Indian Journal of Traditional Knowledge
Vol 21(1), January 2022, pp 55-64

Evaluation of bioavailability of three extracts of a less known ethno-medicinal plant, *Thottea tomentosa* from Assam

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Received 23 January 2020; revised 17 July 2021

Plants of the genus *Thottea* have been established as wild ethno-medicinal plants. The plant, *Thottea tomentosa* also has the illness recuperative property. However, there are no such reports so far on the solvent polarity based performances of the plant. Hence, the current work deals with the assessment of bioavailabilities like qualitative phyto-chemical screening, total phenol contents, total flavonoid contents, antioxidant efficiency, and antimicrobial efficiency using three solvents of diverse polarities like hexane, chloroform, and methanol. The phenolic compositions of methanol extract of stem and leaf; 374.99±3.84 and 260.55±4.00 mg GAE per gram of dry weight of extract respectively and correlate by linking antioxidant activities of methanol extract of the stem and leaf (DPPH; IC_{50} 202.39±0.92 and 254.37±1.47 µg/mL respectively). Thus, probable antimicrobial activities of methanol extract of stem and leaf were also study, than that of other extracts. Methanol stem extract against the microbes; *Bacillus subtilis* (MTCC 1427), *Escherichia coli* (MTCC 1195), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* susp. *aureus* (MTCC 1430), *Streptococcus pneumonie* (MTCC 2672), *Aspergillus niger* (Lab isolates) and *Candida albicans* (MTCC 4748) exhibited highest zone of inhabitation (ZOI). Therefore, these results indicate that *T. tomentosa* can be satisfying the dietetic demand for the food and drug production.

Keywords: Antimicrobial well diffusion activities, Antioxidant properties, Phytochemical profiling, *Thottea tomentosa*, Total polyphenol contents

IPC Code: Int Cl.[22]: A01P 1/00, A61P 17/18, A61P 39/06, C08K 5/107

In current times, medicinal plants get a distinctive importance towards the assessment of novel bioactive agents. Phyto products have produced in the plant cells which are miscellaneous categories of chemicals[1]**.** Plant phenolics (PPs); aromatic benzene ring compounds; stillbenes, flavonoids, lignins, tannins and phenolic acids; are carrying one to more hydroxy group substituent. Effective antioxidant properties prevent oxidative stress associated illnesses, atherosclerosis, arthritis, neurodegenerative disorders, aging, cardiovascular and even carcinogenesis[2]. Numerous research studies have demonstrated that Natural Products (NPs) with most diverse groups of secondary metabolites as natural antimicrobials. PPs is continuously receiving growing attention for microbial resistance towards conventional food preservation and processing, nevertheless, have many additional health benefits[3].

The increase of essential knowledge of antimicrobial research of PPs on pathogenic microorganisms, potential new strategies can be designed to combine the synergy of PPs for antimicrobial agents keeping the existing natural antioxidant benefits. With about 1373 species of the Aristolochiaceae family, 207 (15.1%) are un-assessed, *Thottea tomentosa* (Blume) Ding Hou is out of 624 accepted (45.4%) plant of the family owned by eight plant genera[4]. *T. tomentosa* an undershrub wild plant distributed in subtropical, tropical and temperate regions that reach up to a height of 10-25 cm. Leaves exhibit differently on the same plant, oblong, broadly oblong or heart-shaped. Stem creeping below, rooting then ascending, 15-30 cm. Flower maroon in color, ca 1.2-1.8 cm in diameter, bracts oblong persistent.

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Despite the cosmopolitan distribution of the genus *T. tomentosa* is one of the smallest and most distributed species among the members[5] in India, Bangladesh, Malaysia, Myanmar, Peninsular, Thailand and South Vietnam[6].

Our previous work insights the ethnomedicinal importance of leaf decoction of *T. tomentosa* for the treatment of dysentery by *Jaintia* tribe and stem juice as a tonic by *Barman* tribe of Barak valley, Assam[7,8]. Phytochemical screening unveiled the existence of alkaloids, carbohydrates, saponins, phenolic compounds, terpenoids, flavonoids and fix oils and fats[7]. Besides its use as skin disease, snake bites and cough in Jerantut, Pahang; the whole plant is also used in chest pain, headache, cough and cold by a *Jarawas* tribe of Andaman & Nicobar Islands. The roots are utilized as aphrodisiac[9]. Some claims as an antidiabetic plant of the same region[10-12]. Research studies have demonstrated the antioxidant and PPs activity. *T. barberi*, *T. ponmudiana*, *T. siliquosa* and *T. sivarajanii*[13,14] exhibited antimicrobial activity from the genus, *Thottea*. To the best of our knowledge, that convey solvent effect on extraction, optimization of *T. tomentosa* and the determination of their PPs, flavonoid contents, antioxidants and antibacterial and antifungal efficiency of the leaf and stem part were not produced earlier. Thus, this study aims to describe a systematic evaluation and comparison of potential sources of PPs, natural antioxidants, and antimicrobial activity of the plant. Our study also determines a promising relationship between antioxidant activity and PPs of *T. tomentosa* and compares the most effective medicinal part for the first time. Therefore, within the scope of this study the focus is to evaluate the class phytochemicals of *T. tomentosa* those are strongly accountable for antioxidants and antimicrobials as a bioactive representative in food and drug industry for the great societal benefits.

Materials and Methods

Phyto-raw material

T. tomentosa (Voucher specimen No. AUEc05), growing in various parts Barak Valley, Assam, were collected from Assam University, Silchar campus during March and April, 2016, at its flowering stage. Basic morphological reviews have been made and authentication of the plant was done by accessible floristic writing, for example, Flora of Assam Vol.-4[15] and further identification and confirmation made by the BSI, eastern circle, Shillong. Stems and leaves were removed carefully and washed thoroughly in water for multiple times. Next materials were air dried and made fine powder.

Extraction

Around 70 g of materials of each plant parts were soaked individually with 200 mL of hexane, chloroform and methanol for 72 h. Then filtration process was carried out for 3-4 times. Finally extracts were made solvent free with the help of Rotary evaporator. Respective codes for six extracted materials are equally assigned stem extract using hexane (TSH), chloroform extract of the stem (TSC) and methanol extract of stem (TSM), hexane extract of the leaf (TLH), chloroform extract of the leaf (TLC) and methanol extract of the leaf (TLM).

Extract yield

The following formula was applied to quantify the extract yield of different plant extracts.

$$\frac{\text{Solvent weight}}{\text{Dry weight of material}} \times 100\%$$

Phyto-chemical screening

Ten phyto-chemical tests were applied to find out the existence of chemical constituents like, alkaloids, carbohydrates, glycosides, saponins, proteins and amino acids, phenolic compounds, flavonoids, terpenoids, fixed oils and fats and phytosterols for the extracts of TSH, TSC, TSM, TLH, TLC and TLM (Table 1) following the standard protocols[16].

Total phenolic content (TPC)

Folin–Ciocalteucolorimetric technique[17] (adding few adaptations) was applied for the investigation of TPC of *T. tomentosa.* Briefly, an aliquot of 50 µL of stem and leaf extract samples was mixed with Folin–Ciocalteuphenol reagent (2.5 mL). 5-10 min later, 2.5 mL (7%, w/v) Na_2CO_3 was poured then solution was kept in incubation at 40^0C for 50 min. Then spectrophotometer (UV -1601 PC, Shimazu, Japan) was used at 725 nm to detect the optical density (OD). The results of phenolic contents (Table 2), of all the samples (mg GAE/g DW) were calculated as milligrams of Gallic acid equivalents of dry weight.

Total flavonoid content (TFC)

TFC of the plant extracts were investigated by applying aluminum chloride colorimetric assay[18] with some modifications using quercetin as the standard. Briefly, 150 µL of aliquots and 150 µL standard quercetin solutions were placed in different test tubes.

Table 1 — Phyo-chemical detection of *T. tomentosa*.

Phyto-Constituents	Extracts	*T. tomentosa* leaf	*T. tomentosa* stem
Alkaloids	Ext1	–	–
	Ext2	+	++
	Ext3	++	++
Reducing Sugars	Ext1	–	+
	Ext2	–	++
	Ext3	+	+++
Anthraquinones	Ext1	–	–
	Ext2	–	–
	Ext3	–	–
Saponins	Ext1	–	+
	Ext2	–	++
	Ext3	–	++
Proteins and Amino acids	Ext1	–	–
	Ext2	–	–
	Ext3	–	–
Phenolic compounds	Ext1	+	+
	Ext2	+	++
	Ext3	+ +	+ +
Flavonoids	Ext1	+	+
	Ext2	++	++
	Ext3	+++	+++
Terpenoids	Ext1	–	–
	Ext2	–	–
	Ext3	–	–
Fixed oils and fats	Ext1	–	+
	Ext2	++	++
	Ext3	+ + +	+ + +
Phytosterols	Ext1	–	+
	Ext2	+	+
	Ext3	++	+++

Ext1: Hexane, Ext2: Chloroform, Ext3: Methanol

Made a solution by mixing 0.80 mL of distilled water, 1 mL of 70% methanol, 0.2 mL of 10% $AlCl_3$ and 0.2 mL of CH_3CO_2K (1 M). In the same manner, a blank solution using 1 mL distilled water, 70% methanol (1 mL), 1 M CH_3CO_2K (0.2 mL) was prepared instead of the sample or standard. Tubes were then allowed for 50 min incubation at 40^0C. The absorbance was taken at 540 nm. The results of flavonoid contents (Table 2), of all the samples (mg QTE/g DW) were expressed as milligrams of quercetin equivalent of dry weight.

Antioxidant activities

DPPH assay

Brand-Williams *et al.*[19] method was applied for the DPPH scavenging activity of the test samples with few adaptations. The extract(s) capable of reducing DPPH by donating hydrogen atom more, during the course of reaction color would changes from deep violet to light yellow. Briefly, this reaction is monitored by adding 2 mL of methanolic solution of the test samples in ten test tubes at ten different concentrations, with 3 mL of DPPH solution in each. DPPH reacts with the photons of visible light, hence all the test tubes after adding DPPH solution must kept in dark for 30 min of incubation period, after that OD was observed at 520 nm on UV spectrophotometer. Here, a blank methanol was used for calibration. The percentage of inhibition for DPPH radical scavenging assay by the following formula was calculated and the IC_{50} value for all the extracts were inferred.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

$ABTS^{\cdot+}$ assay

The method of Miller *et al.*[20] (with a little modification) was used to assess $ABTS^{\cdot+}$ of all test samples. Two stock solutions, 7.0 mM $ABTS^{\cdot+}$ solution (prepared in methanol) and 145 mM potassium persulfate solution (prepared in distilled water) were mixed then kept for incubation for 12 h at room temperature. Next of $ABTS^{\cdot+}$ (1 mL) mixed with methanol and kept for dilution to obtain an

Table 2 — Yield of extracts, antioxidant activities, TPC, TFC of *T. tomentosa*.

Plant part	Name of extracts	Extract yield (%)	DPPH IC_{50} in µg/mL	ABTS IC_{50} in µg/mL	TPC mg GAE /g	TFC mg QTE /g
Stem	Hexane	3.12	372.95±1.37	359.73±3.08	49.44±2.93	95.66±5.36
	Chloroform	3.75	271.31±1.34	283.04±0.97	161.66±3.84	230.00±3.28
	Methanol	4.62	202.39±0.92	166.73±1.15	374.99±3.84	579.00±3.38
Leaf	Hexane	4.28	390.95±3.04	401.59±2.17	20.55±2.93	53.33±0.88
	Chloroform	5.14	312.29±1.48	305.34±2.75	128.33±3.85	128.33±3.38
	Methanol	6.71	254.37±1.47	190.61±1.03	260.55±4.00	458.00±3.05

absorbent 0.702±0.001 at 735 nm. To the 20 µL of each test sample $ABTS^{\cdot+}$solution (1 mL) was added and result was observed at 735 nm. In this assay, a standard, ascorbic acid was used.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Antimicrobial activity

Five bacterial strains, *Escherichia coli* (MTCC 1195), *Bacillus subtilis* (MTCC 1427), *Staphylococcus aureus* subsp. *aureus* (MTCC 1430), *Pseudomonas aeruginosa* (MTCC 1688) and *Strptococcus pneumoniae* (MTCC 2672) and two fungi; *Candida albicans* (MTCC 4748) and *Aspergillus niger* (Lab isolates) were applied for the study of antimicrobial activity. The strains were obtained from IMTECH, Chandigarh, India. The *A. niger* was cultured and provided from Downtown Hospital, Dispur (781006), India.

Antibacterial test was performed with the help of well diffusion technique[21] with a little modification. MHA i.e., Mueller-Hinton Agar (for bacterial study) and PDA i.e., Potato Dextrose Agar (for fungal study). Briefly, molten agar media were decanted in the Petri dish and kept for preparation of solid media for microbial culture. Inoculums with (1.5×10^8 CFU/mL) of bacterial culture were spread by sterilized cell spreader on the solidified media. Similarly, fungal culture also spread on PDA plates. In the three wells filled with 50 µL of test samples using three concentrations. A standard antibiotic susceptibility test was also performed. Standard antibiotics; Ciprofloxacin, Ceflexin, Azithromycin and Metronidazole were used for the susceptibility test. Also, two antibiotics; Nystatain and Ketoconazol were used for fungi. Here, standard antibiotics are used as positive control. After that incubation was carried out at 37°C for bacterial culture and 25°C for fungal cultures respectively. Antimicrobial activity was calculated by measuring the ZOI (Zone of Inhibition) around the wells and measured in millimeter (mm).

Minimum Inhibitory Concentration (MIC)

The MICs were determined by the methods[22] with some adaptation. Serial dilutions were carried out in micro plates. 40 µL of each concentration and 40 µL of the overnight broth culture of bacteria (1.5×10^8 CFU/mL) were added to individual wells and was allowed for incubation at 37°C for 24 h. Similarly, extracts (40 µL) were loaded with 40 µL of broth culture of fungi to the wells and allowed for incubation for 25°C for 48 h. Finally, the minimum concentration was detected in the well plates, where no turbidity found.

Minimum Bactericidal Concentrations (MBC)

The MBC determination required the following steps, spreadsing the 12 µL of liquid medium on the solid nutrient agar dishes, kept them for incubation next observed the minimal concentration with lack of visible colony of bacteria was taken as MBC[23].

Minimum Fungicidal Concentrations (MFC)

12 µL of liquid medium was spread over sterile PDA plates for incubation. After the incubation, the minimal concentration that did not possess any noticeable culture was recorded as MFC[23].

Statistical analysis

The tests were performed with their respective controls (if any) in triplicate except MIC, MBC, and MFC, determinations± standard error, at the significance level of $p<0.05$.

Results and discussions

Extract yield

The yield percentage was maximum 4.62% for TSM, followed by TSC, 3.75% and the minimum for TSH, 3.12% was observed. The similar phenomena were noticed for the extracts of *T. tomentosa* leaf 6.71%, 5.14% and 4.28% yields were achieved for TLM, TLC and TLH respectively, presented in

Table 2. The acquired dry weight and yield of each extract proved the highest yield with 6.71%. On the contrary, TSH exhibited the lowest (3.12%) among all other extracts. Of course, high polarity solvent can extract highly polar compounds which are in accordance with our present result. Similar findings for the plant, *Matricaria pubescens* were observed (methanol= 24.34%, ethanol = 9.48% and acetone = 5.02%)[24]; some fractions, aqueous (33.23%), ethyl acetate (6.11%), chloroform (4.71%) of *Sasa quelpaertensis*[25] were also found. Hence, the yield was increased towards the high polarity, hexane to methanol extracts and different yield was certainly due to the nature and type of plant parts, temperature, and storage duration and the existence of polar and non-polar phytochemicals in the plant material[25]. Phenolics, alkaloids, steroids, saponins, glycosides, terpenoids etc. are the large group of naturally produced phytochemicals which are diverging in solubility which set hurdles to acquire sufficient yield in single extraction although it is present ample in nature[26]. Besides, solvent's polarity, temperature, duration of storage and nature and type of plant parts as well as the procedure of extraction, directly extracted or partitioned are also equally accountable for the % of the yield of each extract[25].

Qualitative chemical profiling analysis of the extracts

Secondary metabolites are biologically synthesized non-nutritive phyto-compounds; committed to manifest 'defense mechanism' that hinders different nagging activities for diversified pathogens[27]. These are many traditional medicinal plant species which have multiple therapeutic properties[28]. However our current work exhibited the qualitative chemical profiling of stem and leaf of *T. tomentosa* and displayed the existence of such secondary phytochemicals like alkaloids, carbohydrates, phenolic compounds, flavonoids, fixed oils and fats and phytosterols. Proteins and amino acids were absent in both stem and leaf extracts. However, carbohydrates and phytosterols were observed significantly in stem part than that of the leaf. Saponins were absent in all the extracts of leaf, briefly illustrated the presence and absence of different groups phytochemical in Table 1. The study reveals that the stem part of *T. tomentosa* contains a variety of phytochemicals than that of the leaf parts.

Total Phenolic Content

The TPC values of six extracts are displayed (Table 2) where indicates that TSM demonstrated the highest amount of TPC (374.99±3.84 mg GAE /g) and conversely TLH shows the lowest amount (20.55±2.93 mg GAE /g). However, minimum quantities of phenolic compounds were observed for the less polar solvent, hexane; TSH and TLH (49.44±2.93 and 20.55±2.93 mg GAE /g, respectively), each value expressed in mean ± SE of three independent readings.

PPs have an enormous number of pharmacological activities such are antioxidant activity, anti-diabetic activity, cardiovascular disorder deterrence**,** hepatic disorder deterrence**,** antimicrobial etc.[2,9,30]. Keeping in view of the above-mentioned virtues of PPs the estimation of TPC and TFC of the plant, *T. tomentosa* was considered.

Total Flavonoid Content

TFC of six extracts of *T. tomentosa* were significantly ($p<0.05$) measured. $AlCl_3$ can form an acid complex with C3 or C5, -OH group, or C4, keto group of flavonoid, if present in plant extracts[31]. $AlCl_3$ can also form ortho-metallation with dihydroxide groups of flavonoids, spectrophotometric measurements. The methanol extract, TSM presented the high flavonoid content (579.00±3.38 mg QTE /g), followed by TSC and TSH (230.00±3.28 mg QTE /g and 95.66±5.36 mg QTE /g respectively) of the stem of *T. tomentosa*. The lowest content observed in the hexane extract of leaf, TLH (53.33±0.88 mg QTE /g), the moderate content of chloroform extract, TLC; 128.33±3.38 mg QTE /g. Whereas, 458.00±3.05 mg QTE /g was the highest measured value of the methanol extract of the leaf, in Table 2. The study implies the stem part contains more heterocyclic flavonoid rather than a glycones[16].

Antioxidant activities

The antioxidant efficiency of both stem and leaf of *T. tomentosa* was evaluated using a stable free radical, DPPH. IC_{50} value measures, the extent of scavenging ability of the six extracts of *T. tomentosa*. Higher antioxidant efficiency shows lower IC_{50} value[32]. The stem part of methanol extracts showed lowest IC_{50} value 202.39± 0.92 µg/mL; whereas the moderate value was witnessed for chloroform extract, 271.31±1.34 µg/mL and hexane showed the minimum value, 372.95±1.37 µg/mL. Similar phenomena were noticed for the leaf extracts with IC_{50} values for TLH, TLC and TLM; 390.95±3.04, 312.29±1.48 and 254.37±1.47 µg/mL, respectively. IC_{50} of quercetin exhibited as 6.14 µg/mL. Therefore present study indicated that TSM has the highest antioxidant property and the TLH has the lowest.

Present study exhibited a remarkable ABTS·+ efficiency of the plant extracts as IC_{50} values were observed as, leaf extracts; TLH (401.59±2.17 µg/mL), TLC (305.34±2.75 µg/mL) and TLM (190.61±1.03 µg/mL), the stem extracts exhibited; TSH (359.73±3.08 µg/mL), TSC (283.04±0.97 µg/mL) and TSM (166.73±1.15 µg/mL) respectively with ascorbic acid IC_{50}, 8.12 µg/ml in Table 2. Thus, again proves that TSM has the highest antioxidant activity and the TLH has the lowest. Different values of IC_{50} ($p<0.05$) were detected for the six extracted materials, shows a similar trend of declination in IC_{50} values from hexane, chloroform to methanol[33]. The antioxidant activity, shown by the six extracts of *T. tomentosa*, is doubtlessly related to the total polyphenolic content which is also supported by $ABTS^{\cdot+}$scavenging activities. Natural PPs are reported to have multiple therapeutic effects like Alzheimer's, atherosclerosis, kidney and liver diseases, arthritis, neurodegenerative disorders, fibrosis, aging, cardiovascular and even carcinogenesis[2], including antioxidant activity and excellent natural antimicrobials[3]. Needless to say, the presence of PPs even in various derivatised form plays an important role as far as their medications and nutritional aspects are concerned. The presence of PPs (act as hydrogen donors in the biochemical reactions) mostly shows the antioxidant activity[34]. Nitric oxide scavenging property of the methanolic extract of the same plant has an IC_{50} range between 65.5±1.55 to 148±3.09 µg/mL[14]. Our result suggests a promising relationship between antioxidant activity and PPs of *T. tomentosa*. However, different extracts carrying different phyto-compounds therefore, the methanolic extract of the steam; TSM showed highest antioxidant efficiency. Hence, TSM and TLM could contribute to further understanding of pharmacological examination.

Antimicrobial activity

Well diffusion assay

The present study discloses *T. tomentosa* as a broad-spectrum antimicrobial agent for the seven pathogenic microbes. Results ($p<0.05$) exhibited that all six samples have potential antimicrobial activities except TLH against the bacterium, *S. aureus* susp. *aureus* and fungi, *C. albicans* and *A. niger* (Fig. 1 a, b).

57.14% (4 out of 7), 100% (7 out of 7) and 100% of the microbes were susceptible to the three concentrations (5 mg/mL, 10 mg/mL and 20 mg/mL respectively) of TSM showed in Figure 2 (a, b). Amongst all, TSM showed higher activity against the bacterium *S. pneumoniae* with all three concentrations. ZOI were observed as 11.33±1.33, 16±1.73 and 19.33±0.66 mm, respectively; however, lowest activity of the same extract showed against the fungus *C. albicans* where ZOI were measured as 0, 8.66±0.33 and 14.66±1.20 mm, respectively. TSC showed medium activity against all the pathogens. 28.57%, 57.14% and 100% of microbes were susceptible to all three concentrations. The lowest activity of the sample was observed against the bacterium *S. pneumoniae* with ZOI, 0, 0 and 8.66±0.66 mm. Further, 0, 28.57% and 100% of microbes were susceptible to the three concentrations of TSH. Of the three concentrations, TSH, highest efficacy against *E. coli* was noticed with ZOI, 0, 13.66±0.66 and 15.33±0.33 mm respectively, represented in Figure 2 (a). Though, lowest activity showed against the bacterium, *S. aureus* susp. *aureus*, ZOI were 0, 0 and 8.66±0.66 mm recorded. In Figure 2 (b), 0%, 71.42% and 100% microbes were susceptible to the three concentrations of TLM. It showed higher activity against the bacterium *S. pneumoniae* with all three concentrations, ZOI were observed as 0, 14.66±0.33, 16.33±0.66 mm, respectively; lowest activity showed against the fungus *C. albicans*, with ZOI, 0, 0 and 8.66±0.33 mm, respectively. On the other hand, 0%, 28.57% and 100% of microbes were susceptible to the three concentrations of TLC. Whereas 0%, 14.28% and 57.14% microbes were susceptible for TLH and exhibited the highest activity against the bacterium *E. coli* with 0, 13.33±0.33 and 14.33±0.33 mm ZOI respectively. Similar activity against three bacteria *B. subtilis*, *P. aeruginosa* and *S. pneumonia* were observed with 0, 0 and 8.66±0.33 mm ZOI in Figure 2 (b). Additionally, the bacterium *S. aureus* subsp. *aureus*, two fungi *C. albicans* and *A. niger* did not show any activity for TLH. The broad-spectrum antimicrobial activity of the plant *T. tomentosa* could be due to the high content of alkaloids, saponin**,** some PPs, Flavonoids and phytosterols from plant origin[35-38]. The lowest concentrations of the test samples that inhibit visible growth of a microorganism to test are the best antimicrobials[24]. The present results are in accordance with the previous studies of the genus.

Ciprofloxacin at 5 µg/mL showed activity against five bacteria *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus* susp. *aureus* and *S. pneumonie* (ZOI, 27.66±0.33, 27.33±1.45, 32.66±1.33, 26.33±0.66 and 27.33±1.45 mm respectively). Nystatain at 5 µg/mL showed maximum ZOI against the species of

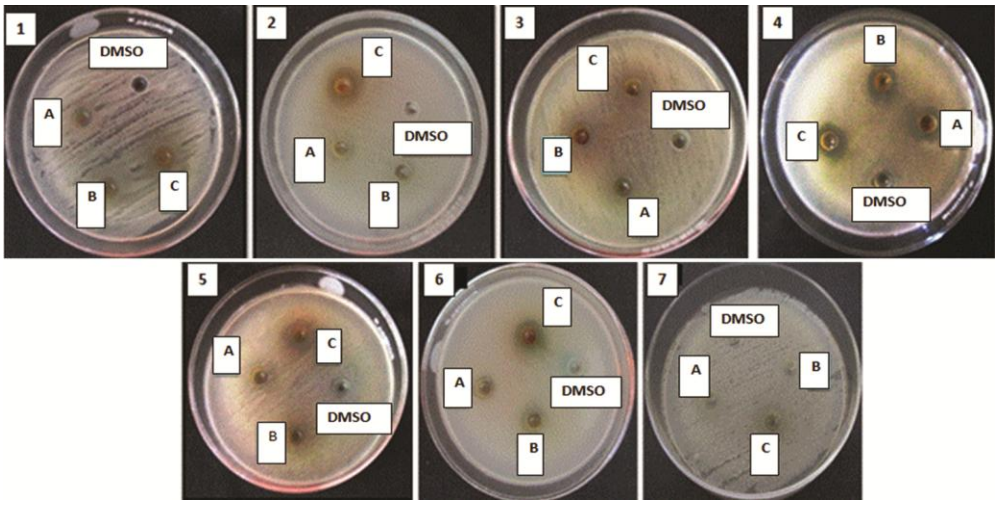

Fig. 1 (a) — Zone of inhibition of TSM.
1: *B. subtilis*, 2: *E.coli*, 3: *P. aeruginosa*, 4: *S. aureus* susp.*aureus* 5: *Streptococcus pneumonia* 6: *A. niger* and 7: *C. albicans*; A: 5 milligram/milliliter B: 10 milligram/milliliter and C: 20 milligram/milliliter.

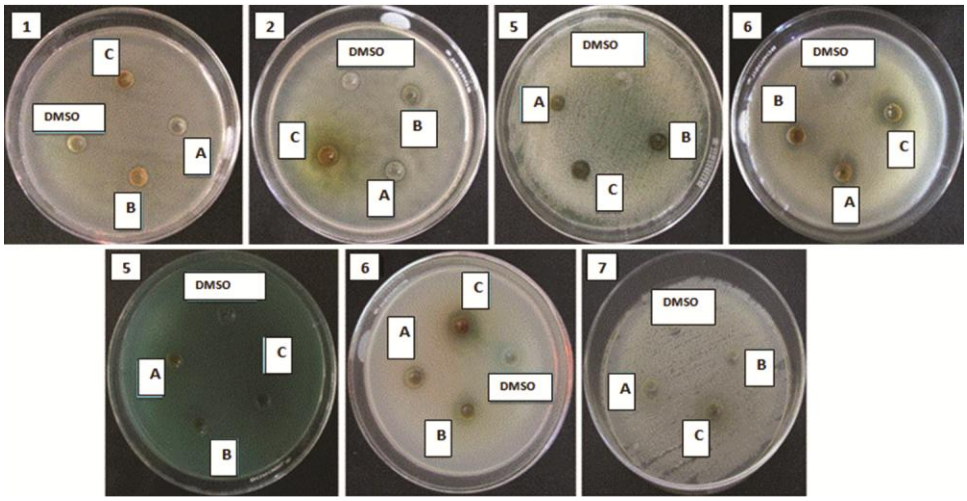

Fig. 1 (b) — Zone of inhibition of TLM.
1: *B. subtilis*, 2: *E. coli*, 3: *P. aeruginosa*, 4: *S. aureus* susp. *aureus* 5: *Streptococcus pneumonia* 6: *A. niger* and 7: *C. albicans*; A: 5 milligram/milliliter B: 10 milligram/milliliter and C: 20 milligram/milliliter.

Aspergillus (13.66 mm) and the species of *Candida* (14.33 mm)

MIC, MBC, and MFC of T. tomentosa

MIC was analyzed for six test samples of *T. tomentosa*, the lowest MIC value of TSM, 0.57±0.20 mg/mL, MBC value was 1.05±0.15 mg/mL against the bacterium *S. pneumoniae* and MFC was 5.80±0.30 mg/mL against *A. niger*. The lowest MIC and MBC values of TSC were 1.40±0.20 mg/mL and 3.60±0.10 mg/mL respectively against the bacterium *S. aureus* susp. *aureus*. The MFC, 5.95±0.25 mg/mL against the fungus *C. albicans* was noticed. Meanwhile, the lowest MIC value of SHT was 5.50±0.10 mg/mL, MBC was 6.00±0.10 mg/mL against *E. coli* and MFC was 12.90±0.20 mg/mL against *A. niger* was witnessed. TLM and TSM displayed the highest activity against the *S. pneumonia* whereas TLC and TSC displayed maximum efficiency against the bacterium *S. aureus* susp. *aureus*. Hence, present study indicates methanol extracts of both plant parts (stem and leaf) have highest antimicrobial activity and shows 100% of microbes susceptible to these extracts. Anilkumar *et al.*[17] studied the *in vitro* antimicrobial activity of methanol extract of four Thottea species, *Thottea. barberi*, *T. ponmudiana*, *T. siliquosa* and *T. sivarajanii* where, highest MIC= 0.064 mg/mL and lowest MIC > 2 mg/mL among those have been observed in

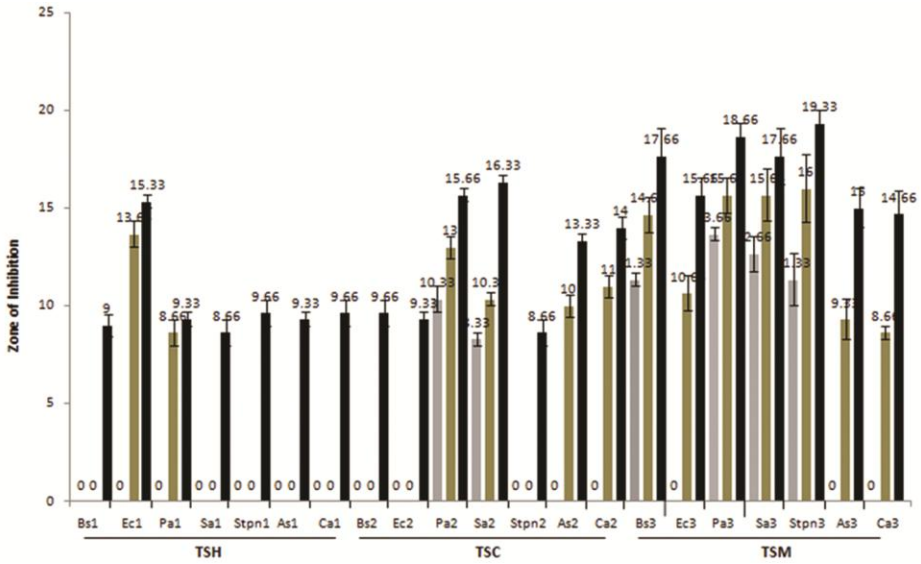

Fig. 2 (a) — Antimicrobial activity of three extracts (TSH, TSC, and TSM) of stem of *T. tomentosa*. Bs= *Bacillus subtilis*; Ec= *Escherichia coli*; Pa= *Pseudomonas aeruginosa*; Sa= *Staphylococcus aureus* susp. *aureus*, St pn= *Streptococcus pneumonia*; As= *Aspergillus niger* Ca= *Candida albicans.*

Bs1, Bs2 and Bs3 are ZOI against *B. subtilis* formed by TSH, TSC and TSM respectively.

Ec1, Ec2, and Ec3 are ZOI against *E. coli* formed by TSH, TSC and TSM respectively.

Pa1, Pa2 and Pa3 are ZOI against *P. aeruginosa* formed by TSH, TSC and TSM respectively. Sa1, Sa2, and Sa3 are ZOI against *S. aureus* susp. *aureus* formed by TSH, TSC and TSM respectively.

St pn1, St pn2, and St pn3are ZOI against *S. pneumonia* formed by TSH, TSC and TSM respectively.

As1, As2 and As3 are ZOI against *A. niger* formed by TSH, TSC and TSM respectively.

Ca1, Ca2 and Ca3 are ZOI against *C. albicans* formed by TSH, TSC, and TSM respectively. The results are expressed in mean ± SE.

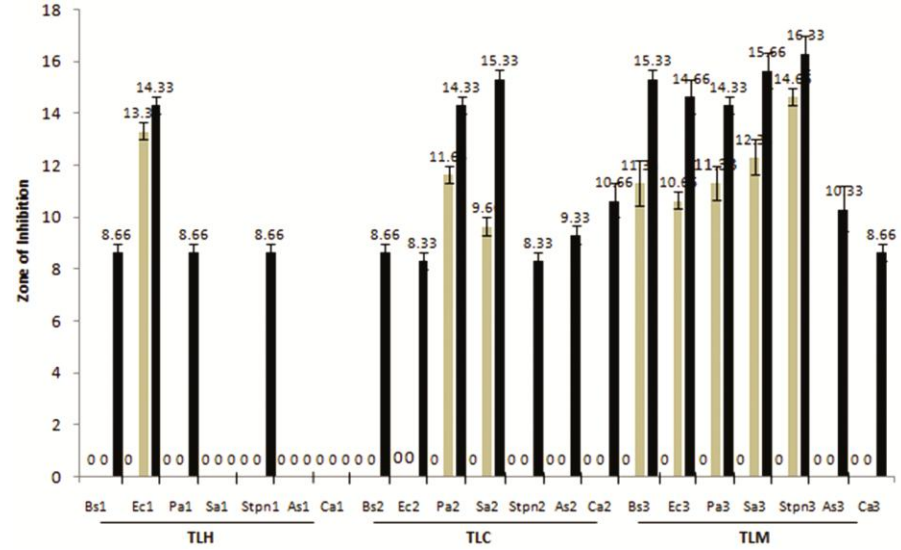

Fig. 2 (b) — Antimicrobial activities of three extracts (TLH, TLC, and TLM) leaf of *T. tomentosa*. Bs= *Bacillus subtilis*; Ec= *Escherichia coli*; Pa= *Pseudomonas aeruginosa*; Sa= *Staphylococcus aureus* susp. *aureus*, St pn= *Streptococcus pneumonia*; As= *Aspergillus niger* Ca= *Candida albicans.*

Bs1, Bs2 and Bs3 are ZOI against *B. subtilis* formed by TLH, TLC and TLM respectively.

Ec1, Ec2, and Ec3 are ZOI against *E. coli* formed by TLH, TLC and TLM respectively.

Pa1, Pa2, and Pa3 are ZOI against *P. aeruginosa* formed by TLH, TLC and TLM respectively.

Sa1, Sa2, and Sa3 are ZOI against *S. aureus* susp. *aureus* formed by TLH, TLC and TLM respectively.

St pn1, St pn2 and St pn3are ZOI against *S. pneumonia* formed by TLH, TLC and TLM respectively.

As1, As2 and As3 are ZOI against *A. niger* formed by TLH, TLC and TLM respectively.

Ca1, Ca2 and Ca3 are ZOI against *C. albicans* formed by TLH, TLC, and TLM respectively. The results are expressed in mean ± SE.

T. sivarajanii and *T. ponmudiana* respectively against *P. aeruginosa*. While in our study, MIC value of methanol extract of stem of *T. tomentosa* has observed as 0.650 mg/mL against the bacterium. Anilkumar *et al*.[13] reported, the highest (MIC 0.125 mg/mL) among those been observed in *T. siliquosa* and the lowest activity, MIC > 2 mg/mL observed in *T. ponmudiana* and *T. sivarajanii* against *S. aureus*. While in our current study MIC value of methanol extract of the stem was found to be 0.890 mg/mL against the sub-species of the same bacterium.

Acceleration of multi-drug resistant microbes in the environment is the awful to a human health and it is associated with nosocomial infection[39]. Consequently, a requirement has observed for the effectual antibiotic agents which are cordial to the human body and also conquer such negative health consequences. Eventually, PPs are receiving a colossal attention by the scientists and hence, a large number of researchers have been introducing to investigate certain noble antimicrobial phyto-drugs.

Conclusion

Our results, for the first time explores, the extracting solvent polarity influences on the extraction yield, qualitative chemical profiling, antioxidant, polyphenol contents, flavonoid contents, and antimicrobial properties of the plant *T. tomentosa*. Subsequently, the present study confirms the variable content of polyphenols most likely influenced the plant to be an excellent antioxidant and antimicrobial agent. The manifestation of such polyphenols hopefully has contributed the inherent medicinal folk claims of *T. tomentosa*. However, it is an interesting exercise for isolation, purification and characterization of the bio-active compounds for such study and for the further guidance of their existing and/or future therapeutic properties which may be emphasized the expectation of the use of the plant in food and drug industry.

Acknowledgements

RB is thankful to Assam University, Silchar for institutional financial support and Biotech Hub, Dakshin Kamrup College, Mirza for accessing laboratory facility.

Conflicts of Interest

The authors declared no conflicts of interest.

Authors' Contributions

Conception and design of the research: RB, AKD, PPA, SBP; Interpretation of data: RB; and revising the manuscript: RB, NR, GDS.

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