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## Antimicrobial and Phytochemical Analyses of Bioactive Compounds of *Butea monosperma* (Lam.) Taub. and *Butea superba* Roxb. from Jharkhand

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### ABSTRACT

*Butea monosperma* and *Butea superba* belonging to the family, Fabaceae are most exploited medicinal plants by different tribal groups of Jharkhand. They are commonly found in the hills and jungles of Jharkhand and are used against arthritis, osteoarthritis, diarrhoea, dysentery, snakebite, male sexual debilities, sunstroke, leucorrhoea, anthelmintic and filariasis. Ethanolic extracts of the barks and flowers of both the plants did not exhibit significant antibacterial and antifungal activities. Phytochemical analyses revealed a total of 14 bioactive compounds from the barks and flowers of *B. monosperma* and *B. superba*. Successful management of several diseases among the ethnic groups of Jharkhand, is indicative of presence of curative drugs without toxicity and side effects, and it could further the isolation and purification of active compounds contained in them.

**Keywords:** *Butea monosperma*, *Butea superba*, Phytochemicals, HPLC, GC-MS, Antibacterial, Antifungal, Latehar, Jharkhand

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## INTRODUCTION

*Butea monosperma* (Lam.) Taub. locally called as “Murka (Oraons)”, “Parsa (Sadri)”, “Murud (Mundari)”, “Murut’ (Santali)” and *Butea superba* Roxb. called as “Larang murka (O)”, “Larang parsa (N)”, “Nări murud (M)”, “Nări murut’ (S)” belonging to the family Fabaceae are most exploited medicinal plants by the different tribal groups of Jharkhand. According to the informants, root bark paste of *B. monosperma* is applied on the fractured bone of osteoarthritic patient; stem bark is used against diarrhoea and dysentery, and bark paste with zinger against snakebite. Although the bark is reported to be erectogenic and aphrodisiac<sup>1</sup>, the informants did not report to be using it for the same. Instead, gum-resin is used against the male sexual debilities. Flowers are used for sunstroke, while flower decoction with *misri* is given against leucorrhoea. Seed powder is used as anthelmintic, while the seed paste is applied on the pelvic region to expel a dead foetus from the womb. On the other hand, the decoction of root bark or stem bark of *B. superba* is used with zinger as a cure for filariasis; flowers have the same uses as that of *B. monosperma*. Other communities use the tuber of *B. superba* for purposes of rejuvenating, maintaining sexual performance or prevent erectile dysfunction<sup>2,3</sup>. None of the informants from Jharkhand reported to be using it for the similar purpose. It could be due to the easy availability of other herbal alternatives.

Literature reviews reveal that *B. monosperma* has numerous pharmacological activities such as anthelmintic, anticonceptive, anticonvulsive, antidiabetic, antidiarrhoeal, antiestrogenic and antifertility, anti-inflammatory, antifungal, antibacterial, antistress, anticancer, antioxidant, chemopreventive, haemagglutinating, hepatoprotective, thyroid inhibitory, antiperoxidative, hypoglycemic effects, wound healing activities, anti-giardiasis, antifertility, chemo preventive activities and radical scavenging activities<sup>4,5, 6,7,8</sup>. Likewise, the tuber of *B. superba* is mostly exploited for the promotion of sexual performance under the name “Red Kwao Krua”<sup>1</sup>.

It is evident from the reviews that there has neither been phytochemical nor pharmacological studies on *B. monosperma* and *B. superba* from Jharkhand. For the present study, the barks and flowers were selected because these plant parts are highly used as ethnomedicine among the tribals of Jharkhand.

## MATERIALS AND METHODS

### Collection of plant materials

The specimens of *B. monosperma* and *B. superba* were collected from Chipadohar jungles of Latehar district, Jharkhand, India. The voucher specimens were processed and deposited in the

Rapinat Herbarium of St. Joesph's College, Trichy, Tamilnadu, India whose accession numbers are RHT 66427, 67081 and RHT 66390, 67074 respectively. The barks and flowers of *B. monosperma* and *B. superba* were collected in April 2015.

### **Extraction of plant material**

Barks and flowers of *B. monosperma* and *B. superba* were chosen for the phytochemical and antimicrobial studies as they are mostly used as ethnomedicine in Jharkhand. The plant materials were 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 (90%) and distilled water. The extraction was done in a rotary shaker for 72 hrs after which they were filtered. The filtrates were concentrated and dried to powdered form. The dried extracts were weighed and dissolved in 10ml of respective solvents and kept in specimen bottles for further analyses.

### **Preliminary phytochemical investigations**

The bioactive secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, saponins, proteins, phenols, glycosides and carbohydrates were screened by adopting the standard procedures of phytochemical investigations<sup>9-14</sup>.

### **Antimicrobial activities**

#### **a) Antibacterial studies**

The twelve bacterial strains consisting of four Gram positive and eight Gram negative 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*, *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 and flower extracts. The antibiotic Streptomycin (200µg/disc) was used as positive control. The experiments were carried out in triplicates for 12 pathogenic bacterial species and the mean and standard deviations were calculated using standard formulae.

#### **b) Antifungal studies**

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

water per well. The experiments were carried out in six replicates for four pathogenic fungi and the mean and standard deviations were calculated using standard formulae.

### **High Performance Liquid Chromatography (HPLC)**

The phytochemical detection was done through HPLC analysis. 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, Acetonitrile and HPLC grade water were used for the gradient elution. The HPLC analyses were directly performed on ethanolic extracts of barks and flowers of *B. monosperma* and *B. superba*. The bark and flower samples were run for 30 and 45 minutes respectively and the chromatograms were obtained at 254nm.

### **Gas Chromatography—Mass Spectroscopy (GC-MS)**

The ethanolic extracts of bark and flower of *B. monosperma* and *B. superba* were subjected to GC-MS analysis on GC-MS Shimadzu instrument with following conditions - 4.0µl 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 40 minutes and the mass spectra were recorded for the mass range 0-16000 m/z. Identification of compounds were based on comparison of their mass spectra. Interpretation of mass spectra were done using the database of National Institute Standard and Technology research library. The 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.

## **RESULTS AND DISCUSSION**

### **Phytochemical Screening**

Preliminary phytochemical screening of the ethanolic and aqueous extracts of barks and flowers of *B. monosperma* and *B. superba* were carried out. The extracts were diluted 1:1 ratio in their respective solvents and were tested for the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, steroids, tannins, saponins etc. The results are given in **Table 1**. It is evident from the table that the ethanolic and aqueous extracts contain flavonoids, phenols, steroids, tannins, anthocyanins and cardiac and anthracene glycosides in high concentration. Moreover, free amino acids and reducing sugars were found in high concentration only in aqueous extracts of bark and flower of *B. monosperma* and *B. superba*. Also cardiac glycosides were found to be present in very high concentration in the flowers of both the ethnomedicinal plants. Plant steroids were found in very high concentration only in the flowers of *B. monosperma*.

**Table 1: Phytochemicals screening of bark and flower of *B. monosperma* and *B. superba***

S. N.	Ethnomedicinal Plants → Phytochemicals ↓	<i>Butea monosperma</i>				<i>Butea superba</i>			
		Bark		Flower		Bark		Flower	
		EthOH	Aqua	EthOH	Aqua	EthOH	Aqua	EthOH	Aqua
1	Alkaloids	-	-	-	-	-	+	-	-
2	Reducing sugars	-	++	-	++++	+	+++	-	++++
3	Starch	-	-	-	-	-	-	-	-
4	Flavonoids	-	-	+++	+	++++	+	+++	+
5	Fats & Fixed oils	-	+	-	-	-	-	-	-
6	Glycosides	+++	-	+++	++	+++	+	+++	++
7	Cardiac glycosides	+++	+	+++	++++	-	+	+++	++++
8	Anthral glycosides	++	-	+++	+++	-	+	+++	+++
9	Phenols	+++	-	++	+++	++++	+++	+++	+++
10	Tannins	+++	+	+++	+++	++++	+++	++	+++
11	Proteins	-	+	-	++	-	++	+	++
12	Free Amino Acids	-	++++	-	++++	-	+++	-	++++
13	Saponins	+	-	+	+	-	-	-	-
14	Steroids & Terpenoids	+++	-	++++	++	+++	+	++	++
15	Anthocyanins	+	-	++++	-	+	-	++++	-
16	Anthraquinone	+	-	-	+++	++	+	-	+++
17	Quinones	-	-	+	+++	-	-	+	+++

EthOH-Ethanollic extract, Aqua-Aqueous extract, Very high (++++), high (+++), moderate (++) , low (+) and nil (-)

### Antibacterial Activities

The antibacterial activities of the ethanolic extracts of barks and flowers of *B. monosperma* and *B. superba* were tested against four Gram positive bacteria and eight Gram negative bacteria using Streptomycin as control. The results are given in **Table 2**. The study revealed that the barks of both the medicinal plants possess some antibacterial activities. The flower extracts exhibited relatively low inhibition zone in all the tested microbes. The flowers of both the medicinal plants did not show any activities against *Bacillus* species. Moreover, the bark of *B. superba* had no effect on *V. cholerae*. The results support the ethnomedicinal usage of *B. monosperma* against diarrhoea, dysentery and gastric disorders in rural Jharkhand.

**Table 2: Antibacterial activities of bark and flower ethanolic extracts of *B. monosperma* and *B. superba***

S. N.	Bacterial species ↓	<i>Butea monosperma</i>		<i>Butea superba</i>		Control (10mg/ml) (in mm)
		Bark	Flower	Bark	Flower	
1	<i>Bacillus cereus</i>	-	-	-	-	20.6±1.15
2	<i>Bacillus subtilis</i>	-	-	-	-	23.0±2.6
3	<i>Enterobacter aerogenes</i>	14.40±0.40	12.90±0.36	12.20±0.53	11.70±0.50	21.6±2.8
4	<i>Escherichia coli</i>	13.27±0.25	12.40±0.51	13.93±0.51	12.70±0.60	24.0±1.7
5	<i>Klebsiella pneumoniae</i>	12.30±0.30	11.80±0.29	9.43±0.40	11.90±0.80	25.0±0
6	<i>Proteus mirabilis</i>	9.67±0.59	8.77±0.68	7.90±0.52	9.20±0.50	21.6±1.5

7	<i>Proteus vulgaris</i>	9.40±0.40	8.80±0.30	7.37±0.15	8.90±0.10	25.0±0
8	<i>Pseudomonas aeruginosa</i>	8.47±0.42	8.60±0.50	8.13±0.70	9.30±0.10	25.0±0
9	<i>Salmonella paratyphi</i>	11.30±0.30	8.40±0.50	8.97±0.97	7.30±0.20	25.0±0
10	<i>Staphylococcus aureus</i>	10.40±0.36	8.50±0.70	9.20±0.70	7.60±0.60	22.6±2.5
11	<i>Streptococcus faecalis</i>	11.33±0.42	9.70±0.60	7.87±0.60	10.40±0.40	24.0±1.7
12	<i>Vibrio cholerae</i>	10.50±0.30	8.70±0.65	–	9.20±0.60	21.6±2.8

Data given are Mean of triplicates ± Standard Deviation, – indicates no activity.

### Antifungal Activities

The antifungal activities of ethanolic extracts of barks and flowers of *B. monosperma* and *B. superba* were carried out against four pathogenic fungi, namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus lacticoffeatus* and *Mucor indicus*. The results are given in **Table 4**. All the extracts exhibited maximum inhibition against *A. flavus*. The bark and flower extracts of *B. superba* did not show any inhibition against *M. indicus* and *A. lacticoffeatus* respectively. However, the bark of *B. superba* showed highest antifungal activities against *A. niger* and *A. flavus*.

**Table 3: Antifungal activities of ethanolic extracts of barks of *B. monosperma* and *B. superba***

S. N.	Fungal Species ↓	<i>Butea monosperma</i>		<i>Butea superba</i>		Control (d.H <sub>2</sub> O)
		Zone of inhibition in mm				
		Bark	Flower	Bark	Flower	
1	<i>Aspergillus niger</i>	1.08±0.16	1.07±0.08	1.58±0.08	1.10±0.09	–
2	<i>Aspergillus flavus</i>	1.27±0.23	1.42±0.12	1.62±0.21	1.42±0.12	–
3	<i>Aspergillus lacticoffeatus</i>	1.05±0.10	1.03±0.12	1.28±0.08	–	–
4	<i>Mucor indicus</i>	1.08±0.13	1.07±0.08	–	1.22±0.08	–

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

### HPLC Analysis

The preparatory HPLC analyses of ethanolic extracts of barks and flowers of *B. monosperma* and *B. superba* were carried out whose chromatograms are given in Fig. 1-4. The number of peaks, retention time and area % are given in Tables 4-7. HPLC analysis exhibited more peaks in the bark and flower of *B. monosperma* than that of *B. superba* indicating more number of bioactive compounds. The peak 1 of all the extracts indicated higher concentration of one bioactive compound in both the bark and flower of *B. monosperma* and *B. superba*.

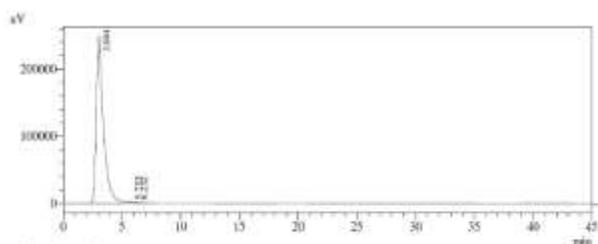


Figure. 1: Chromatogram of *B. monosperma* flower

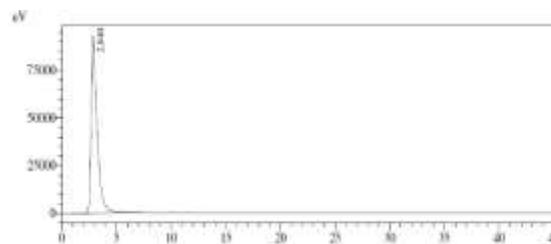
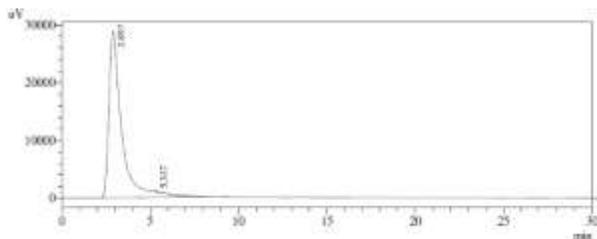
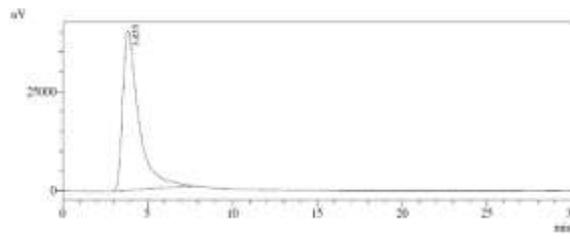


Figure. 2: Chromatogram of *B. superba* flower

Figure. 3: Chromatogram of *B. monosperma* barkFigure. 4: Chromatogram of *B. superba* bark**Table 4: HPLC detection of Flower extract of *B. monosperma***

Peak No.	Ret. Time	Area	Height	Area %	Height %
1	3.044	10713635	249114	99.928	99.872
2	5.733	1864	88	0.017	0.035
3	6.232	5904	230	0.055	0.092

**Table 5: HPLC detection of Bark extract of *B. monosperma***

Peak No.	Ret. Time	Area	Height	Area %	Height %
1	2.897	1456575	28946	99.893	99.768
2	5.317	1558	67	0.107	0.232

**Table 6: HPLC detection of Flower extract of *B. superba***

Peak No.	Ret. Time	Area	Height	Area %	Height %
1	2.840	3471814	93387	100.00	100.00

**Table 7: HPLC detection of Bark extract of *B. superba***

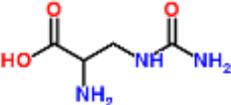
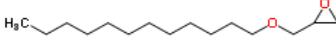
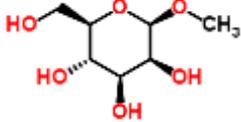
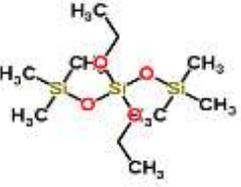
Peak No.	Ret. Time	Area	Height	Area %	Height %
1	3.855	2645434	40264	100.00	100.00

### GC-MS Analysis

The bioactive compounds found through GC-MS analysis of the ethanolic extracts of barks and flowers of *B. monosperma* and *B. superba* are presented in **Tables 8 & 9**. The bark of *B. monosperma* was found to two compounds - 3-[(Aminocarbonyl)amino]- *L*-alanine and 1-(4,5-Dimethoxy-2-nitrophenyl)-4-methylpiperazine of which the latter was consisted of high retention time and area percentage, i.e. 31.31 and 131.98 respectively. The bark of *B. superba* was found to contain three compounds - [(Dodecyloxy) methyl]-Oxirane, Methyl  $\beta$ -*D*-mannopyranoside and Diethyl bis(trimethylsilyl) orthosilicate. The latter two compounds consisted higher retention time and area percentage – 17.31, 187.00; 35.55, 333.82 respectively.

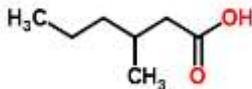
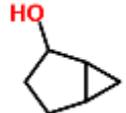
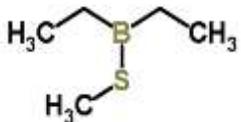
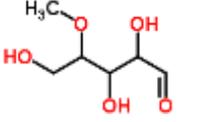
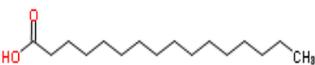
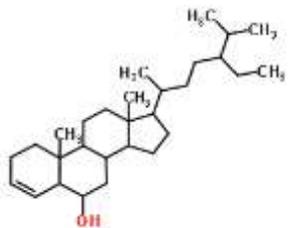
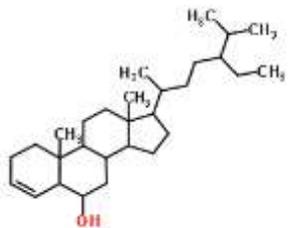
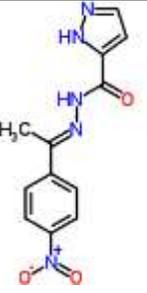
The major compounds in the flowers of *B. monosperma* are 3-Methyl-N-(5-methyl-4,5-dihydro-1,3-thiazol-2-yl)-2-pyridinamine, Tris[dimethyl(2-methyl-2-propanyl)silyl] arsenite, 8-[(3-Ethoxypropyl)amino]-1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione, 2-Chloro-4,6-di(1-piperidinyl)-1,3,5-triazine, 1,1,1,3,5,5,5-Heptamethyltrisiloxane, Anthra[2,1-*d*][1,3]thiazole-2,6,11(3*H*)-trione, 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene and Diethyl bis(trimethylsilyl) orthosilicate, whose structures are given in **Table 9**.

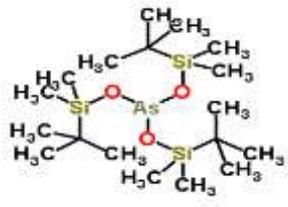
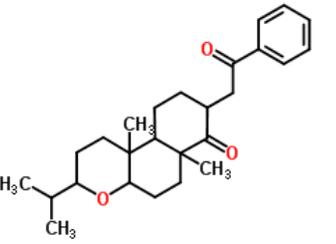
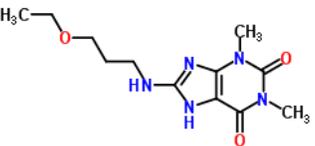
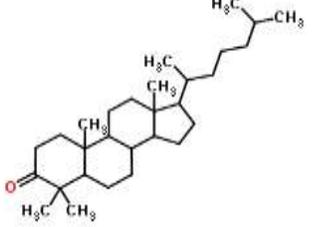
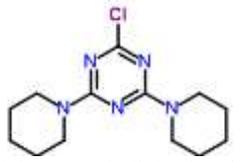
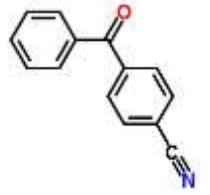
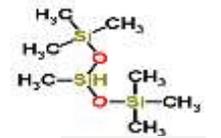
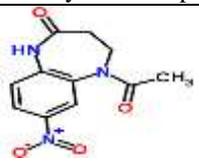
**Table 8: Compounds detected in ethanolic bark extracts of *B. monosperma* and *B. superba* using GC-MS analysis.**

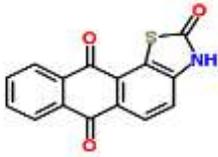
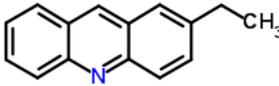
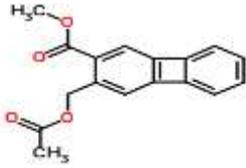
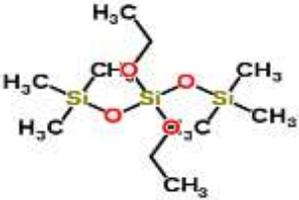
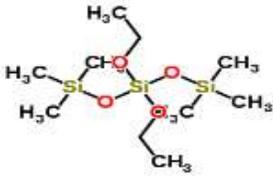
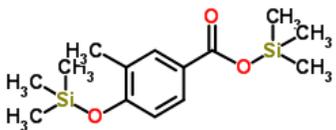
Bark of <i>B. monosperma</i>				Bark of <i>B. superba</i>					
Peak No.	RT	Area %	MF / MW	Structures of Bioactive Compounds	MF / MW	Area %	RT	Peak No.	
1	17.32	31.98	C <sub>4</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub> MW 147.133	 3-[(Aminocarbonyl)amino]- L-Alanine	 [(Dodecyloxy)methyl]-Oxirane	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub> MW 242.40	46.82	12.27	1
2	31.31	131.98	C <sub>13</sub> H <sub>19</sub> N <sub>3</sub> O <sub>4</sub> MW 281.308	 1-(4,5-Dimethoxy-2- nitrophenyl)-4- methylpiperazine	 Methyl β-d-mannopyranoside	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub> MW 194.182	187.00	17.31	2
				 hyl bis(trimethylsilyl) orthosilicate	Diet hyl bis(trimethylsilyl) orthosilicate	C <sub>10</sub> H <sub>28</sub> O <sub>4</sub> Si <sub>3</sub> MW 296.583	333.82	35.55	3

MF = Molecular formula, MW = Molecular weight, RT = Retention time

Table 9: Compounds detected in ethanolic flower extracts of *B. monosperma* and *B. superba* using GC-MS analysis.

Flower of <i>B. monosperma</i>				Flower of <i>B. superba</i>					
Peak No.	RT	Area %	MF / MW	Structures of Bioactive Compounds	MF / MW	Area %	RT	Peak No.	
1	13.45	4.78	C <sub>7</sub> H <sub>14</sub> O <sub>2</sub> 130.185	 3-Methylhexanoic acid	 Bicyclo[3.1.0]hexan-2-ol	C <sub>6</sub> H <sub>10</sub> O 98.143	1.85	13.40	1
2	17.38	17.77	C <sub>5</sub> H <sub>13</sub> BS 116.033	 Diethyl(methylsulfanyl)borane	 4-O-Methyl-d-arabinose	C <sub>6</sub> H <sub>12</sub> O <sub>5</sub> 164.156	4.10	17.48	2
3	23.33	1.93	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> 256.424	 n-Hexadecanoic acid (Palmitic acid)	 Stigmastan-3-en-6-ol	C <sub>29</sub> H <sub>50</sub> O 414.707	2.69	23.33	3
4	26.55	1.47	–	– Borane, 2,3-dimethyl-2-butyl- (dimer)	 Stigmastan-3-en-6-ol		20.70	25.68	4
5	33.44	20.66	C <sub>10</sub> H <sub>13</sub> N <sub>3</sub> S 207.295	 3-Methyl-N-(5-methyl-4,5-dihydro-1,3-thiazol-2-yl)-2-pyridinamine	 Acetophenone, 4-nitro-, 6-pyrazolylcarbonylhydrazone	C <sub>12</sub> H <sub>11</sub> N <sub>5</sub> O <sub>3</sub> –	21.55	27.02	

6	34.52	11.44	$C_{18}H_{45}AsO_3Si_3$ 468.726	 Tris[dimethyl(2-methyl-2-propanyl)silyl] arsenite	 3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2-phenyl-ethyl)-dodecahydro-benzo[f]chromen-7-one	$C_{26}H_{36}O_3$ 396.562	2.68	31.17	6
7	36.06	7.06	$C_{12}H_{19}N_5O_3$ 281.311	 8-[(3-Ethoxypropyl)amino]-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione	 5 $\alpha$ -4,4-Dimethylcholestan-3-one	$C_{29}H_{50}O$ 414.707	4.76	31.78	7
8	36.60	5.59	$C_{13}H_{20}ClN_5$ 281.784	 2-Chloro-4,6-di(1-piperidinyl)-1,3,5-triazine	 Cyanobenzophenone	$C_{14}H_9NO$ 207.227	17.45	34.67	8
9	38.27	8.18	$C_7H_{22}O_2Si_3$ 222.505	 1,1,1,3,5,5,5-Heptamethyltrisiloxane	 5-Acetyl-7-nitro-1,3,4,5-tetrahydro-2H-1,5-benzodiazepin-2-one	$C_{11}H_{11}N_3O_4$ 249.223	12.24	36.11	9

10	38.95	7.98	C <sub>15</sub> H <sub>7</sub> NO <sub>3</sub> S 281.286	 Anthra[2,1-d][1,3]thiazole- 2,6,11(3H)-trione	 Ethylacridine	C <sub>15</sub> H <sub>13</sub> N 207.270	2.32	38.19	10
11	40.81	3.22	C <sub>17</sub> H <sub>14</sub> O <sub>4</sub> 282.291	 2-(Acetoxymethyl)-3- (methoxycarbonyl)biphenylene	 Diethyl bis(trimethylsilyl) orthosilicate	C <sub>10</sub> H <sub>28</sub> O <sub>4</sub> Si <sub>3</sub> 296.583	2.70 0.76 1.36 0.28	38.71 39.73 43.15 44.37	11 12 14 15
12	42.24	9.93	C <sub>10</sub> H <sub>28</sub> O <sub>4</sub> Si <sub>3</sub> 296.583	 Diethyl bis(trimethylsilyl) orthosilicate	 Trimethylsilyl 3-methyl-4- [(trimethylsilyl)oxy]benzoate	C <sub>14</sub> H <sub>24</sub> O <sub>3</sub> Si <sub>2</sub> 296.510	4.56	40.37	13

MF = Molecular formula, MW = Molecular weight, RT = Retention time, – Not available

On the other hand, the major bioactive compounds detected in the flowers of *B. superba* are *Stigmastan-3-en-6-ol*, *Acetophenone*, *4-nitro-*, *6-pyrazolylcarbonylhydrazone*, *3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2-phenyl-ethyl)-dodecahydro-benzo[f]chromen-7-one*, *4,4-Dimethylcholestan-3-one*, (*5α*)-, *Cyanobenzophenone*, *5-Acetyl-7-nitro-1,3,4,5-tetrahydro-2H-1,5-benzodiazepin-2-one*, *Diethyl bis(trimethylsilyl) orthosilicate* and *Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy]benzoate*. The structures of the above compounds are given in **Table 9**.

It was interesting to note that *Diethyl bis(trimethylsilyl) orthosilicate* which was detected in the flower of *B. monosperma*, was also detected in the flower and bark *B. superba*.

The bark, flowers, gum and seeds of *B. monosperma* possess medicinal properties like astringent, antidiarrheal, antidysenteric, febrifuge, aphrodisiac, purgative and anthelmintic, which is not legally restricted and does not require any toxicity precautions<sup>15</sup>. The present phytochemical analyses are supportive of these medicinal properties and also validate the indigenous usage. Flowers of *B. monosperma* are used in diarrhoea, diuretic, depurative and tonic while the bark is used in indigenous medicine for the treatment of dyspepsia, diarrhoea, dysentery, ulcer, sore throat and snake bite which are effectively treated due to the presence of many of the above phytochemicals<sup>4,7,16</sup>.

Due to medicinal properties like enhancing sexual stamina, sensitivity, and sexual performance, the tuber of *B. superba* has been extensively studied<sup>2,3,17,18,19,20</sup>. However, the literature reviews do not report any studies on the bark and flower of *B. superba*. In this regard, the antimicrobial and phytochemical analyses are done first time by us. *Stigmastan-3-en-6-ol* and *5α-4,4-Dimethylcholestan-3-one*, the derivatives of *Stigmasterol*, usually found in the bark of *B. monosperma*, were detected in the flowers of *B. superba*, which may possess thyroid inhibitory, antiperoxidative and hypoglycemic properties<sup>7</sup>.

## CONCLUSION

Phytochemical analyses revealed a total of 14 bioactive compounds from the barks and flowers of both *B. monosperma* and *B. superba*. However, the antibacterial and antifungal activities were quite low when compared to the number of phytochemicals present in them. Successful management of arthritis, osteoarthritis, leucorrhoea, male sexual debilities, filariasis, etc by the ethnic groups of Jharkhand with no side effects and toxicity, is indicative of presence of curative and harmless drugs in the ethnomedicinal plants, especially in *B. superba*. Moreover, it points to do further research for the isolation and purification of active compounds contained in them.

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