EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 6
166-172
Year: 2015

PHYTOCHEMICAL, ANTIBACTERIAL AND ANTIFUNGAL STUDIES OF BARK EXTRACTS OF *BOSWELLIA SERRATA* ROXB. AND *SOYMIDA FEBRIFUGA* (ROXB.) JUSS. FROM JHARKHAND

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Article Received on 24/07/2015 **Article Revised on 14/08/2015** **Article Accepted on 08/09/2015**

ABSTRACT
Phytochemical screening of ethanolic, chloroform and aqueous extracts of the barks of *Boswellia serrata* Roxb. and *Soymida febrifuga* (Roxb.) Juss. revealed that the bark of *S. febrifuga* contain higher concentration of secondary metabolites. The HPLC and GC-MS studies of the ethanolic extract of *B. serrata* recorded two bioactive components. They are – 3,7,11-Trimethyl-14-(1-methylethyl) -[S-(E,Z,E,E)]- 1,3,6,10-cyclotetradecatetraene and 4-Methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-cycloheptane. The ethanolic bark extract of *S. febrifuga* recorded twelve bioactive components. The components with higher concentration are Diethylbis (trimethylsilyl) Silicic acid, 1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethyl amide, Tris(trimethylsilyl) arsenite, Catechol and 5,5-Diethyl-3-heptyne. The greater antibacterial and antifungal activities of the bark of *S. febrifuga* are due to greater number of bioactive compounds contained in it. The bark of *S. febrifuga* could be a good source of noble drugs for the treatment of arthritis and gynaecological infections, disorders and diseases.

KEYWORDS: *Boswellia serrata*, *Soymida febrifuga*, Phytochemicals, HPLC, GC-MS, Antibacterial, Antifungal, Latehar, Jharkhand.

INTRODUCTION
Boswellia serrata Roxb., locally called as "Salhai" or "Salaiya" and *Soymida febrifuga* (Roxb.) Juss., locally called as "Ruhin" or "Rohna" are highly exploited medicinal plants which are commonly found in the hills of Chotanagapur plateau of Jharkhand. According to the informants, the tribal *vaidhyas* use the bark of *B. serrata* as powder or decoction to treat gastric problems, arthritis and inflammations, while the bark of *S. febrifuga* is used for several treatments – decoction for uterus cleaning after delivery and white leucorrhoea; bark powder with honey for haemorrhage and excess menstruation; bark infusion for dysentery and intermittent fever; bark tea with black salt for constipation. The bark boiled water is applied on the body of small babies to treat fever. The tribals of Gujarat too, use the bark decoction of *S. febrifuga* for the management of leucorrhoea, menorrhagia and dysmenorrhea.[1] Several workers have reported various uses of the barks of *B. serrata* and *S. febrifuga*. Traditionally, the oleo-gum-resin of *B. serrata* has been used for a variety of therapeutic purposes such as diarrhoea, dysentery, ring-worm, boils, fevers, skin and blood diseases, cardiovascular diseases, vaginal discharges, hair loss, jaundice, haemorrhoids, syphilis, irregular menses and as liver stimulant. The modern Ayurvedic medicine has found the usage of gum-resin in arthritis, inflammation, pain-reliever, hepatoprotection, cancer, inflammation, asthma, psoriasis, colitis and hyperlipidaemia.[2-5]

The decoction of the bark S. *febrifuga* has been reported as a potent therapy for vaginal infections, rheumatism, stomach-ache, dental diseases, uterine bleeding, haemorrhage, anticancer agent[6], leucorrhoea, menorrhagia, dysmenorrhea, rheumatic inflammations and oedema.[7-9] Gum resin possesses significant activities of analgesic, reducing WBC count in joint fluid, and restoring damaged blood vessels.[10-11] Taxonomically, *Boswellia serrata* Roxb. ex Colebr. belonging to family Burseraceae, is a deciduous tree lavishly full-fledged in dry undulating parts of India.[12] It is a medium or large sized tree possessing thin bark whose surface is greenish, grey or yellowish and the blaze pinkish or red exuding resin from the cracks. The bark exfoliates into thin, papery, smooth flakes.[13] While, *Soymida febrifuga* A. Juss. belongs to family Meliaceae, which is a lofty deciduous tree found on dry stony hills and on laterite soil endemic to India. The

heartwood is dark blood red to reddish brown while the sapwood whitish. The bark is reddish brown possessing astringent and antiperiodic properties.[14] The bark exudes bitter resin which has therapeutic activity against vaginal infections and uterine diseases. The literature reviews indicate that several studies have been done on the gum resins of *B. serrata* and *S. febrifuga* reporting several similarities in their therapeutic usages. Therefore, the present work deals with the comparative studies of the barks of both the medicinal trees.

MATERIALS AND METHODS

Collection of plant materials

The specimens of *B. serrata* and *S. febrifuga* were collected from Putrungi hills of Mahuadanr and Chhipadohar jungles of Latehar district, Jharkhand, India. The voucher specimens were duly processed and deposited in the Rapinat Herbarium of St. Joesph's College, Trichy, Tamilnadu, India whose accession numbers are RHT 66393 and RHT 66386 respectively. The barks of *B. serrata* and *S. febrifuga* were collected in April 2015.

Extraction of plant material

The barks were washed and dried under shade over a period of two weeks. They were chopped into small pieces and powdered mechanically. 10g of each of the powders were extracted in 100ml of ethanol, chloroform and distilled water. The extraction was carried out in a rotary shaker for 72 hrs after which the extracts were concentrated and dried to powdered form. The dried extracts were weighed and dissolved in 10ml of respective solvents and kept in sterile specimen bottles for further analyses.

Preliminary phytochemical investigations

The behaviour of powders of the barks of *B. serrata* and *S. febrifuga* and their basic phytochemical investigations were carried out by adopting the standard methods.[15-17] The bioactive secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, saponins, proteins, phenols, glycosides and carbohydrates were screened according to the prescribed methods for phytochemical investigations.[18-24]

Antimicrobial activities

a) Antibacterial studies

The thirteen bacterial strains consisting of four G^{+ve} and nine G^{-ve} were used in this study. They are *Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella paratyphi, Serratia marcescens, Staphylococcus aureus, Streptococcus pneumoniae* and *Vibrio cholerae*. The disc diffusion method was used for antibacterial studies in Nutrient agar medium. The sterilized discs of 6mm diameter were impregnated with 200µg/disc of the bark extracts. The antibiotic Streptomycin (200µg/disc) was used as the control.

b) Antifungal studies

Four fungal species were used for antifungal studies in Potato Dextrose agar medium. They are *Aspergillus niger, Aspergillus flavus, Aspergillus lacticoffeatus* and *Mucor indicus*. The well diffusion method was adopted for this study with the size of well as 6mm. The concentration of the extracts was 200µg/well while that of the control 100µl/well.

High Performance Liquid Chromatography (HPLC)

HPLC is a popular method for the analysis of herbal medicines because of its accuracy, preciseness and un-limitedness by the volatility or stability of the sample compounds.[25] For the HPLC studies, the following conditions were applied - 2ml of extract was filtered through 0.2µm filter and 10µl was injected into the Shimadzu HPLC equipped with auto-sampler and diode array detector. The solvents used for gradient elution were Acetonitrile and HPLC grade water. The HPLC analyses were directly performed on ethanolic extracts of barks of *B. serrata* and *S. febrifuga*. The samples were run for 30 minutes and the chromatograms were obtained at 254nm.

Gas Chromatography—Mass Spectroscopy (GC-MS)

The ethanolic extracts was subjected to GC-MS analysis on GC-MS Shimadzu instrument with following conditions - 4.0 µl of sample was injected for analysis while the flow rate of helium gas was set to 1.5 ml/min. The samples were run for about 45 minutes and the mass spectra were recorded for the mass range 40-1000 m/z. Identification of compounds were based on comparison of their mass spectra. Interpretation of mass spectrum GC-MS was done using the database of National Institute Standard and Technology (NIST) research library. The spectrum of unknown compound was compared with the spectrum 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[26] and ChemSpider.[27]

Statistical Analysis

The antibacterial experiments were carried out in triplicates for 13 pathogenic bacterial species. The antifungal experiments were carried out in six replicates for four pathogenic fungi. The results are given as the mean ± standard deviation. The Mean value and the Standard deviation (S) were calculated by the formula: - Mean = Sum of x values / n, where n = number of values; Standard deviation =

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

RESULTS AND DISCUSSION

Phytochemical Screening

The bark powders of *B. serrata* and *S. febrifuga* were treated with different chemical reagents and the behaviours of the powders were observed based on

which the inferences were drawn for the presence of the phytochemicals (Table 1). The powder studies indicated the presence of phenols, tannins, proteins, anthraquinone and flavonoids in both the barks of *B. serrata* and *S. febrifuga*. However, alkaloids were found to be present only in the bark of *B. serrata* and Quinones were found to be present only in the bark of *S. febrifuga*.

Table 1: Behaviour of bark powders of *B. serrata* and *S. febrifuga* with different chemical reagents

S. N.	Chemical tests	*Bark of B. serrata*		*Bark of S. febrifuga*	
		Observation	**Inference**	**Observation**	**Inference**
1	Powder + Conc. HCl	Brown colour	Leucoanthycyanins & Quinone absent	Yellow ppt.	Quinone present
2	Powder + Conc. H_2SO_4	Reddish brown colour	Steroids present	Reddish brown colour	Steroids present
3	Powder + Conc. HNO_3	Yellow colour	Proteins present	Yellow colour	Proteins present
4	Powder + Picric acid	Yellow colour	Alkaloids present	Golden colour	Alkaloids absent
5	Powder + Aq. $FeCl_3$	Greenish-black colour	Phenols & tannnis present	Bluish-black colour	Phenols & tannnis present
6	Powder + I_2 solution	Pale brown	Starch absent	Pale brown colour	Starch absent
7	Powder + NH_3 solution	Deep blood red colour	Athraquinones present	Deep blood red colour	Athraquinone present
8	Powder + Aq. KOH	Yellow colour	Athraquinones present	Yellow colour	Athraquinone present
9	Powder + Aq. NaOH	Yellow colour	Flavonoids present	Yellow colour	Flavonoids present

Preliminary phytochemical screening of the ethanolic, chloroform and aqueous extracts of barks of *B. serrata* and *S. febrifuga* were carried out. The extracts were diluted in the ratio of 1:1 and were tested for the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, steroids, tannins, saponins etc. The results are given in Table 2. The data in the table indicate that the ethanolic and aqueous extracts contain most of bioactive phytochemicals. Moreover, the aqueous extracts were found to be contain the higher concentrations of phytochemicals. The bark of *S. febrifuga* was found to possess higher concentrations of bioactive compounds than the bark of *B. serrata*.

Table 2: Phytochemical screening of bark extracts of *B. serrata* and *S. febrifuga*

	Plant part	**Bark of *B. serrata***			**Bark of *S. febrifuga***		
S.N.	**Phytochemicals**	Ethanol	Chloroform	Aqueous	Ethanol	Chloroform	Aqueous
1	Alkaloids	+++	–	+++	+	–	+
2	Carbohydrates	++	–	+++	++	–	+++
3	Flavonoids	++	+++	++	+++	+	+
4	Anthral glycoside	–	–	+	–	+	+
5	Cardiac glycoside	+++	++	++	+++	++	++
6	Phenols	++++	–	+++	++	–	++
7	Proteins	+	–	–	–	–	++
8	Amino acids	–	–	–	–	–	–
9	Saponins	++	–	+	++	–	++
10	Steroids	+++	+	+	++	++	+
11	Tannins	++++	–	+++	+++	–	+++
12	Terpenoids	+++	+	+	+++	+	++
13	Anthraquinone	++	–	++	+++	+	++
14	Anthocyannin	–	–	–	–	–	–
15	Leucoanthocyannin	+	–	–	++	–	–
16	Plobatannin	–	–	–	–	–	–
17	Emodin	++	–	++	+++	+	++
18	Coumarin	–	+	–	–	–	–
19	Quinone	–	–	–	++	–	++

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

Antibacterial activities

The antibacterial activities of the ethanolic, chloroform and aqueous bark extracts of *B. serrata* and *S. febrifuga* were tested against four Gram positive bacteria and nine Gram negative bacteria using Streptomycin as control. The results are given in Table 3. The study reveals that both the barks possess potential antibacterial activities. The bark of *S. febrifuga* was found to be more potent than bark of *B. serrata* in antibacterial activities. The aqueous extracts of both the barks showed higher inhibition zones than the ethanolic extracts. Chloroform extracts did not exhibit any inhibition zone.

Table 3: Antibacterial study of bark extracts of *B. serrata* and *S. febrifuga.*

S.N.	Bacterial species	Bark of *B. serrata*			Bark of *S. febrifuga*			Control (10mg/ml) (in mm)
		Zone of inhibition in mm			*Zone of inhibition in mm*			
		EthOH	Chloro	Aqua	EthOH	Chloro	Aqua	
1	*Bacillus cereus*	13±2	–	14±2	14±1.5	–	14±0.5	20.6±1.15
2	*Bacillus subtilis*	13±1	–	13±0.5	17±1.5	–	15±0.5	23.0±2.6
3	*Enterobacter aerogenes*	12±1	–	11±1	14±1	–	14±0.5	21.6±2.8
4	*Escherichia coli*	12±0.5	–	15±1	17±1.5	–	16±0.5	24.0±1.7
5	*Klebsiella pneumoniae*	11±1	–	12±0.5	14±0.5	–	14±1	25.0±0
6	*Proteus mirabilis*	11±1	–	11±0.5	15±1	–	15±0.5	21.6±1.5
7	*Proteus vulgaris*	12±1	–	11±0.5	16±2	–	14±0.5	25.0±0
8	*Pseudomonas aeruginosa*	13±1	–	12±2	14±1.5	–	14±0.5	25.0±0
9	*Salmonella paratyphi*	14±1	–	13±1	13±2	–	15±0	25.0±0
10	*Serratia marcescens*	0±0	–	10±0.5	0±0	–	11±0.5	25.0±0
11	*Staphylococcus aureus*	9±2	–	11±1	13±1	–	15±0.5	22.6±2.5
12	*Streptococous pneumoniae*	14±1	–	12±2	16±1.5	–	14±0.5	24.0±1.7
13	*Vibrio cholerae*	12±1.5	–	12±0.5	17±1.5	–	15±1	21.6±2.8

Data given are Mean of triplicates ± Standard Deviation, – indicates no activity, concentration 200µg/100µl, EthOH=Ethanolic extract, Chloro=Chloroform extract, Aqua=Aqueous extract.

Antifungal activities

The antifungal activities of ethanolic bark extracts of *B. serrata* and *S. febrifuga* were carried out against four pathogenic fungi, namely *Aspergillus niger, Aspergillus flavus, Aspergillus lacticoffeatus and Mucor indicus.* The results are given in Table 4. The bark of *S. febrifuga* exhibited higher antifungal activities than the bark of *B. serrata.*

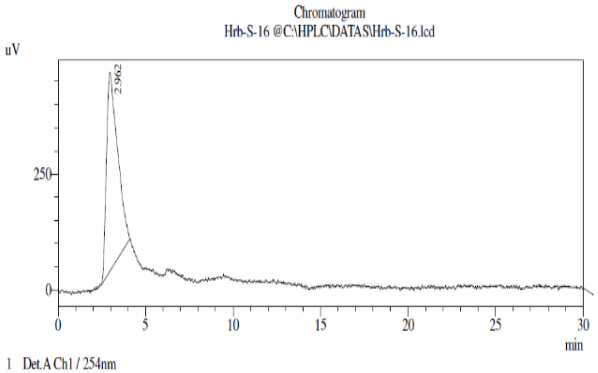
Table 4: Antifungal activities of ethanolic extracts of barks of *B. serrata* and *S. febrifuga*

S. N.	Fungal Species	Bark of *B. serrata*	Control (Ethanol)	Bark of *S. febrifuga*	Control (Ethanol)
		Zone of inhibition in mm		*Zone of inhibition in mm*	
1	*Aspergillus niger*	1.21±0.07	1.0±0.06	1.16±0.27	0.93±0.13
2	*Aspergillus flavus*	1.46±0.45	1.53±0.49	1.15±0.10	1.53±0.18
3	*Aspergillus lacticoffeatus*	0.66±0.12	0.41±0.11	1.60±0.10	0.21±0.19
4	*Mucor indicus*	0.73±0.05	–	1.01±0.07	–

Data given are Mean of six replicates ± Standard Deviation, – indicates no activity

HPLC Analysis

The HPLC studies of the bark of *B. serrata* produced only one peak with retention time 2.962 with an area of 100% revealing high concentration of one phytochemical in the bark which is shown in Fig. 1. The HPLC studies of the bark *S. febrifuga* gave two peaks with retention time 2.946 and 9.919 respectively shown in Fig. 2.

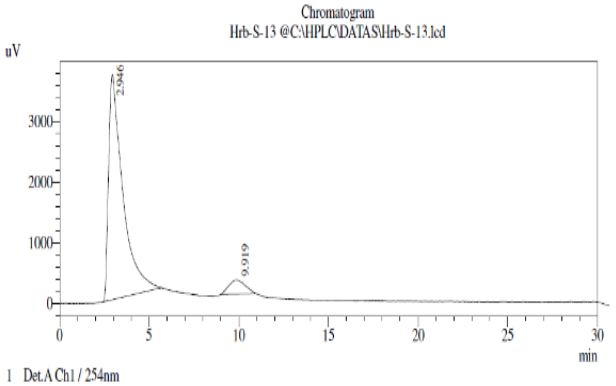


PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.962	18822	428	100.000	100.000
Total		18822	428	100.000	100.000

Fig. 1: HPLC Chromatogram of bark of *B. serrata*



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.946	205573	3720	93.276	94.196
2	9.919	14819	229	6.724	5.804
Total		220392	3949	100.000	100.000

Fig. 2: HPLC Chromatogram of bark of *S. febrifuga*

GC-MS Analysis

The bioactive compounds found through GC-MS studies of the ethanolic bark extracts of *B. serrata* and *S. febrifuga* are presented in Table 5-6. Well known Boswellic acid was not found in the ethanolic extract of

B. serrata whereas 4-Methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-cycloheptane was found to be in very high concentration. The ethanolic bark extract of *S. febrifuga* possess several bioactive compounds in higher amount. They are Catechol, 5, 5-Diethyl-3-heptyne, Diethylbis(trimethylsilyl)Silicic acid, 1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethyl amide and 1-Ethoxy-3-methyl-4-nitrobenzene.

Table 5: Compounds detected in the bark extract of *B. serrata* using GC-MS analysis.

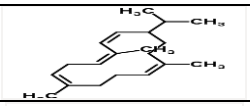
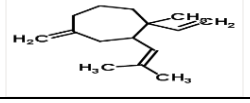
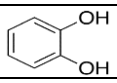
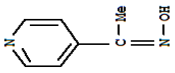
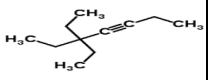
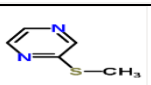
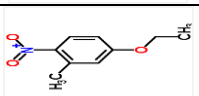
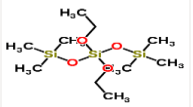
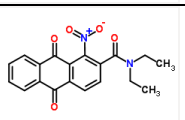
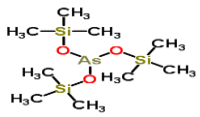
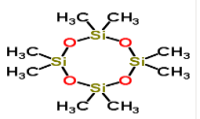
Peak No.	RT	Compound name	*Molecular formula*	*Molecular weight*	*Molecular structure*	*Area %*
1	26.770	3,7,11-trimethyl-14-(1-methylethyl) - [S-(E,Z,E,E)]-1,3,6,10-Cyclotetradecatetraene,	$C_{20}H_{32}$	272.46		9.76
2	27.016	4-Methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-cycloheptane	$C_{15}H_{24}$	204.35		90.24

Table 6: Compounds detected in the bark extract of *S. febrifuga* using GC-MS analysis.

Peak No.	RT	Compound name	*Molecular formula*	*Molecular weight*	*Molecular structure*	*Area %*
1	8.168	Catechol	$C_6H_6O_2$	110.1		23.09
2	13.896	Ethanone, 1-(4-pyridinyl)-, oxime	$C_7H_8N_2O$	136.15		4.44
3	17.392	5,5-Diethyl-3-heptyne	$C_{11}H_{20}$	152.27		14.09
4	19.074	2-Methylthio pyrazine	$C_5H_6N_2S$	126.17		7.09
5	21.437	1-Ethoxy-3-methyl-4-nitrobenzene	$C_9H_{11}NO_3$	181.18		6.18
6	23.337	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42		1.47
7	26.547	N-Cyclooctylidene cyclohexanamine	$C_{14}H_{25}N$	207.35		0.98
8	28.910	(1S,6R,9S)-5,5,9,10-Tetramethyltricyclo[7.3.0.0(1,6)]dodec-10(11)-ene	-	-	-	0.65
9 11 13	33.562 37.905 40.114	Diethylbis(trimethylsilyl) Silicic acid	$C_{10}H_{28}O_4Si_3$	296.58		25.73 3.22 3.09
10	36.698	1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethyl amide	$C_{19}H_{16}N_2O_5$	352.34		3.83
12	38.311	Tris(trimethylsilyl) arsenite	$C_9H_{27}AsO_3Si_3$	342.48		5.14
14	44.108	Octamethyl-cyclotetrasiloxane	$C_8H_{24}O_4Si_4$	296.61		1.06

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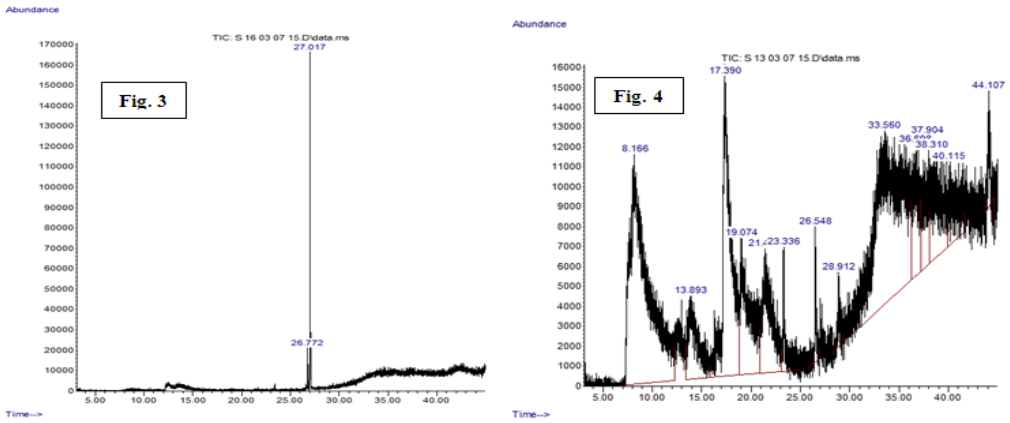
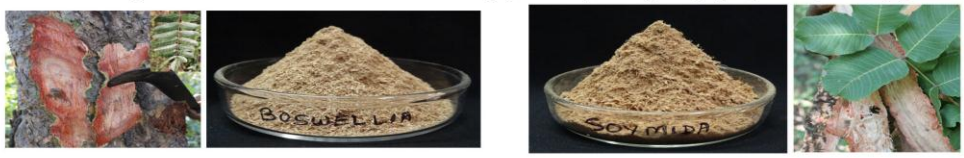
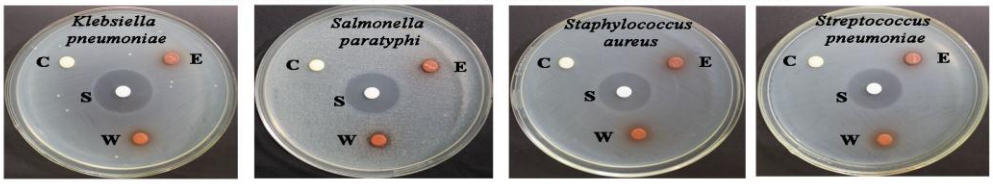


Fig. 3 & 4: GC-MS Chromatogram of bark extracts of *B. serrata* (Left) and *S. febrifuga* (Right)

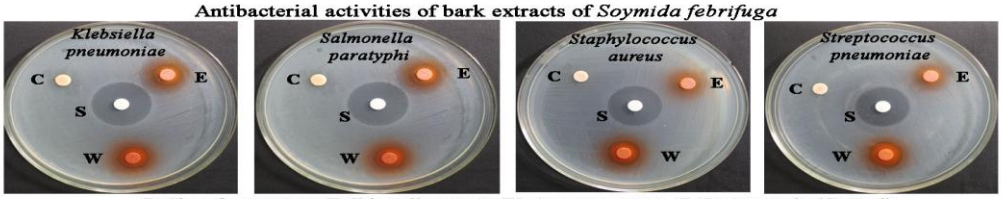
Bark powders of *Boswellia serrata* Roxb. (L) and *Soymida febrifuga* (Roxb.) Juss. (R)



Antibacterial activities of bark extracts of *Boswellia serrata*

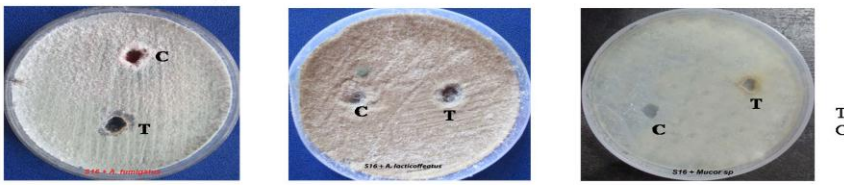


Antibacterial activities of bark extracts of *Soymida febrifuga*



C=Choroform extract, E=Ethanolic extract, W=Aqueous extract, S=Streptomycin (Control)

Antifungal activities of bark extracts of ***Boswellia serrata***



Antifungal activities of bark extracts of ***Soymida febrifuga***

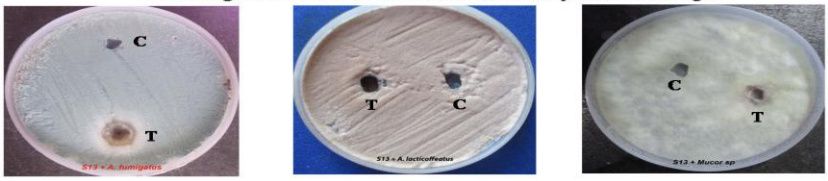


Fig. 5: Comparative study of Barks of *B. serrarta* Roxb. and *Soymida febrifuga* (Roxb.) Juss.

CONCLUSION

Preliminary phytochemical screening revealed that the bark of *S. febrifuga* contain higher concentration of secondary metabolites. The HPLC and GC-MS studies also exhibited the presence of higher concentration of bioactive components in the bark of *S. febrifuga* than that of *B. serrata*, which contribute to the higher antibacterial and antifungal activities. The aqueous extract of barks of

B. serrata and *S. febrifuga* were found to possess higher antimicrobial activities than that of the ethanolic extracts. Moreover, the bark of *S. febrifuga* was found to possess greater kinds of bioactive compounds which could give a lead to further research for noble drugs for the treatment of arthritis, vaginal and uterine disorders and diseases.

ACKNOWLEDGMENTS

The author is grateful to Dr. S. John Britto, the Director and Head of Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Trichy, Tamilnadu, India. He is also grateful to the informants of Jharkhand and to CSIR, New Delhi for supporting through Rajiv Gandhi National Fellowship (RGNF).

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