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PHARMACOGNOSTICAL, PHYTOCHEMICAL, AND IN VITRO BIOASSAY STUDIES OF OSBECKIA STELLATA BUCH-HAM. LEAVES

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ABSTRACT

Background: *Osbeckia stellata* (Os) is a medicinally significant herb that is consumed for the treatment of various diseases, including skin diseases, diabetes, diarrhea, cancer, asthma, arthritis, dysentery, leukoderma, hypertension, jaundice, malaria, rheumatism, spondylitis, and tuberculosis, as well as inflammation and wound healing. **Methodology:** This study standardizes the plant of Os by accepted practices. Os leaves have been examined physicochemically, phytochemically, microscopically, and morphologically. Extracts were reviewed for both qualitative and quantitative phytochemical examination, and in vitro bioassays were also evaluated. **Results:** Diagnostic traits, such as xylem arteries, trichomes with cover, and anomocytic stomata, were identified in the histological study. Nutritional profiling revealed fiber content (48.1 ± 0.99 mg/100 g). Heavy metal analysis revealed that Pb, Hg, Sn, Sb, Cd, Cu, and As were within the permissible limits. Pesticide residues were verified with ICP-MS analysis. The in vitro antioxidant studies of different extracts show IC_{50} values 1003.35±0.23, 152.11±0.1, 192.12±0.14, 111.79±0.06, and 982.49±0.31 (µg/ml) as compared to standard 130.54±0.03 and 330.86±0.09 (µg/ml). Antimicrobial assay studies show the Zone of Inhibition by different extracts is 26.00 ± 1.20, 17.00 ± 0.60, 18.66 ± 0.58, 22.33 ± 1.52, 6.33 ± 0.58 (mm) as compared to the standard 38.00 ± 1.00, 35.00 ± 1.35, 22.00 ± 1.00, 41.00 ± 1.00, 30.66 ± 1.54(mm). **Discussion:** The methanol extract of Os has total phenols and total tannins of 120.04±5.97 and 123.0±1.52 (mg/g TAE), respectively, which is high in quantity and is reported to possess high antimicrobial and antioxidant properties. **Conclusion:** This study concludes that the quality control parameters for Os are essential for promoting its use in pharmaceutical applications.

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INTRODUCTION

The *Osbeckia* genus was first described by Linnaeus in 1753, in honor of Pehr Osbeck (1723–1805), who brought this plant specimen from China to Sweden. *Osbeckia* is easily identified by its ribbed capsules, bristles, hairs, emergences, and stamens [1]. With roughly 50 species, the genus *Osbeckia* L. belongs to the Melastomataceae family and includes herbs, shrubby plants, and shrubs. West Africa and Asia are home to a large population of the *Osbeckia* plant [2]. Historically, *Osbeckia* species have been used for various therapeutic purposes. In Sri Lanka, Ayurvedic doctors have prescribed for viral hepatitis with *Osbeckia octandra* and *Osbeckia aspera* extracts for viral hepatitis. Additionally, some plant extracts showed hepatoprotective properties [3]. In the China region, doctors utilized *Osbeckia chinensis* for the treatment of inflammatory and pyretic conditions. In *Osbeckia aspera*, phenols were the primary antioxidants [4].

Few studies on *Osbeckia nepalensis* have reported its effect on diabetes. Ethanolic and aqueous extracts have shown maximum antihyperglycemic effect in diabetic rats initiated with alloxan [5]. *Osbeckia stellata* Buch-Ham. ex D. Don (*O. stellata*) is a shrub with upright branches that range in height from 0.5 to 2.5 meters, with 4-angled stems with strigose hairs. The flowers are pink to purple in colour, terminal, with four petals, sepals, 8 stamens with anthers, carpels with an inferior 4-lobed ovary, and the pore is apical on the fruit capsule. The leaves are opposite, entire, subcoriaceous, and strigose, with three to five nerves [6]. This plant grows from August to November at altitudes between 600 and 2300 meters in the Darjeeling district, known by local practitioners as "Sacred Forests" for the treatment of different ailments. *O. stellata* has been used for the treatment of skin diseases, diabetes, diarrhea, cancer, asthma, arthritis, dysentery, leukoderma, hypertension, jaundice, malaria, rheumatism, spondylitis, tuberculosis, and as a toothache remedy [7-8].

The leaves of *O. stellata* were traditionally used by the Apatani tribe in northeastern India to alleviate toothaches [9], and they were also used to heal wounds in Nepal [10]. More than 50% of medicinal plants used in India's herbal businesses and for export are derived from their natural habitat [11]. Herbs have long been utilized and trusted by the indigenous populations of India, particularly those living in the northeast region, which is rich in biodiversity, and have been used to treat a variety of diseases [12]. The taxonomy of *Osbeckia* in India has already been documented in literature; the most common species in this genus, *O. stellata* (Melastomataceae), has a complete chloroplast genome sequence measuring 156,372 bp [7]. Mature leaves of the plant *O. stellata* were collected from the field and tested for the presence of different chemical compounds in the Darjeeling District. The petroleum ether extract demonstrated superior antimicrobial and antioxidant activity [13]. In LPS-treated RAW 264, methanol extracts of *O. stellata* (Os-ME) were used at 99% to reduce NO and PGE2 release [14]. It was reported that an endophytic fungus was isolated from the *O. stellata* plant, which is found in the pine forests of Meghalaya [8].

Despite the studies above, numerous other evaluation parameters remain to be studied for new herbal materials. To use a herb for medicinal purposes, a thorough standardization is required according to WHO guidelines, which is still insufficient for quality control of *O. stellata*. From the traditional knowledge, *O. stellata* is a potent medicinal agent for wound healing activity. Therefore, the goal of the current study is to optimize the methods for aerial portions of the plant by assessing the morphological, microscopical (powder analysis and histology), phytochemical, antioxidant, and antimicrobial activities of *O. stellata* extracts.

MATERIALS AND METHODS

Plant material and processing

O. stellata Buch-Ham. ex D. Don. were collected in August–September (2020) from the Jaintia Hills district, Meghalaya. The plant specimen was identified as *O. stellata* Buch-Ham. (Melastomataceae) at the Botanical Survey of India, Shillong, India, with reference no. BSI/ERC/Tech/2020/1245. For future reference, a herbarium voucher specimen (GIPS/MS/2020/BP-002) was submitted to the Museum (Pharmacognostical Section) of the Girijananda Chowdhury University, Guwahati-781017, Assam. Fresh leaves of *O. stellata* were collected and washed thoroughly under tap water, followed by mechanical tray drying for three days at 37 °C. The crude drugs were pulverized into coarse powders using a grinder machine, sieved through a #60 mesh sieve, and stored inside an airtight container.

Macroscopic and microscopic evaluation

The morphology, as well as the qualitative and quantitative microscopy of the leaves of O. stellata, were examined using standard methods [15-16]. Various morphological parameters, including colour, odour, taste, shape, and size, were evaluated

for the leaves. For further histological evaluation, *O. stellata* sliced leaves were fixed for 24 hours in a formalin solution (5 mL acetic acid, 5 mL formalin, and 90 mL 70% ethyl alcohol). The specimens were removed after a specific period and dehydrated using different concentrations of tertiary-butyl alcohol, followed by fixation in melted paraffin wax blocks. Thin sections obtained from a rotary microtome with a thickness of 5-10 µm were subjected to deparaffinization and stained with a mixture of HCl and phloroglucinol (1:1), dilute iodine solution [17], and monitored under a digital binocular microscope (Model LX-200, Labomed Inc, USA). Stomatal number, vein-islet number, stomatal index, vein termination number, and palisade ratio were the leaf constants evaluated using a quantitative microscopic technique.

Physicochemical evaluation

The air-dried material of *O. stellata* aerial leaves was examined for various physicochemical properties using standard methods described in the Indian Herbal Pharmacopoeia and WHO guidelines [17]. Parameters were evaluated, including ash values, foreign matter, extractive values, loss on drying, foaming index, and swelling index. The data were presented in triplicate to obtain mean ± standard deviation (SD) values.

Fluorescence powdered drug analysis

Fluorescence powder analysis of the *O. stellata* plant material was evaluated based on the standard procedure [18]. Pulverized powdered material of *O. stellata* was treated with a variety of chemical reagents, and the fluorescence pattern was visualized in a UV-Visible chamber cabinet made by Aetron Pvt. Ltd. in Mumbai, India.

Heavy metals and pesticide residue content

A heavy metal assessment of plant powdered material was performed using an Inductively coupled plasma mass spectrometry (ICP-MS) instrument, following the guidelines of the Food Safety Standards Authority of India [19]. Cadmium, arsenic, chromium, lead, copper, mercury, antimony, nickel, and tin were examined as heavy metals with commercially available standard solutions. The *O. stellata* sample was digested with concentrated HNO_3 in a microwave digestion equipment at a temperature of 200°C and a pressure of ≥300 psi [20]. The LC-MS/MS standard method was employed to determine multiple pesticide residues in the O. stellata sample, estimating pesticide residues [21].

PHYTOCHEMICAL EVALUATION

Extraction Procedure

Coarsely powdered plant material of 1 Kg was extracted with a Soxhlet apparatus (2 L) by treating successive solvents (2.5 L) of petroleum ether, chloroform, ethyl acetate, and methanol. An aqueous extract was collected using the maceration method [22]. All five successive extracts, namely OSPE (Osbeckia stellata Petroleum Ether), OSCH (Osbeckia stellata Chloroform), OSEA (Osbeckia stellata Ethyl Acetate), OSME (Osbeckia stellata Methanol Extract), and OSAQ (Osbeckia stellata Aqueous), were separately filtered, evaporated, and kept in desiccators.

Phytochemical Qualification

Qualitative phytochemical screening of the dried *O. stellata* extracts was determined and analyzed by the thin-layer chromatographic technique (TLC) [23]. Application of the sample (10-50µL) to an Aluminum plate coated with silica Gel GF254, 10×10 cm, the stationary phase was operated using both spot application and band application. The TLC chromatogram plates coated with silica were detected under UV-visible cabinet in the UV range of 254 nm and 365 nm, followed by derivatization with various chemical reagents, viz. 1% fast blue salt reagent (for the detection of polyphenolic) and vanillin-H_2SO_4 reagent were used for the detection of triterpenoid and steroidal saponins.

Phytochemical Quantification

The quantity of total phenolic and tannin phytochemical components [25], flavonoid phytochemical components [26], and saponin phytochemical components [27] was measured for all the successive extracts using UV-visible spectrophotometric (Shimadzu-AA6300) methods. The results of the quantitative study were performed in triplicate, and statistical data were evaluated to express the mean ± SD value using standard linear regression. The data obtained were plotted using external standards, namely tannic acid (for total phenolics & tannins), quercetin (for total flavonoids) & diosgenin (for total saponins), against concentration (µg/mL).

***IN-VITRO* ASSAYS**

***In-vitro* antioxidant studies**

Antioxidant studies were conducted on the five different extracts of O. stellata, which were evaluated for their ability to scavenge DPPH radicals [28] at varying concentrations ranging from 20 to 200 µg/mL. Furthermore, the total antioxidant capacity

(phosphomolybdenum method) at a concentration of 100 µg/mL [29] and the reducing power assay at the same concentration [30] were examined for all subsequent extracts. Aliquots with different dilutions 20-200 µg/mL (for DPPH) and 100 µg/mL were prepared (for reducing power assay & total antioxidant assay) from the stock solution with a volume of 1 µg/mL in methanol and standard as ascorbic acid in a volume of 1 µg/mL in methanol. The reaction process was measured in duplicate using a UV-visible spectrophotometer, and the results were evaluated and presented as Mean ± SD. IC_{50} values were found by following the regression equation and plotting the standard calibration curve [31].

In-vitro anti-microbial studies

In vitro assays for microbial studies were evaluated using the agar well diffusion method on Mueller-Hinton Agar (MHA) plates [32]. Plant extracts were prepared at a concentration of 50 µg/ml and dissolved in Dimethyl Sulfoxide (DMSO) solvent. Media plates were bored in four wells using a cork borer (6 mm in diameter), and the bored wells were filled with 50 µL of different plant extracts. Ampicillin, Vancomycin, and Cephalosporin were used at 50 µg/ml as the standard (positive) control for bacterial strains, and DMSO at a 4% concentration was used as the negative control, respectively. All the extracts and standard positive and negative control chemicals were allowed to spread through the wells for 30 minutes at room temperature, and the plates were then incubated for 18-24 hours at 37°C. Incubated Plates with wells were examined for a clear zone after incubation, which indicates the active antimicrobial capacity of the tested extracts. The zone of inhibition (ZOI), or the clear zone remaining near the wells, was measured in millimeters and analyzed.

RESULTS AND DISCUSSION

Pharmacognostical standardization

Morphological and microscopic characters of *O. stellata* leaf

The *O. stellata* plant is a prevalent, erect, branched shrub that grows 0.5-2.5m tall. The stem was observed to be 4-angled and covered with hairs. Leaves were entire, opposite to each other, subcoriaceous, and covered with 3-5 nerves. Flowers were found to be pink to purple in colour, terminal in position, and prominent in Figure 1. Petioles were 1-15 mm in length, lamina present were ovate to elliptic in shape or sometimes narrowly ovate to lanceolate in shape, apex is acute, margins entire, ciliate, and glabrous on both surfaces of the leaf. The leaf upper surface was observed to be dark green in colour, and the lower surface is light green. Odour and taste were characteristic of the plant. The texture was observed as smooth, with a velvet-like feel. From the microscopical examinations of a leaf thin section, the lamina of O. stellata showed the presence of spongy and palisade mesophyll parenchyma on each side. T.S. also has shown epidermal cells, cuticles, vascular bundles, xylem and phloem, trichomes, and palisade cells. Stomatal numbers were observed 22±6/mm², vein-islet numbers were observed 10±4/mm², stomatal indices were observed 30±3 (%), vein termination numbers were observed 7±5/mm², and palisade ratios were observed 8±3/mm² of leaf constants using a quantitative microscopic technique. Powder evaluation of *O. stellata* leaf has revealed the presence of multicellular trichomes, spiral-shaped xylem vessels, concentric starch grains, and fragments of parenchymatous cells **[Figure 1]**.

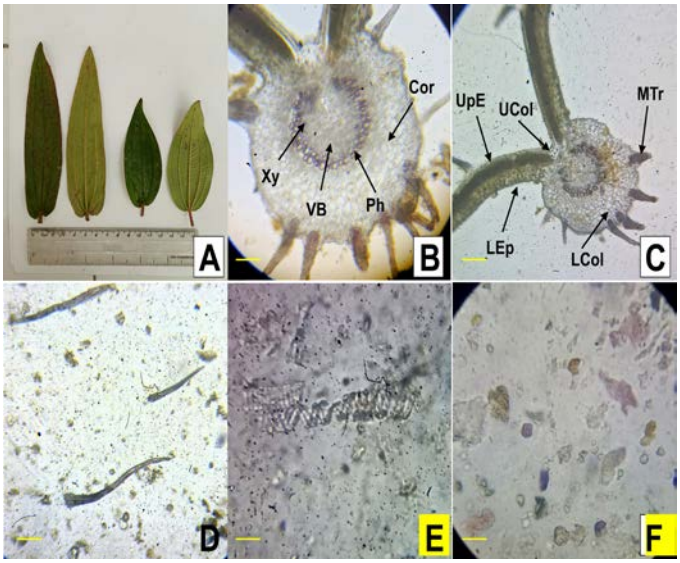


Figure 1: Morphology of Os leaves (A), Scale bars of 100µm (yellow line) in histological (B-C) and microscopic powdered drug (D-F) features of Osbeckia stellata leaf.

UEp- Upper epidermis; LEp-Lower Epidermis; UCol- Upper Collateral cells; LCol- Lower Collateral cells; MTr: Multicellular trichome; Cor- Cortex; VB- Vascular Bundle; Xy- Xylem; Ph- Phloem; D- Multicellular trichromes; E- Xylem element with spiral thickening; F- Starch grains.

Physicochemical evaluations of *O. stellata* leaf

By following the Indian Herbal Pharmacopoeia (2002) and the WHO guidelines (2002), the dried crude drug was found to contain a minimal amount of foreign matter, at 0.22 ± 0.012% w/w, indicating no contamination or unwanted substances. The moisture content of the dried powder was found to be 7.0 ± 0.4% w/w. The amount of inorganic substances expressed by the total

ash value and the total ash can be differentiated into two types of ashes, namely acid-insoluble ash and water-soluble ash. The results of ash values were found to be 11 ± 0.1% w/w, 7.46 ± 0.02% w/w, and 4.73 ± 0.1% w/w, respectively. This test detected the presence of extraneous matter, such as clay and sand [16]. The alcohol and water-soluble extractable matter were reported to be in considerable amounts, representing 17±0.54% w/w and 19±0.27% w/w, respectively [33]. According to the WHO, both the foaming and swelling indices are additional physicochemical indicators for determining the presence of water-soluble substances [17]. Our results revealed that the foaming index of O. stellata was found to be less than 100 cm, specifically 0.4±0.22 cm, indicating that the plant may have a low saponin content. In contrast, the swelling index reported was 4.5 ± 0.23 mL, suggesting the presence of carbohydrates.

Fluorescence powdered drug analysis of *O. stellata* leaf

The fluorescence patterns of the powdered leaf sample of O. stellata are represented in **[Table 1]**. It was observed that when the powder material was exposed to different reagents, it formed a fluorescent pattern, visible both in daylight and under ultraviolet light (254 and 365 nm). Our findings are consistent with previous studies, which indicate that O. stellata exhibits a similar light pattern, suggesting the presence of chlorophyll and other plant pigments, as well as phytoconstituents with active fluorophores [18].

Table 1: Fluorescence analysis of the powdered crude of *O. stellata* leaf

Powder+Reagent	Day light	254 nm	365 nm
	O. Stellata	*O. stellata*	*O. stellata*
Powder as such	Light green	Dark green	Violet
Powder+1N NaOH in H_2O	Light Brown	Dark Brown	Dark Violet
Powder+1N NaOH in MeOH	Light green	Dark green	Orange
Powder+1N HCl in H_2O	Light pink	Light green	Dark Violet
Powder+1N HCl in MeOH	Dark Brown	Dark Brown	Orange
Powder+1N HNo_3 in H_2O	Light pink	Dark green	Light orange
Powder+1N HNo_3 in MeOH	Dark pink	Dark green	Dark orange
Powder+ I_2 (5%)	Dark Brown	Dark green	Black
Powder+ $FeCl_3$ (5%)	Dark green	Black	Dark Violet
Powder+ KOH (50%)	Dark Brown	Dark green	Dark Violet
Powder+ NH_3 (25%)	Orange	Dark green	Dark green
Powder+ Acetic acid	Light green	Light green	Light violet

Fiber content of *O. stellata* leaf

It was observed that the plant has a considerable amount of fiber content, which was found to be 48.2 ± 0.98 mg/g. Many functional foods, herbs & nutraceuticals contain dietary bioactive substances that can treat or even prevent illnesses [34]. Fibers present in plants play a crucial role in determining the nutritional status of plants, and a diet rich in fiber is beneficial for the digestive system [35-36].

Analysis of heavy metals in *O. stellata* leaf

The heavy metals (in ppm) present in the plant extracts of O. stellata were evaluated using Inductively Coupled Plasma Mass Spectrometry, a precise technique that adheres to WHO and FSSAI guidelines [37]. The test was performed at the State Public Health Laboratory in Guwahati, Assam. All the heavy metals analyzed are below the limit of official WHO guidelines [38], except for chromium and nickel, which indicate that O. stellata plant species are safe for consumption as a medicinal aid. Thus, Chromium and nickel were found in high amounts; they have some other health benefits. Chromium metal has been shown to have health benefits in diabetes, glucose tolerance, polycystic ovary syndrome, metabolic syndrome, and dyslipidemia, among other conditions. Nickel metal has several advantages in treating anemia, osteoporosis, nickel deficiency,

and other health conditions [39], as summarized in **[Table 2]**. Pesticide residues were examined in 96 samples, which were found to be within the prescribed limits, with a maximum residual limit (MRL) of 0.01 mg/kg. The study was conducted according to AOAC 2007.01 guidelines and confirmed that the results were below the specified detection limit (SDL), indicating that the plant is free from pesticide contamination.

Table 2: Heavy metal analysis of *O. stellata* leaf

SN	Name of Metals	Method	Metal Content parts per million (mg/kg) *Osbeckia stellata*	Maximum Limit in parts per million as per regulation 2.1.1 (mg/Kg)
1	Arsenic	FSSAI Manual	0.219	1.1
2	Cadmium	-do-	0.127	1.5
3	Chromium	-do-	2.39	1.0
4	Copper	-do-	11.605	30
5	Lead	-do-	2.49	2.5
6	Mercury	-do-	0.213	1.0
7	Nickel	-do-	4.637	1.0
8	Antimony	-do-	0.602	1.0
9	Tin	-do-	0.134	250

Phytochemical evaluation and analytical studies

Preliminary phytochemical screening

Different successive extracts of *O. stellata* were obtained by the Soxhlet extraction method, and the extracts were treated with chemicals for preliminary phytochemical screening. The result was obtained for the extracts of O. stellata leaf. It demonstrated the presence of active phytochemicals, including glycosides, steroids, polyphenolics (phenolic compounds, tannins, and flavonoids), saponins, cardiac glycosides, alkaloids, and carbohydrates. However, no coumarins were found in any of the five successive extracts. All the respective results are shown elaborately in **[Table 3]**.

Quantification of phytochemical classes

Consecutive extracts of *O. stellata* have a significant number of polyphenols. This result demonstrates that the entire plant can be a rich source of polyphenolics, which are naturally present in plants and offer therapeutic benefits [40]. A preliminary test for polyphenols also helps determine the quality and presence of polyphenols in plant extracts. In addition to evaluating the polyphenols, the total quantity of tannin, flavonoid, and saponin content was measured using UV-visible spectrophotometric analysis. Before isolating compounds from plant extracts, it is necessary to recognize that the quantity of active phytoconstituents is a crucial stage in Phytochemistry [41]. Using the UV-Visible spectrophotometer of the Shimadzu-AA6300, the phytochemicals such as phenolic content, flavonoid content, tannin content, and saponin content were estimated and presented in **[Table 4]**. A similar study has already been published for O. stellata, which was collected from the Manipur state, and it found high concentrations of total phenolic components in the methanol extract, at 1266.25±36.11 mg GAE/g, and total flavonoid content in the methanol extract, at 1273.11±46.84 mg QE/g [7].

Table 3: Preliminary Phytochemical Testing of various extracts of *O. stellata* leaf

Parameters	OSPE	OSCH	OSEA	OSME	OSAQ
Carbohydrate					
Molish Test	+	+	+	++	+
Fehling Test	-	-	+	+	-
Benedict Test	-	-	+	++	-
Barfoed's Test	-	-	+	++	+
Iodine Test	-	-	-	-	-
Protein					
Biuret Test	-	-	-	-	+
Millon Test	+	-	+	-	-

Parameters	OSPE	OSCH	OSEA	OSME	OSAQ
Amino acid					
Ninhydrin Test	-	+	-	-	-
Steroid					
Salkowski	-	-	+	-	-
Glycoside					
Cardiac Legal's Test	-	-	-	+	-
Anthraquinone	-	-	++	+	-
Saponin	-	-	-	+	-
Coumarin	-	-	-	-	-
Flavonoids					
Shinoda's Test	-	-	++	+	-
Alkaloid					
Dragendorff	-	-	+	-	-
Mayer Test	-	+	-	-	-
Wagner's Test	-	+	-	-	-
Tannin and Phenolics					
$FeCl_3$	-	-	-	++	-
Dil HNO_3	-	-	-	+	-
Acetic acid					

Table 4: Quantification of Phytoconstituents in various successive extracts of *O. stellata* leaf

Phytochemical classes	OSPE	OSCH	OSEA	OSME	OSAQ
Total flavonoid (mg/g RE)	21.02±0.39	32.0±0.21	76.02±1.76	68.01±2.45	14.08±0.78
Total saponin (mg/g DE)	9.03±0.97	4.02±1.23	10.0±1.32	23.08±1.41	2.90±0.94
Total phenolic (mg/g TAE)	54.05±1.87	76.2±4.54	111.02±3.12	120.04±5.97	45.0±5.32
Total tannin (mg/g TAE)	46.02±0.57	65.09±1.13	109.01±1.09	123.0±1.52	51.03±2.43

TAE: Tannic acid equivalent; RE: Rutin equivalent; DE: Diosgenin equivalent

Thin Layer Chromatography profile

TLC presents consecutive extracts of *O. stellata*, and their corresponding Rf values were calculated [42]. Retention factor or Rf value represents the ratio of migration distance of the sample substance to the migration distance of the sample carrying solvent, which shows specific different compounds separated from the mixture of components. The solvent front used in the TLC process plays the most crucial role in the separation of components. The results of TLC analysis for consecutive extracts of *O. stellata* are shown in **Figure 2**.

***In vitro* antioxidant and anti-microbial studies**

***In vitro* antioxidant studies**

The *O. stellata* plant's OSME extract had the highest total antioxidant capacity, showing the highest reducing ability and the highest scavenging activity compared to standard controls. The observation of the DPPH scavenging capacity and IC_{50} value of all different solvent extracts from *O. stellata* was observed 1003.35±0.23, 152.11±0.1, 192.12±0.14, 111.79±0.06, and 982.49±0.31 (µg/ml) as compared to standard 130.54±0.03 and 330.86±0.09 (µg/ml) [19]. Except for OSME, all of the examined solvent extracts showed a significant result ($p < 0.05$) when compared with the standard ascorbic acid and Quercetin, which is considered a positive control. The standard control findings were compared with different solvent extracts; the OSME extract showed statistically non-significant observations ($p<0.05$), indicating that it may be able to scavenge the DPPH radical. Overall, it was confirmed that among all the different solvent extracts, the methanol solvent extract of the plant O. stellata exhibited the highest antioxidant capacity when statistically compared with the standard ascorbic acid and quercetin. *O. stellata* collected from Manipur state has shown DPPH scavenging activity, with the methanol extract having the lowest IC_{50} of 24.76±2.78 µg/ml [7]. Similarly, Reducing power of different extracts were shown 0.065±0.001, 0.075±0.001, 0.240±0.001, 0.441±0.002, 0.092±0.002 in compare with standard control Ascorbic acid and rutin 2.214±0.252 and 1.325±0.259 (µg/ml) accordingly with concentration of 100µg/ml and total antioxidant assay shows 0.019±0.001, 0.044±0.002, 0.142±0.002, 0.169±0.002, 0.081±0.002 (µg/ml) in compare with standard control ascorbic acid with concentration of 100µg/ml. The results are shown in **[Figure 3]**.

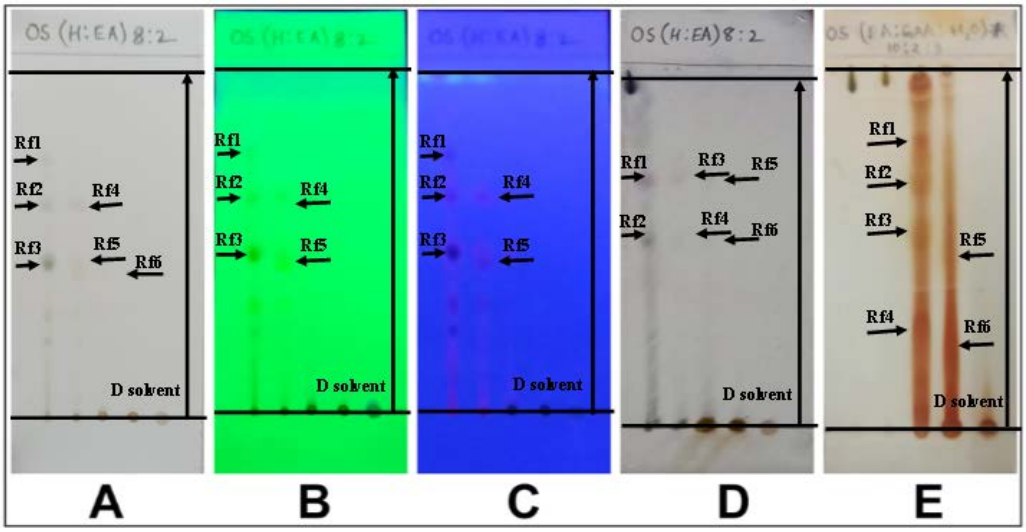
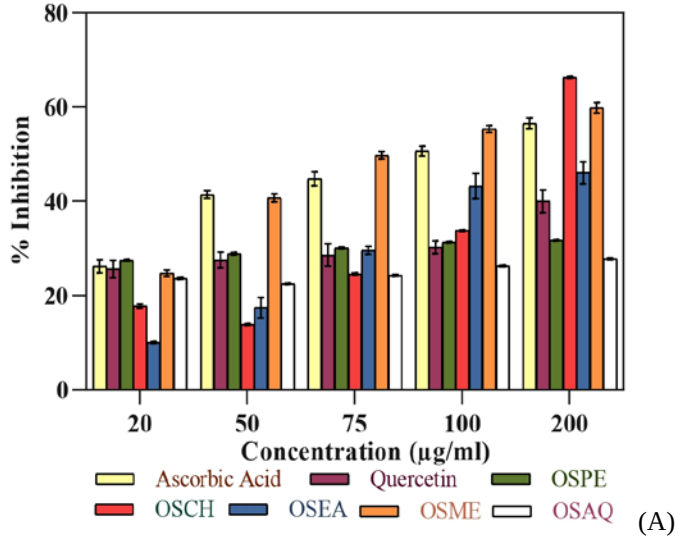
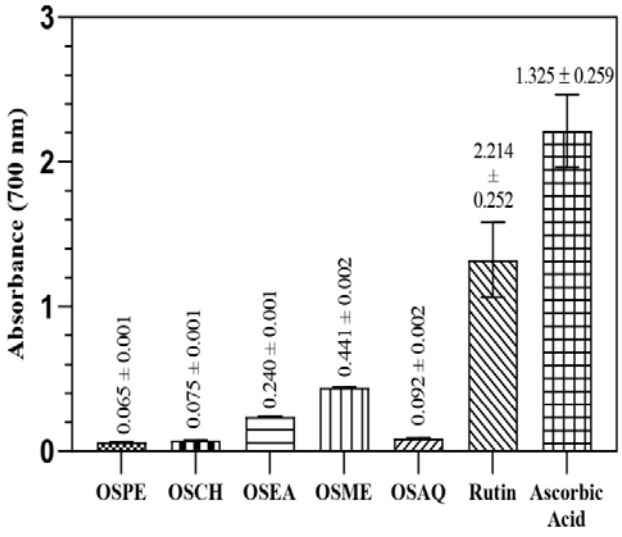


Figure 2: TLC profiling of the five different solvent extracts; visualized under daylight (A); Short UV wavelength under 254 nm (B), and Long UV wavelength under 365 nm(C); Steroidal compound spots in OSPE, OSCH, and OSEA extracts (D); Polyphenolic compound spots in OSEA and OSME extracts (E).

Rf- Retention factor of components, D solvent- Distance travelled by the solvent.



(A)



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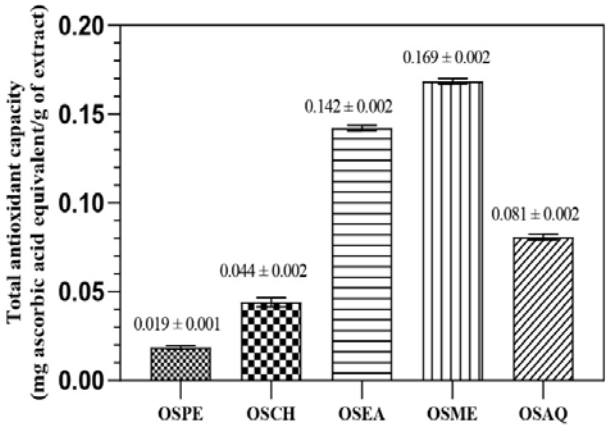

(C)

Figure 3: In vitro antioxidant potential of various extracts of *Osbeckia stellata* leaves with triplicate results (A) DPPH radical scavenging assay of different extracts in comparison with standard control Ascorbic acid and Quercetin (mg/g); (B) Reducing power assay of different extracts in comparison with standard control Ascorbic acid and Rutin (mg/g); (C) Total antioxidant capacity of different extracts in comparison with standard control Ascorbic acid (mg/g).

***In-vitro* antimicrobial studies**

The antimicrobial activity of *O. stellata* leaf extracts is illustrated in [Figure 4], which also displays measurable zones of inhibition produced by the extracts against Gram-positive bacteria, such as Bacillus subtilis & Staphylococcus aureus, and Gram-negative bacteria, including *E. coli* & *P. aeruginosa* [43]. The ZoI of Inhibition of Ampicillin was found to be 27±1.01, as compared with the OSPE (50 µL) extract, and 17±0.9 against *E.*

coli bacteria. As compared to standard antibiotic vancomycin solution with ZoI of 35.00 ± 1.35 mm, OSPE (50 µL) has shown prominent activity against *S. aureus* bacteria with ZoI of 17.00 ± 0.60 mm. Compared to vancomycin (38.00 ± 1.00 mm), the OSEA extract (50 µL) demonstrated excellent antimicrobial activity against *B. subtilis* bacteria with its ZoI capacity of 26.00 ± 1.20 mm. The growth of *P. aeruginosa* was suppressed by OSCH with a ZoI of 22.33±1.52 mm, which was lower than the standard Cephalosporin ZoI (41.00±1.00 mm). In addition, OSME considerably reduced *S. aureus* growth with a ZoI of 18.66±0.58 mm, as compared to vancomycin (22.00±1.00 mm). Similarly, in comparison to standard Ampicillin ZoI (30.66±1.54 mm), OSAQ showed minimal antimicrobial activity against the bacterium E. coli, with a Zone of Inhibition of 6.33 ± 0.58 mm [44].

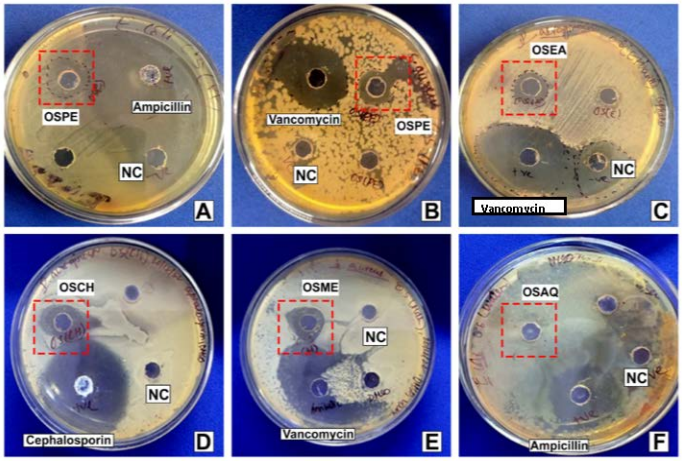

Figure 4: In vitro antimicrobial potential of various extracts of *Osbeckia stellata* leaf at a dosage of 50µL. (A) OSPE against *E. coli*, (B) OSPE against *S. aureus*, (C) OSEA against *B. subtilis*, (D) OSCH against *P. aeruginosa*, (E) OSME against *S. aureus*, (F) OSAQ against *E. coli*. Abbreviations: NC- Negative Control (DMSO 4%), Ampicillin, Vancomycin, and Cephalosporin (50µL) – Positive control.

CONCLUSION

Evaluation of every aspect of a medicinal plant is necessary because it standardizes the essential procedures and ensures the correct validation of the true characters of the crude medicinal plants. The World Health Organization (WHO) reports indicate that the majority of the global population still relies on medicinal plants for their health treatments. This respective study creates a distinctive marker for the *O. stellata* plant, which has shown a 4-angled stem with covering hairs and dark green leaves, presence of xylem, phloem, trichomes and palisade cells, considerable extractive matter and low foaming index, presence of active fluorophores, high fiber content, low limit heavy metals, high flavonoid and polyphenolic contents. Methanolic extract of the plant has a high quantity of phenolics and tannins; spots were observed for ethyl acetate and methanol extracts in the TLC plate. The highest antioxidant activity, and a considerable reduction of *S. aureus* microbe growth with a ZoI of 18.66±0.58 mm, were observed compared to standard vancomycin (22.00±1.00 mm). Therefore, in future studies, the OSME extract can be utilized for the isolation of active phytochemical constituents to identify important compounds with prominent antioxidant and anti-microbial activity. Methanol extract of *O. stellata* has been used for the isolation of flavonoid-rich quercetin compounds, which have been published in a previous paper, which can also be helpful for further isolation studies [14].

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

D. Laloo supervised and guided the whole study of the article above. C. Bordoloi prepared the manuscript and edited the first draft. N. Sharma Bora has drafted the tables and figures of the above article. All the authors have finally approved the article manuscript.

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