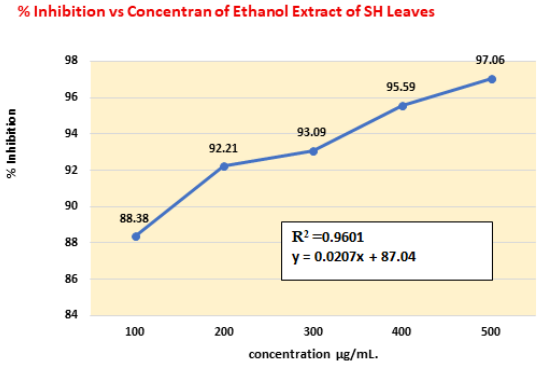

Fig 19: -Graph of % Inhibition vs concentration of Ethanol Stem extract

The antioxidant activity of leaf and stem extracts was evaluated using the DPPH free radical scavenging assay and compared with the standard antioxidant, ascorbic acid. All tested extracts exhibited strong DPPH radical scavenging activity in a concentration-dependent manner. Ethyl acetate leaf extract showed percentage inhibition ranging from 87.21% to 94.85%, while ethyl acetate stem extract exhibited inhibition between 90.15% and 93.68% across the tested concentrations (100–500 µg/mL). Linear regression analysis revealed good correlation between concentration and percentage inhibition, with R^2 values of 0.9055 and 0.8670 for leaf and stem extracts, respectively.Ethanol extracts demonstrated comparatively higher and more consistent antioxidant activity. Ethanol leaf extract showed inhibition values ranging from 87.94% to 96.76% with a high correlation coefficient (R^2 = 0.9730). Similarly, ethanol stem extract exhibited inhibition from 88.38% to 97.06%, with an R^2 value of 0.9601, indicating a strong linear relationship between concentration and scavenging activity.

Since all extracts showed more than 50% DPPH inhibition at the lowest tested concentration, the IC_{50} values were found to be greater than 500 µg/mL. The high percentage inhibition and strong regression coefficients confirm the potent antioxidant potential of both ethyl acetate and ethanol extracts, particularly from stem and leaf parts.

CONCLUSION:

The present study provides a systematic pharmacognostic and phytochemical standardization of *Syzygium hemisphericum* leaves and stem in accordance with WHO guidelines. Distinct morphological, microscopic, physicochemical, and fluorescence characteristics were established as reliable diagnostic markers for quality control. Quantitative analyses demonstrated solvent-dependent variation in phytoconstituents, with ethanol extracts showing the highest phenolic content and petroleum ether/ethyl acetate extracts

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exhibiting notable flavonoid levels. Both leaf and stem extracts displayed strong, concentration-dependent DPPH radical scavenging activity, correlating well with their phenolic and flavonoid content. These findings scientifically validate the antioxidant potential of *S. hemisphericum* and provide a robust foundation for future molecular studies, bioactive compound isolation, and standardized phytopharmaceutical development.

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