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PHYTOCHEMICAL PROFILING, ANTIBACTERIAL SCREENING AND ANTIOXIDANT PROPERTIES OF THE SACRED TREE (*SHOREA ROBUSTA* GAERTN.) OF JHARKHAND

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ABSTRACT: *Shorea robusta* Gaertn., commonly called as Sal tree, is revered as a sacred tree by most of the tribals of Jharkhand, India. They do not worship it rather believe it to the abode of tribal goddess. The plant parts are used as a remedy against various ailments in combination with other ingredients. Preliminary phytochemical screening of the whole plant exhibited the presence of high concentration of bioactive components. The plant extracts also exhibited good antibacterial activities. The HPLC and GC-MS analysis showed the presence of very high concentrations of several phytochemicals such as Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy] benzoate, D-Mannitol, Sorbital, Phytol, Hexamethylcyclotrisiloxane, β-Caryophyllene, 1,2,4-Benzenetriol, etc. which are pharmaceutically and industrially very important. Several of these phytochemicals have been determined to be anti-cancerous and antioxidant, antidiarrheal, anti-dysenteric, antibacterial, stimulant, diuretic, styptic and anti-gonorrhoeal. The study revealed that the sacred tree of Jharkhand could be a real blessing for the world in order to harvest several phytochemicals in large quantities and to produce drugs at the low cost to heal several human ailments.

INTRODUCTION: Sal is considered to be a sacred tree by most of the tribals of Jharkhand, India. They do not worship it but they believe it to the abode of tribal goddess *Chalapachcho* or *Jaher era* or *Jaherburhi*. Every tribal village possess a sacred grove (collection of Sal trees) at one end of the village in which the village deities dwell under the chieftainship of *Chālāpachcho*or *Jaher era*.

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Scientifically, a Sal tree is called as *Shorea robusta* Gaertn. Which belongs to the family Dipterocarpaceae. It is called by different names by various ethnic groups as follows –*Makka* (Oraons), *Sakhua* (Sadri), *Serga* (Kharias) and *Sarjom* (Mundas, Santals and Hos). The sacred grove is called as *Chālā* (Oraons), *Sarnatharo*(Kharias), *Jayar* (Mundas), *Jaher* (Santals) and *Sarna* (Hos).

The entire tree of *S. robusta* is used for varied purposes such as timber in house construction, agriculture tools, firewood, twig as toothbrush, leaves for making leaf-plates and cups, flowers are offered to deities in *Sarhul*or *Baha* festival and the seeds are used as a food supplement with the boiled flowers of *Mandhu calatifolia*. According to the

informants, the bark decoction or seed powder or gum-resin with jaggery is given to treat diarrhoea and dysentery. The bark decoction with black salt is given to manage diabetes and also to women against burning sensation during urination. The tender leaves are chewed against indigestion.

Literature review reveals that several works have done on the oleo resin of *S. robusta* regarding its anti-diarrhoeal, anti-dysentery, anti-skin allergic, emulsifying and antibacterial properties [1, 2]. It acts as stimulant, expectorant, diuretic, styptic and also has been used against gonorrhoea, bleeding piles, bronchitis, and leucorrhoea, menorrhagia, enlargement of the spleen [3]. All the parts of the tree has been studied by different workers taking a single part – bark [4-6], leaves [7-9], flower [10] and seeds[11]. No cumulative works of all the parts have been reported, hence the need of the present study. Moreover, despite being the sacred and state tree of Jharkhand, no extensive works have been done on *S. robusta* of the state with respect to phytochemical and antibacterial studies.

Taxonomy:

The taxonomy hierarchy of *S. robusta* is given in **Table 1** with its common and vernacular names. *S. robusta* is a large, deciduous tree up to 50 m tall bearing epicormic branches and spreading or spherical crowns (**Fig. 1a**). The trunk girth is up to 5m consisting of thick and dark brown bark with longitudinal fissures. The trunk becomes shallow in aged trees. Leaves are simple, shiny, glabrous, about 10-25 cm long and broadly oval at the base, with the apex tapering into a long point (**Fig. 1b**).

The new leaves are reddish which turn delicate green and finally dark green. Flowers are yellowish-white which are arranged in large terminal or axillary racemose panicles. Fruits are capsules, ovoid, and about 1.3-1.5 cm long and 1 cm in diameter. They are winged with enlarged sepals of 5 unequal sizes and lengths (**Fig. 2c**). Seeds and greenish and fleshy with unequal cotyledons.

TABLE 1: TAXONOMY OF *SHOREA ROBUSTA*

Botanical Name in full	***Shorea robusta* Gaertn.**
Kingdom	Plantae
Class	Magnoliopsida
Order	Malvales
Family	Dipterocarpaceae
Genus	Shorea
Species	*Shorea robusta*
Common Names	Sal tree
Vernacular Names	*Makka*(Oraon), *Sakhua*(Sadri), *Serga* (Kharia) and *Sarjom*(Munda, Santal and Ho)

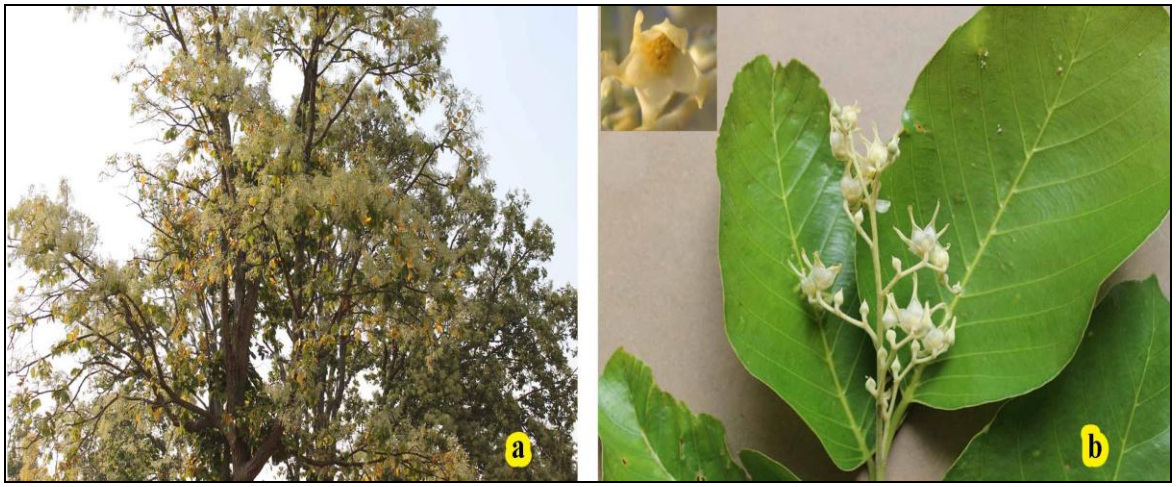


FIG.1: A) *SHOREA ROBUSTA* GAERTN. (HABIT- TREE IN FULL BLOOM B) CLOSE UP LEAVES AND FLOWERS OF *S. ROBUSTA*

FIG.2: A) *SHOREA ROBUSTA* BARK OUTER SURFACE. B) *SHOREA ROBUSTA* BARK INNER SURFACE C) FRUITS AND SEEDS OF *S. ROBUSTA*

MATERIALS AND METHOD:

Collection of Plant Materials:

The voucher specimens and the plant materials such as bark, leaves, flowers and seeds of *S.robusta* were collected from the jungles of Balumath, Jharkhand, India. The herbarium specimens were authenticated by Dr. S. John Brittoand were deposited in the Rapinat Herbarium of St. Joseph's College, Trichy, Tamilnadu, India with the accession number RHT 67036 and RHT 67059. The different habits, flowers and seeds of the tree were photographed and deposited in the same herbarium. The plant parts for the experiments were collected during the months of April-May, 2015.

Extraction of Phytochemicals:

The plant parts such as bark, leaves, flowers and seeds of *S.robusta* were dried under shade at room temperature for a period of two weeks. The dried plant materials were powdered mechanically and kept in the air-tight containers. 10g of the powder of each plant part was extracted in a rotary shaker for 72 hours with 90% ethanol and distilled water. The extracts were concentrated and dried by evaporation.

Preliminary Phytochemical Investigations: [12-19]

A pinch of powders of bark, leaf, flowers and seeds of *S.robusta* taken on a test tube and added with a few drops of chemical reagents such as strong acids, strong bases and other reagents. The characteristic colours produced by the reactions were observed and recorded as per the standard methods.

Qualitative phytochemical analysis of ethanolic and aqueous extracts of bark, leaves, flowers and seeds of *S.robusta* was carried out by adopting standard methods from various sources. The bioactive compounds such as alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, tannins, terpenoids, etc. were screened by doing three different tests for each phytochemical to ascertain the presence.

Microscopic Study of Powders: [20]

A pinch of powders of bark, leaves, flowers and seeds of *S.robusta* was taken on a slide and added with few drops of safranin and glycerine. It was mixed well and then observed under microscope and photographed with Nikon Eclipse 80i. The photographs were edited with NIS Elements F 3.00 SP7and Adobe Photoshop CS6 softwares for labelling.

Antibacterial Screening of Sacred Tree:

Twelve bacterial pathogens consisting of four Gram^{+ve} and eight Gram^{-ve} were selected for the antibacterial study of the sacred tree. The selected Gram $^{+ve}$ pathogens were *Bacillus cereus*(ATCC #4342), *Bacillus subtilis* (MTCC # 441), *Staphylococcus aureus* (MTCC # 3163) and *Streptococcus pneumonia* (ATCC # 7066), while the Gram^{-ve} pathogens included *Enterobacter aerogenes* (MTCC # 2990), *Escherichia coli* (MTCC# 199), *Klebsiella pneumonia* (MTCC # 3040), *Proteus mirabilis*(MTCC # 1429), *Proteus vulgaris* (MTCC # 1771), *Pseudomonas aeruginosa* (MTCC # 2474), *Salmonella paratyphi* (MTCC #734) and *Vibrio cholerae* (ATCC # 14104).

The ethanolic extracts of bark, leaf, flower and seed of *S. robusta* were tested for susceptibility against all the given pathogens adopting standard disc diffusion method in Nutrient agar medium. The concentrations of 200µg/disc was taken for the extracts and also the control (streptomycin). The experiments were done in triplicates from which the mean and standard deviations were calculated by using standard formulae.

HPLC and GC-MS Analysis of Sacred Tree:

Ethanolic extracts of bark, leaf, flower and seed of *S. robusta* was subjected to analytical HPLC adopting the standard procedures and conditions [21]: 2ml of extract was filtered through 0.2µm filter and 20µl was injected into the Shimadzu HPLC equipped with auto-sampler and diode array detector. The solvents Acetonitrile and HPLC grade water were used for gradient elution and the running time consisted of 30 minutes, while the chromatogram was obtained at 254nm. For the GC-MS analysis, the same extracts were subjected to GC-MS Shimadzu instrument by adopting standard procedure and conditions [22, 23]. Identification and interpretation of compounds were done by the comparison of mass spectra of the samples using the database of NIST research library. Spectra of unknown compounds were compared with the spectra of known compounds stored in the NIST library. The names, molecular formula, molecular weight and molecular structures of the compounds of the test extracts were ascertained from the databank of PubChem[24] and Chem Spider [25]. The biological activities of the compounds were obtained from various sources which have been referenced.

Antioxidant Activity by DPPH Radical Scavenging Assay: [26, 27, 28]

Free radical scavenging activity of ethanolic extracts of bark, leaf, flower and seed of *S. robusta* was determined by DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay with slight modification.3ml of DPPH (30mg/L) solution was added to 1ml of sample solution at different concentrations (100-500µg/ml). The reaction mixture was mixed well and kept in the dark at room for 30minutes. The absorbance was measured at 517 nm by using Lambda 35 UV/VIS Spectrometer. The absorbance of the samples were compared with that of the control standard (Ascorbic acid). The IC_{50} value of samples (concentration of sample required to inhibit 50% of the DPPH free radical) was calculated using Log dose inhibition curve. The lower absorbance of the reaction mixture indicated higher free radical activity. The ability of the plant extracts to scavenge DPPH radical was calculated by the following formula:

DPPH scavenging effect (%) or Percent inhibition

$= A_0 - A_1 / A_0 \times 100.$

Where A_0= Absorbance of control and A_1 = Absorbance of samples.

RESULTS AND DISCUSSIONS:

Phytochemical Screening: The powders bark, leaf, flowers and seeds of *S. robusta*, when treated with different chemical reagents produced specific colour reactions. On the basis of colour reactions, the inferences were drawn for the presence of the phytochemicals (**Tables 2 and 3)**. The powder studies indicated the presence of alkaloids, phenols, tannins, steroids, flavonoids, etc. in the bark, leaf, flower and seed of the sacred tree. However, protein was absent in all parts except the seed. Similarly, anthraquinone absent in bark and leaf, while present in flower and seed.

TABLE 2: BEHAVIOUR OF BARKAND LEAFPOWDERS OF *S. ROBUSTA* WITH DIFFERENT CHEMICAL REAGENTS

S. N.	Chemical Tests	Bark		Leaf	
		Observation	Inference	Observation	Inference
1	Powder + Conc. HCl	Brick red	Leucoanthycyanins present	Yellowish	Leucoanthycyanins present
2	Powder + Conc.H_2SO_4	Reddish brown	Steroids present	Reddish brown	Steroids present
3	Powder + Conc. HNO_3	Reddish yellow	Proteins absent	Reddish yellow	Proteins absent
4	Powder + Picric acid	Yellow	Alkaloids present	Yellow	Alkaloids present
5	Powder + Aq. $FeCl_3$	Bluish green	Phenols &Tannnis present	Bluish green	Phenols &Tannnis present

6	Powder + I_2 solution	Pale brown	Starch absent	Pale yellow	Starch absent
7	Powder + NH_3 solution	Pale blood red	Athraquinone present	Brownish yellow	Athraquinone absent
8	Powder + Aq. KOH	Reddish	Athraquinone present	Yellowish brown	Athraquinone absent
9	Powder + Aq. NaOH	Intense yellow	Flavonoids present	Yellow	Flavonoids present

Abbreviations: Conc. – concentrated;, Aq.-Aqueous

TABLE 3: BEHAVIOUR OF FLOWER AND SEED POWDERS OF *S. ROBUSTA* WITH CHEMICAL REAGENTS

S. N.	Chemical Tests	Flower		Seed	
		Observation	Inference	Observation	Inference
1	Powder + Conc. HCl	Yellow	Quinonepresent	Yellowish	Quinonepresent
2	Powder + Conc.H_2SO_4	Reddish brown	Steroids present	Reddish brown	Steroids present
3	Powder + Conc. HNO_3	Reddish	Proteins absent	Yellowish	Proteins present
4	Powder + Picric acid	Yellow	Alkaloids present	Intense yellow	Alkaloids present
5	Powder + Aq. $FeCl_3$	Dark blue	Phenols &Tannnis present	Bluish green	Phenols &Tannnis present
6	Powder + I_2 solution	Brownish yellow	Starch absent	Bluish black	Starch present
7	Powder + NH_3 solution	Reddish	Athraquinone present	Reddish	Athraquinone present
8	Powder + Aq. KOH	Reddish	Athraquinone present	Reddish	Athraquinone present
9	Powder + Aq. NaOH	Yellowish	Flavonoids present	Yellow	Flavonoids present

Abbreviations: Conc. – concentrated;, Aq.-Aqueous

Preliminary phytochemical screening of the ethanolic and aqueous extracts of bark, leaf, flower and seed of *S. robusta* were carried out. The dried extracts were dissolved in 15ml of respective solvents and were tested for the presence of bioactive compounds such as, alkaloids, carbohydrates, flavonoids, glycosides, phenols, steroids, tannins, saponins etc. The results are given in **Table 4**. The data in the table indicate that both the ethanolic and aqueous extracts contain most of bioactive phytochemicals. Moreover, carbohydrates, reducing sugars, phenols and tannins were found to be present in quite high concentrations. The bark was found to possess good concentration of flavonoids, cardiac glycosides, steroids and terpenoids. The starch and fixed oils were not detected in any plant part of *S. robusta.*

TABLE 4: PHYTOCHEMICAL SCREENING OF DIFFERENT PARTS OF *S. ROBUSTA*

S.N.	Plant parts →	Bark		Leaf		Flower		Seed	
	Phytochemicals ↓	EthOH	Aqua	EthOH	Aqua	EthOH	Aqua	EthOH	Aqua
1	Alkaloids	++	–	+++	–	+++	++	++	++
2	Carbohydrates	+++	+++	++	+++	++	++++	+++	++++
3	Reducing sugars	+++	+++	+	+++	+++	++++	+	++++
4	Starch	–	–	–	–	–	–	–	–
5	Flavonoids	++++	+++	+++	++	++	+	++	++
6	Fixed oils	–	–	–	–	–	–	–	–
7	Anthral glycosides	++	++	–	–	–	+	–	++
8	Cardiac glycosides	+++	+++	+	++	++	++	+++	+
9	Phenols	++++	+++	++++	+++	++++	+++	++++	++++
10	Proteins	–	–	–	–	–	–	+	+
11	Amino acids	–	–	–	–	+++	–	–	–
12	Saponins	++	++	++	–	–	–	++++	+
13	Steroids	++++	+++	+	++	++	+	++	+
14	Tannins	+++	+++	++++	+++	++++	+++	++++	++++
15	Terpenoids	++++	+++	–	+	++	+	++	+
16	Anthraquinone	+	+	–	–	–	+	–	+
17	Anthocyanin	+	+	–	–	–	–	–	–

18	Leucoanthocyanin	+	+	–	–	–	–	–	–
19	Plobatannins	++	+	–	–	–	–	–	–
20	Emodin	+	+	–	–	–	+	-	+
21	Coumarin	–	–	++	++	++	+	+	–
22	Quinone	+	+	–	–	+	+	+	++

Very high (++++), high (+++), moderate (++), low (+) and nil (–)

Fluorescent analysis of Extracts: [29, 30]

A small quantity of the extract was placed inside the UV viewer chamber and viewed in visible light and short ultraviolet radiations (254 nm). The ethanolic and aqueous extracts were observed under the visible light and UV light for their characteristic colours and the colour data were recorded (**Table 5**).

TABLE 5: FLUORESCENT ANALYSIS OF ETHANOLICAND AQUEOUS EXTRACTS OF *S. ROBUSTA*

S.N.	Plant parts	Ethanolic extract		Aqueous extract	
		Visible light	UV light (254nm)	Visible light	UV light (254nm)
1	Bark	Brownish yellow	Pale brick red	Reddish brown	Pale brown
2	Leaf	Greenish black	Brick red	Yellowish brown	Pale yellow
3	Flower	Golden yellow	Pale aqua	Brownish yellow	Pale brown
4	Seed	Yellowish brown	Pale brick red	Yellowish cream	Yellowish cream

Microscopic Analysis of Powders:

The components observed in the bark powder of *S. robusta* are presented in **Fig. 2a-j**. The pharmacognostic markers were thick walled cork cells with wavy walls, starch grains, thick walled stone cells with broad lumen, sclereids, gum ducts, prismatic and druse crystals, crystal fibres and heterogeneous medullary rays filled with starch grains. On the other hand, the leaf powder of *S. robusta* exhibited unicellular trichomes, resin crystals, druse crystals of calcium oxalate, stomata, simple starch grains and oil globules (**Fig. 3a-i**). The flower powder of *S. robusta* consisted of glandular and unicellular trichomes, pollen grains, resin crystals, few starch grains and oil globules (**Fig. 4a-i**). The seed powder of *S. robusta* was found to contain abundance of spherical starch grains, oil globules and a few resin crystals **(Fig. 5a-f).** was interesting to note that all parts of the sacred tree consisted of resin crystals and oil globules. Even the calcium oxalate crystals were observed in all parts but the seed. A spherical structure with a nucleus in the centre was observed, which is a mystery to be identified in the sacred tree (**Fig.3g** & **4c**).

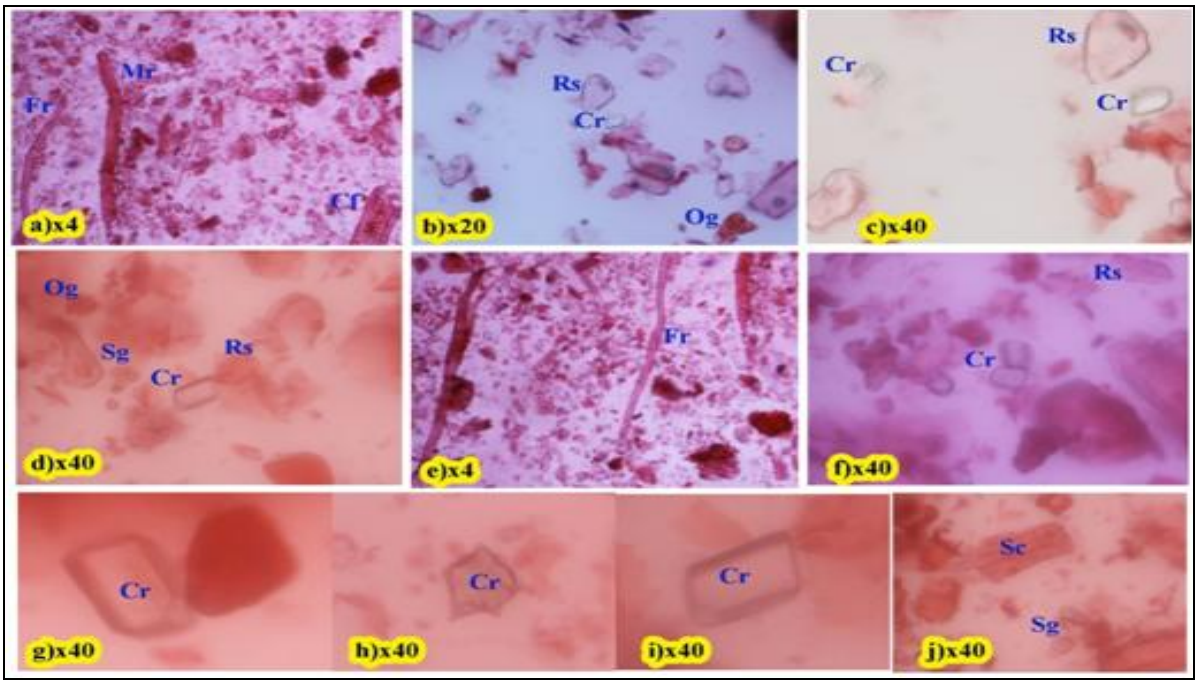


FIG.2: a-j) BARK POWDER MICROSCOPY OF *S. ROBUSTA*; Mr Medullary rays; Cf- Crystal fibre; Rs Resin crystal; Og- Oil globule; Cr- Calcium oxalate crystal; Sg- Starch grain; Fr- Fibre; Sc- Sclereid

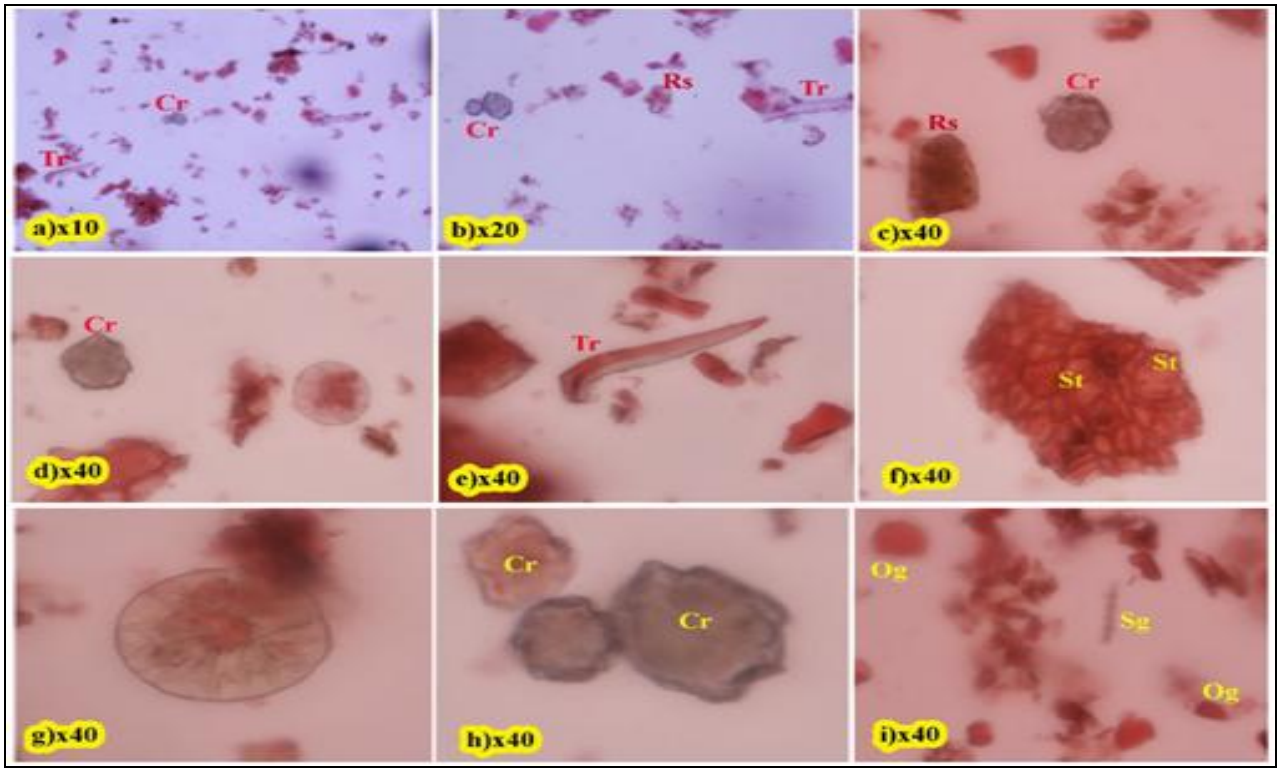


FIG.3 a-i) LEAF POWDER MICROSCOPY OF *S. ROBUSTA*; Tr- Trichome; Cr- Calcium oxalate crystal; St Stomata; Rs Resin crystal; Og- Oil globule; Sg- Starch grain;

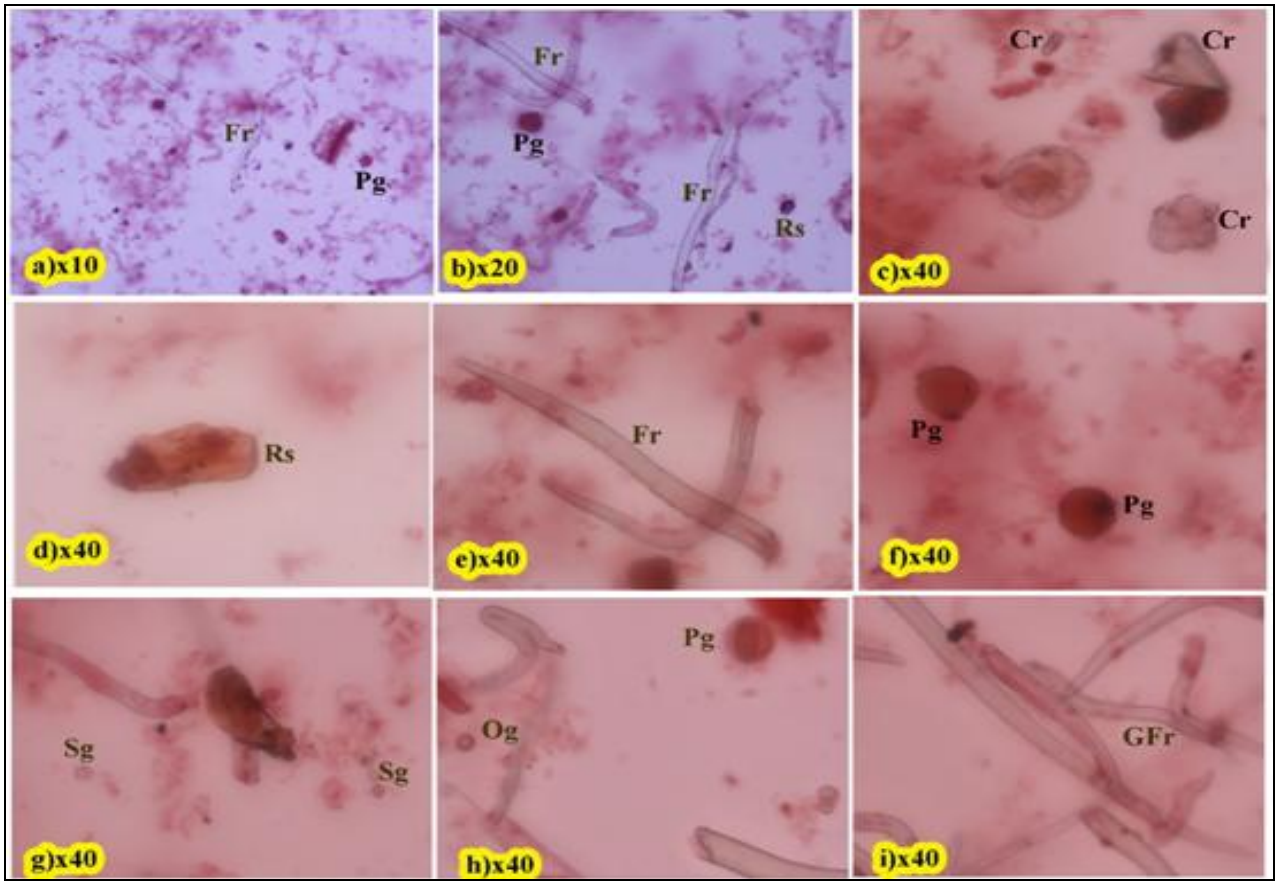


FIG.4: a-i) FLOWER POWDER MICROSCOPY OF *S. ROBUSTA*; Fr- Floral hairs; Pg- pollen grains; Cr- Calcium oxalate crystal; Rs Resin crystal; Og- Oil globule; Sg- Starch grain; GFr- Group of floral hairs

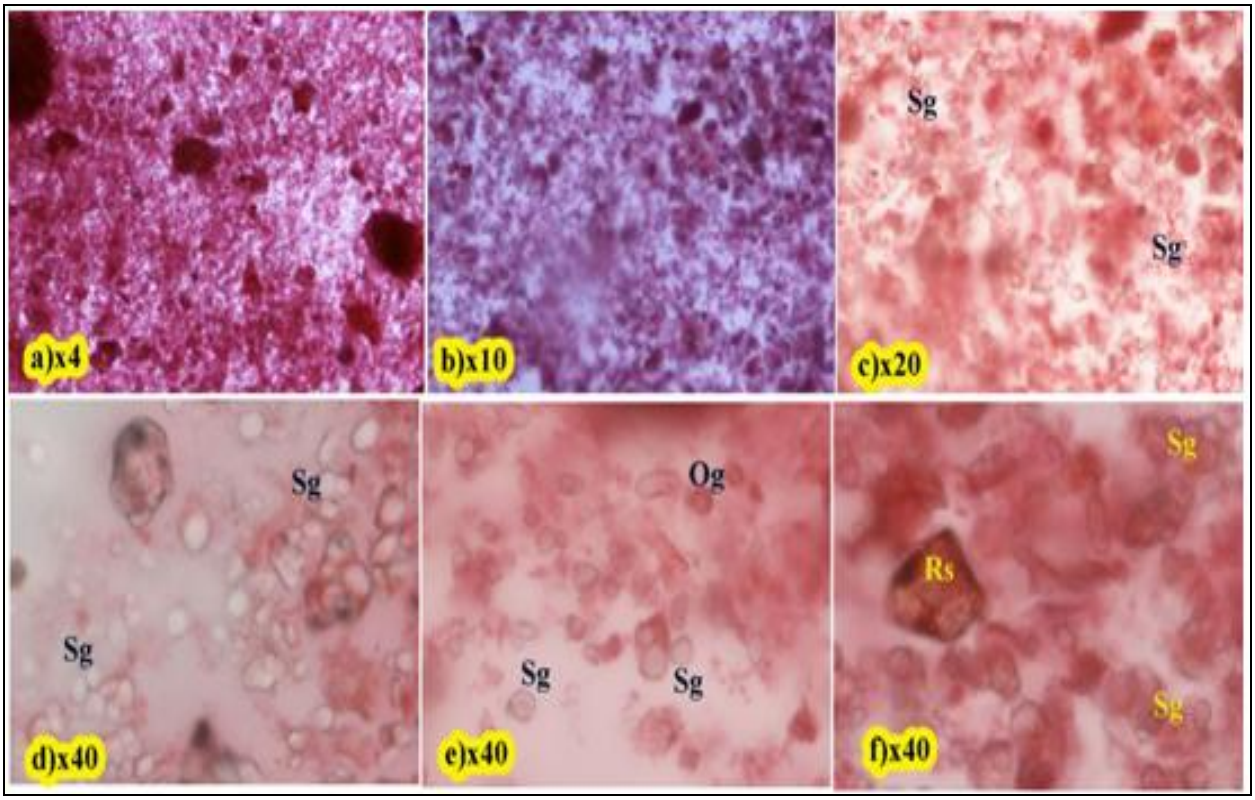


FIG.5: a-f) SEED POWDER MICROSCOPY OF *S. ROBUSTA*; Rs Resin crystal; Og- Oil globule; Sg- Starch grain;

Antibacterial Activities:

The antibacterial activities of the ethanolic extracts of bark, leaf, flower and seed of the sacred tree were tested against 4 Gram $^{+ve}$ bacteria and 8 Gram^{-} ^{ve} bacteria using Streptomycin as control. The results are presented in **Table 6** and **Chart 1.** All the plant parts exhibited activities all the tested microorganisms. However, they showed minimum inhibition zones against *B. Subtilis* and *P. aeruginosa.* It is clear from the analytical chart that the bark and seed possess higher antibacterial activities followed by leaf and flower. Moreover, leaf exhibited considerable sizes of zones against *S. aureus* and *S. faecalis*. The higher antibacterial activities exhibited by the seed validates its ethnic usage against diarrhoea, dysentery and gastritis.

TABLE 6: ANTIBACTERIAL ACTIVITIES OF DIFFERENT PARTS OF. *SHOREA ROBUSTA*

S. No.	Bacterial species	*Zone of inhibition in mm*				Control
		Bark	Leaf	Flower	Seed	
1	*Bacillus cereus*	13.8±0.25	12.5±0.51	8.7±0.12	14.5±0.70	25±0.0
2	*Bacillus subtilis*	8.4±0.36	9.5±0.50	8.2±0.35	10.8±0.72	23.6±0.5
3	*Enterobacter aerogenes*	10.2±0.29	13.6±0.53	9.3±0.26	12.2±0.35	25±0.0
4	*Escherichia coli*	10.0±0.45	11.5±0.50	10.2±0.23	12.4±0.69	25±0.0
5	*Klebsiella pneumoniae*	13.8±0.21	12.2±0.29	10.1±0.17	13.0±1.0	24±1.0
6	*Proteus mirabilis*	11.9±0.36	11.2±0.20	9.4±0.38	12.2±1.0	24.6±0.5
7	*Proteus vulgaris*	11.1±1.01	11.1±0.23	11.3±0.42	13.2±0.25	24.6±0.5
8	*Pseudomonas aeruginosa*	8.5±0.50	8.0±0.50	7.5±0.50	14.4±0.50	25±0.00
9	*Salmonella paratyphi*	11.1±0.23	10.2±0.25	10.4±0.40	12.4±0.40	25.6±0.5
10	*Staphylococcus aureus*	13.1±1.03	13.5±0.50	11.3±0.60	15.3±0.75	24.3±1.1
11	*Streptococous faecalis*	12.5±0.50	13.2±0.20	12.3±0.40	14.9±0.12	24.3±0.5
12	*Vibrio cholerae*	12.5±0.56	10.3±0.20	10.6±0.61	11.3±0.71	24±1.0

Data given are Mean of triplicates ± Standard Deviation

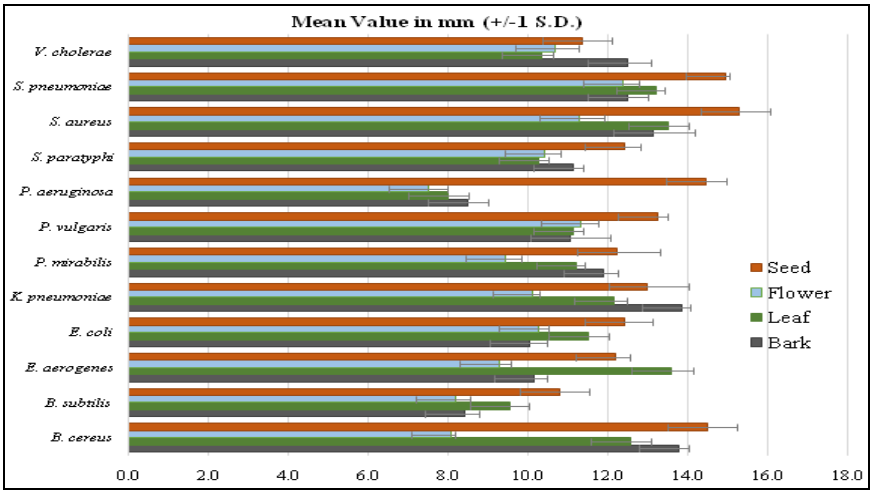


CHART 1: COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITIES OF *SHOREA ROBUSTA*

HPLC Analytical Examination of Sacred Tree: The HPLC analysis of ethanolic extract of bark, leaf, flower and seed *S. robusta* produced 1, 1, 4 and 2 peaks respectively (**Fig.6-8**). The details such as retention time and area percentage are given in **Table 7**. It was interesting to note that the flower produce higher number of peaks followed by seed, leaf and bark. The results indicated that the flower and seed possess higher number bioactive compounds.

Bark extract of ***S. robusta:***

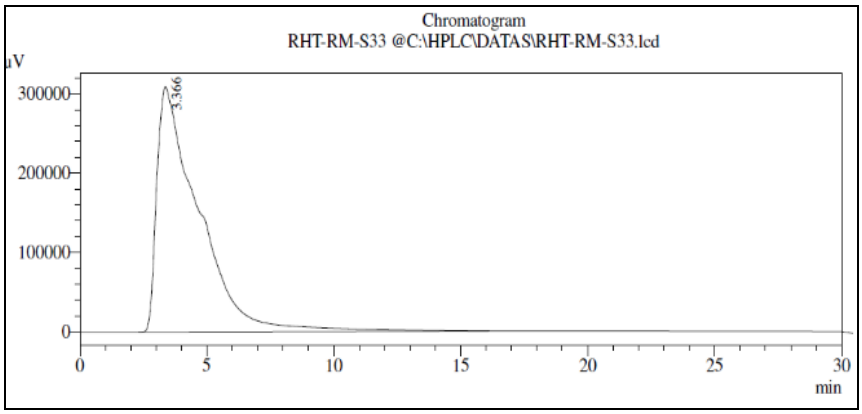


FIG. 6: HPLC CHROMATOGRAM OF ETHANOLIC

Leaf extract of ***S. robusta:***

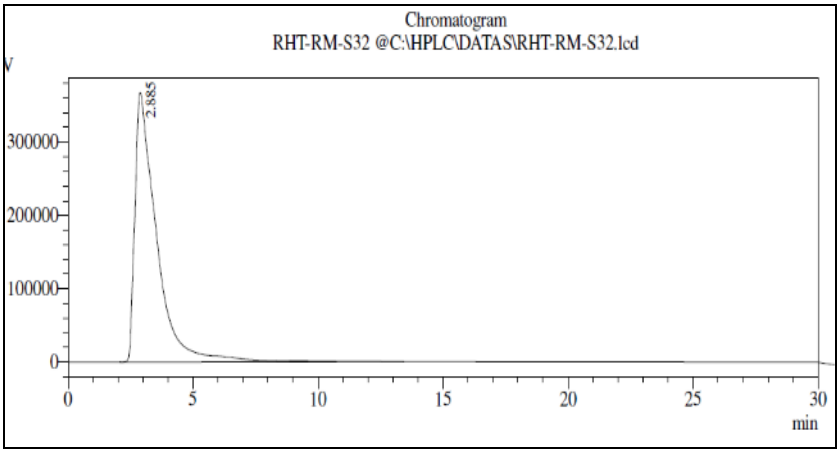


FIG. 7: HPLC CHROMATOGRAM OF ETHANOLIC

Flower extract of ***S. robusta:***

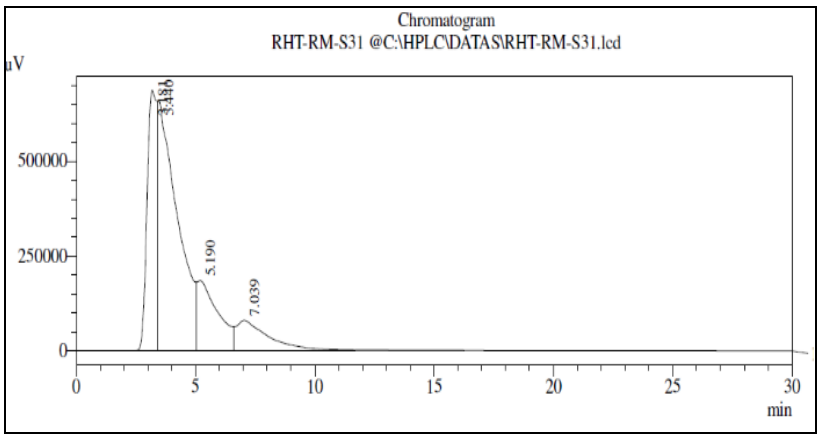


FIG. 8: HPLC CHROMATOGRAM OF ETHANOLIC

Seed extract of ***S. robusta:***

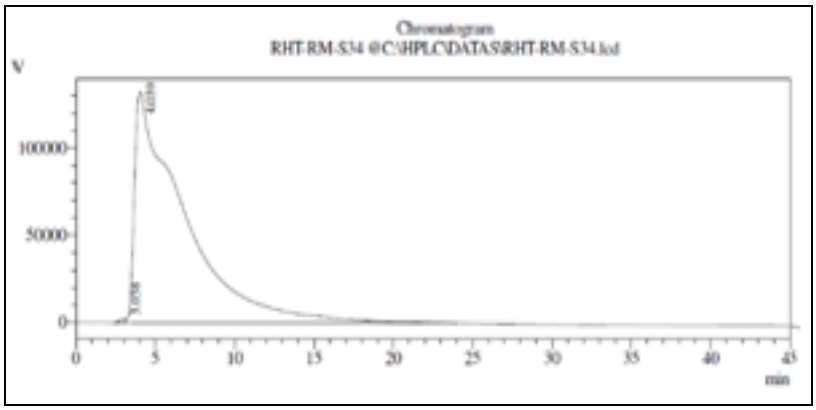


FIG. 9: HPLC CHROMATOGRAM OF ETHANOLIC

TABLE 7: HPLC DETECTION OF DIFFERENT PARTS OF SACRED TREE

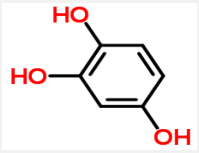
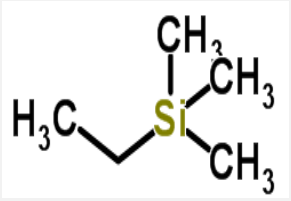
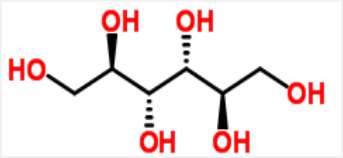
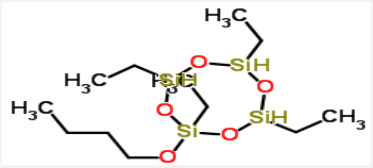
Plant part	Peak#	Ret. Time	Area	Height	Area %	Height %
Bark	1	3.366	35380579	308913	100.00	100.00
Leaf	1	2.885	22351005	367488	100.00	100.00
Flower	1	3.181	18634704	686313	24.522	42.621
	2	3.440	38187554	659444	50.252	40.952
	3	5.190	11340940	184423	14.924	11.453
	4	7.039	7828412	80100	10.302	4.974
Seed	1	3.058	61182	2280	0.210	1.689
	2	4.039	29045256	132717	99.790	98.311

GC-MS Data Analysis of Sacred Tree:

The bioactive compounds detected through GC-MS analysis of ethanolic extracts of bark, leaf, flower and seed of the sacred tree are presented in **Table 8, 9, 10** and **11**. The number of peaks produced and the compounds detected are: bark – 4, leaf – 7, flower -6 and seed – 4. The industrial uses and the biological activities of the phytochemicals are given in the respective tables. The bark of *S. robusta* possess high concentration of 1,2,4-Benzenetriol, Ethyl(trimethyl)silane and D-Mannitol with the area percentage 35.20, 19.97 and 42.65 respectively. The leaf of the sacred tree possess good concentration of Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy]benzoate, Propyl octan-2-yl carbonate and n-Hexadecanoic acid with area percentage 52.26, 21.23 and 10.11 respectively. The flower showed heavy concentration of β-Caryophyllene, Undecanal, 4a-Methyl-3,4,4a,5,8,8a-hexahydro-2(1H)-

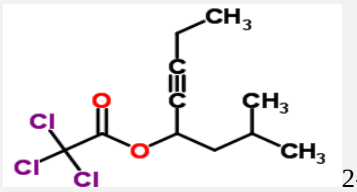
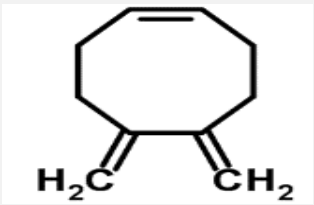
naphthalenone and Sorbitol with the area percentage 39.41, 13.74, 10.58 and 27.17 respectively while the seed exhibited the presence of high concentration of Hexadecyltrichloroacetate, Cyclooctane, methyl-2-Decanol and Hexamethyl cyclotrisiloxane with area percentage as 67.91, 25.37 and 100.00 respectively.

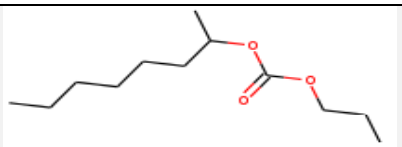
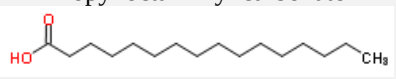
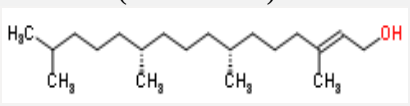
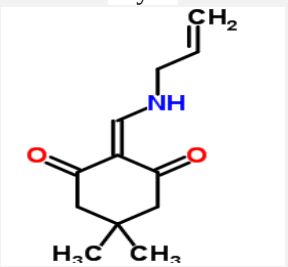
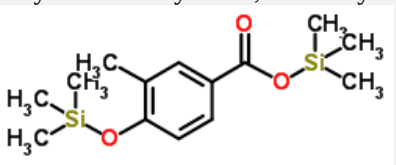
TABLE 8: GC-MS ANALYSIS OF BARK ETHANOLIC EXTRACT OF *S.ROBUSTA*

Peak No.	RT	Area %	M.F. & M.W.	Molecular structure	Uses / Bioactivity
1	12.328	35.20	$C_6H_6O_3$ 126.110	 1,2,4-Benzenetriol	Hair dye formulations [31]
2	17.295	19.97	$C_5H_{14}Si$ 102.250	 Ethyl(trimethyl)silane	In semi-conductor industry as etchant in plasma phase [32]
3	19.566	42.65	$C_6H_{14}O_6$ 182.172	 D-Mannitol	Diuresis treatment and acute kidney failure [33]
4	33.436	2.17	$C_{12}H_{32}O_5Si_4$ 368.721	 1,3,5,7-Tetraethyl-1-butoxycyclotetrasiloxane	Additives to plastics, coatings, sealants and as lubricants for food processing machinery, heat-resistant coatings [34]; magnetic fluid in eye surgery [35]

RT-Retention Time, M.F.-Molecular Formula, M.W.-Molecular Weight

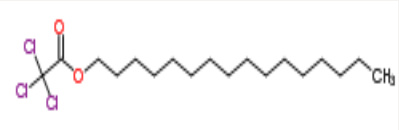
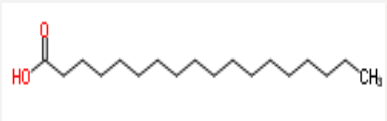
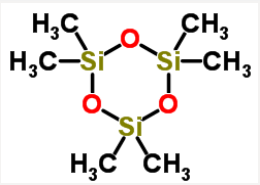
TABLE 9: GC-MS ANALYSIS OF LEAF ETHANOLIC EXTRACT OF *S.ROBUSTA*

Peak No.	RT	Area %	Molecular formula	Molecular structure	Uses/Bioactivity
1	12.477	3.45	$C_{11}H_{15}Cl_3O_2$ 285.595	 2-Methyloct-5-yn-4-yl 2,2,2-trichloroacetate	-
2	15.561	6.47	$C_{10}H_{14}$ 134.218	 Cyclooctene, 5,6-dimethylene-	Aroma chemical & aroma precursor[36]

3	16.310	21.23	$C_{12}H_{24}O_3$	 Propyl octan-2-yl carbonate	-
4	23.320	10.11	$C_{16}H_{32}O_2$ 256.424	 n-Hexadecanoic acid (Palmitic acid)	Soaps, cosmetics, release agent, processed foodstuffs, in napalm for military actions
5	26.141	4.43	$C_{20}H_{40}O$ 296.531	 Phytol	Manufacture of vitamins E and K[37, 38]; cancer preventive, antiinflammatory, diuretic[39]
6	26.507	2.06	$C_{12}H_{17}NO_2$ 207.269	 Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl-	-
7	39.942	52.26	$C_{14}H_{24}O_3Si_2$ 296.510	 Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy]benzoate	Protects human cells againstoxidative damage and cancer[40]

RT-Retention Time, M.F.-Molecular Formula, M.W.-Molecular Weight, -no reference

TABLE 11: GC-MS ANALYSIS OF SEED ETHANOLIC EXTRACT OF *S.ROBUSTA*

Peak No.	RT	Area %	M.F. / M.W.	Molecular structure	Uses / Bioactivity
1	12.740	67.91	$C_{18}H_{33}Cl_3O_2$ 387.812	 Hexadecyltrichloroacetate	-
2	17.466	25.37	-	Cyclooctane, methyl-2-Decanol	-
3	27.016	6.73	$C_{18}H_{36}O_2$ 284.477	 Octadecanoic acid (Stearic acid)	Production of hormones that regulate blood pressure, blood clotting and immune response and manufacture of soaps, cosmetics, detergents, lubricants, softening and release agents
4	42.185	100.00	$C_6H_{18}O_3Si_3$ 222.462	 Hexamethylcyclotrisiloxane	Adhesives, sealant chemicals, synthesis of silicone rubber products, polymers, silicone polymers, used to prepare many chemicals and intermediates

Pharmaceutically important phytochemical such Ursolic acid, α-Amyrenone, α-Amyrin, β-Amyrin, Shoreaphenol, Hopeaphenol, Friedelin, β-Sitosterol, Dihydroxyisoflavone, Asiatic acid, Benthamic acid and Uvaol [47, 48] have already been isolated from different parts of the plant. Preliminary phytochemical analysis of plant from other parts of India revealed the presence of leucoanthocyanidin, hopeaphenol, triterpenoids and a terpene alcohol, furfural, monomethylether, dimethylether of homocatechol, alkybenzene derivatives, pentosans, lignan, tannin, amino acids, fatty acids, triterpenoids, ellagic, chebulinic, gallic, phenolic and shorbic acids [49].

However, the GC-MS analysis of same plant from Jharkhand revealed several new phytochemicals which are pharmaceutically and industrially important, eg. Trimethylsilyl 3-methyl-4-[(trimethylsilyl) oxy] benzoate, D-Mannitol, Sorbital, Phytol, Hexamethyl cyclotrisiloxane, etc. Moreover, these phytochemicals were detected in a very high concentration indicating that the plant parts could be rich sources of good harvest.

DPPH Radical Scavenging activities:

The scavenging activity on DPPH radicals was used to determine the free radical-scavenging activity of different parts of the sacred tree. The results are presented in the form a chart (**Chart 2**). The reduced antioxidant became pale yellowish in colour, which was used to evaluate the antioxidant activity of bark, leaf, flower and seed of *S. robusta*. It was found all the parts of the sacred tree consist of very high free radical scavenging activities. The concentrations of 100-500 µg/ml exhibited higher free radical scavenging activity than the control (Ascorbic acid). Moreover, the bark was found to possess higher antioxidant activity than the other parts of the sacred tree. Jeyadoss *et al.* (2014) [50] and Nethaji *et al.* (2014) [51] also determined the high antioxidant capacity of *S. robusta* leaf. The whole plant free radical-scavenging activity is being reported for the first time.

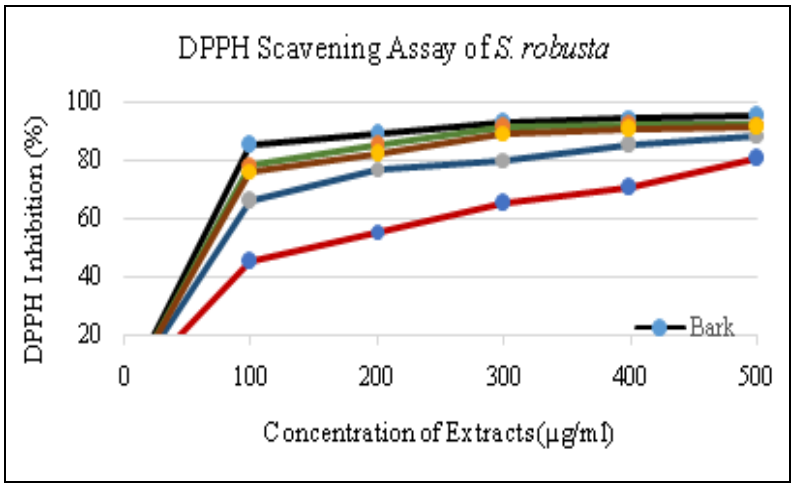


CHART 2: DPPH FREE RADICAL SCAVENGING ACTIVITY OF *S. ROBUSTA*

CONCLUSION: It is first time report of the whole plant study with various scientific parameters. The results of phytochemical tests, antibacterial screening and antioxidant assay are supportive of the usage of the sacred tree to heal various ailments among different ethnic groups of Jharkhand. The antibacterial results are evident that the different parts of the tree has curative power against diarrhoea, dysentery, diabetes, burning sensation, indigestion, skin allergies, expectorant, diuretic, gonorrhoea, bleeding piles, bronchitis, leucorrhoea, menorrhagia and enlargement of the spleen. Preliminary phytochemical screening exhibited the presence of very high concentration of bioactive components such as alkaloids, glycosides, phenols, tannin, steroids and terpenoids which contribute to high antibacterial activities. The HPLC and GC-MS analysis determined the presence of very high concentrations of several phytochemicals such as Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy] benzoate, D-Mannitol, Sorbital, Phytol, Hexamethylcyclotrisiloxane, β-Caryophyllene, 1,2,4-Benzenetriol, etc. which are pharmaceutically and industrially very important. Moreover, the phytochemical, Trimethylsilyl 3-methyl-4-[(trimethylsilyl) oxy] benzoate, found in a very

high concentration in leaf of the *S. robusta*, has been determined to be anti-cancerous and a powerful antioxidant. This could open a new pathway for further research. Hence, the sacred tree of Jharkhand could be a real blessing for the world in order to harvest several phytochemicals in large quantities and to produce drugs at the low cost to heal several human ailments.

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