Sex-specific variations in phytochemicals and antimicrobial potentiality of *Dioscorea*

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**Abstract**

**Background:** The monocotyledonous herbaceous crop *Dioscorea* is native to tropical and temperate regions of the world. *Dioscorea* tubers are dioecious in nature, have colossal therapeutic potentiality, and are frequently used in traditional medical practices throughout the world. Most of the research works are aimed to determine the medicinal property, nutrition, antinutrients, and biological activities of *Dioscorea* spp. without specifying the sexes of *Dioscorea* which promoted us to carry out this current research work.

**Results:** Sex-specific variation of phytoconstituents, antioxidants, and antimicrobial efficiency in tubers was appraised. The results obtained from this study divulge existence of significant quantitative variation between the male and female tubers. The female tubers are superior in acquiring phytochemicals compared to male counterparts and acquired maximum antioxidant and antimicrobial potentiality.

**Conclusion:** This study will offer an apposite baseline for further sex-specific assessment which can be directed towards both qualitative and quantitative amelioration of medicinally important noble compounds by exploiting modern scientific strategies leading to their active participation in nutraceutical industries.

**Keywords:** Sex-specificity, Phytoconstituents, Antioxidant, Baseline, Nutraceutical industry

**Background**

The genus *Dioscorea* earlier positioned under order Liliales [1] but later included under Dioscoreales [2]. The highly medicinal dioecious *Dioscorea* of Dioscoreaceae contains more than 600 species globally [3–5]. The *Dioscorea* tubers are renowned for their ethnobotanical, nutritive, antioxidant, and biological potentiality that ensure the quality of daily nourishment of the indigenous people [4, 6, 7]. *Dioscorea* (Yam) is a staple food for the people of tropical countries of Asia, Africa, Caribbean, and the Pacific region [8]. The long-term storage potentiality of these tubers ensures seasonal food security in developing countries [9]. Diosgenin is a phytosteroidal saponin and a major bioactive compound found in the roots of wild yam [10]. It is the main precursor in the manufacture of synthetic steroids in the pharmaceutical industries [11].

Dioecy is attributed to seven percent of total plant taxa although most of the medicinal plants are monoecious [12]. Knowledge of the dioecious nature of plants has existed since Babylonian times but their consequences in the traditional medical system are not recognized appropriately [13]. Insinuation of dioecy in chemical and pharmacological properties has been pointed out [14–16]. The sex-specific biological activities of *Piper betle*, *Carica papaya*, and *Tinospora cordifolia* was recorded [17–19]. Sex determination in *Dioscorea* has not yet been fully elucidated although [20–22] have favored male as the heterogametic sex; Smith [22] and Meurman [23] emphasized the occurrence of an extra chromosome for the male expression, while [23–25] have reported absence of sex chromosomes. Literature survey concerning the biological potentiality and phytoconstituents’ availability underpin

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that evaluation of sex-specific variation concerning phyto-
constituents and biological efficacy of Dioscorea spp. has
remained unexplored although reports are available [7].

Hence, this present study has entailed to evaluate the
variation in the phytoconstituents and antimicrobial effi-
ciency based on the male and female tubers of Dioscorea.

Methods
Sample collection
Matured male and female tubers of five edible Dioscorea
were collected by using the shrivel and auger and packed
into marked zipped sterile polythene bags from the for-
est bed of Tripura (Figs. 1, 2, 3, and 4). The collected
plant samples were identified by using the Flora of Trip-
ura [26], and two of them are a new addition to the
Flora of the state [27, 28]. Flowers, micro- and macro-
morphological characters were considered during the
identification of the male and female plants and further
authenticated with taking help from the expertise from
Botanical Survey of India (Eastern Regional Centre, Shil-
long). The herbarium prepared for the selected Dios-
corea spp. with their respective voucher numbers were
deposited in the departmental herbarium and depicted
(Table 1). Analysis of International Union for Conserva-
tion of Nature (IUCN) status pointed out that among
five of the selected Dioscorea spp., only Dioscorea walli-
chii is included in least concern category.

Plant samples
The male and female tubers of five Dioscorea spp. were
collected during the flowering phase, and photographs

Fig. 1 Male and female plants of Dioscorea spp. with their respective reproductive structure. a, b Male and female plants of Dioscorea alata. c, d Male and female plants of Dioscorea hamiltonii. e, f Male and female plants of Dioscorea oppositifolia. g, h Male and female plants of Dioscorea pubera. i, j Male and female plants of Dioscorea wallichii
were taken as reference for the identification from the forest bed of three different districts of Tripura (Table 1). Care was taken during the collection of the tubers that the tubers of both male and female plants were available at each of the selected study sites.

Sample extraction
Collected tubers were cleaned in running tap water, shade dried, and pulverized to powder in a mechanical grinder. Twenty grams tuber powder of each of the Dioscorea species was extracted separately with methanol (200 mL) in a shaker at room temperature. After, overnight extracts were filtered through Whatman No. 1 filter paper. The filtrates were subjected to analysis for total phenolic, flavonoid contents, and DPPH radical scavenging activities.

Determination of moisture content
Tubers samples were chopped into small pieces by using sterilized blades. Ten grams of the chopped samples were taken in the previously weighed Petri plates. Then, the sample was kept in a hot-air oven for overnight at 100 ± 2°C. The dried samples were cooled at room temperature and weighed to a constant weight. The loss in weight was considered as the moisture percentage and was calculated by using the following formula:

\[
\text{Percentage of moisture content} \, (\%) = \frac{W_1 - W_2}{W_1} \times 100
\]

where \( W_1 \) = weight of the sample (leaf and rhizome) taken and \( W_2 \) = weight of the oven-dried samples.

Determination of carbohydrate
Carbohydrate was determined [29] from the dried tuber samples. One hundred milligrams of the sample was
Fig. 3 Antimicrobial activity of male and female tubers of *Dioscorea* spp. a, b Antibacterial activity of male and female tubers of *Dioscorea* spp. against *Streptococcus pneumoniae* (MTCC-655) and *Shigella dysenteriae* (MTCC-227). c, d Antifungal activity of male and female tubers of *Dioscorea* spp. against *Candida tropicalis* (Agartala medical college) and *Candida albicans* (MTCC-227).

Fig. 4 Inhibition concentration (IC₅₀) values of male and female tubers of *Dioscorea* spp. and ascorbic acid.
taken into boiling tubes and hydrolyzed with 5 ml of 2.5 N-HCl for 3 h and cooled at room temperature followed by the neutralization with sodium carbonate pellets. The volume is made up to 10 ml and centrifuged at 5000 rpm for 15 min. The supernatant was collected and 1 ml aliquots were taken for analysis. Then, 4 ml of 2% anthrone (w/v in concentrated H₂SO₄) reagent was added and heated in a boiling water bath for 10 min. The absorbance was taken at 630 nm using a spectrophotometer. Glucose was used as a standard.

**Determination of protein**

The protein content was determined [30]. One hundred milligrams of the sample was ground well with a pestle and mortar in 10 ml of the potassium phosphate buffer (0.1 M, pH 7.5) and centrifuged at 5000 rpm for 15 min. The pellet was discarded and the supernatant was used for protein estimation. From the supernatant, 1 ml of sample was taken in dried test tubes and 5 ml of reagent (0.1 M, pH 7.5) and centrifuged at 5000 rpm for 15 min. The supernatant was collected and 1 ml aliquot was taken into boiling tubes and hydrolyzed with 5 ml of 2.5 N-HCl for 3 h and cooled at room temperature flowed by the neutralization with sodium carbonate pellets. The volume is made up to 10 ml and centrifuged at 5000 rpm for 15 min. The supernatant was collected and 1 ml aliquots were taken for analysis. Then, 4 ml of 2% anthrone (w/v in concentrated H₂SO₄) reagent was added and heated in a boiling water bath for 10 min. The absorbance was taken at 630 nm using a spectrophotometer. Bovine serum albumin (BSA) was used as a standard.

**Estimation of total free amino acids**

The amount of total free amino acid was estimated [31]. For this, 100 mg of dried tubers sample was homogenized in 10 ml of 50% aqueous ethanol with a pinch of activated charcoal. The slurry was centrifuged at 5000 rpm for 10 min, and the free amino acid was extracted in the form of a clear supernatant which was used for spectrophotometric estimation. The volume of supernatant was raised to 10 ml with aqueous 50% ethanol. To 1 ml of the supernatant, 2 ml of 2% Ninhydrin (w/v in dehydrated alcohol) was added. The mixture was kept on a water bath at 75 ± 2 °C for 10 min, and after cooling, aqueous alcohol (1:1) was added to make up the volume to 3 ml. The absorbance was measured at 570 nm on a spectrophotometer. Glycine was used as a standard.

**Determination of fat content**

The fat content was determined [32]. Two grams of the sample was taken in dried test tubes, and petroleum ether was added on that and allowed to stand for 16 h. After 16 h, the petroleum ether was evaporated to dryness and weights the flask before after for fat.

**Estimation of total crude fiber**

Crude fiber of the tuber samples was estimated [32]. One gram of dried leaf sample was subjected to acid and subsequent alkali digestion for degradation of native cellulose and lignin. The residue obtained after final filtration was weighed, incinerated, cooled, and weighed again. The loss in weight gives the crude fiber contents.

**Determination of ascorbic acid**

For determination of ascorbic acid content in the tubers, [33] method was employed. Five grams of the sample was weighed into a bottle containing 100 ml of ethylene-diaminetetraacetic acid (EDTA)/tricarboxylic acid (TCA) (2:1 v/v) extraction solution. The mixture was shaken vigorously for 30 min. The solution was transferred into a centrifuge tube, and centrifugation was done at 3000 rpm for 20 min. Then, the preparation was transferred to a 100-ml volumetric flask and 1% starch indicator was added followed by titration with 20% copper sulfate (CuSO₄) and waited until the dark color was developed.

**Determination of riboflavin**

For the determination of tubers riboflavin content, 5 g of the sample was extracted with 100 ml of 50% ethanol and shaken for 1 h followed by the filtration into 100 ml flask. From this preparation, 10 ml of the extract was pipetted into 50 ml volumetric flask and 10 ml of each 5%
potassium permanganate, and 30% H2O2 was added subsequently. This preparation was taken to a hot water bath for 30 min. This was followed by the addition of 2 ml of 40% sodium sulfate. The volume was made up to 50 ml and the absorbance measured at 510 nm [34].

**Determination of thiamine**

Five grams of the tuber sample was homogenized with 50 ml 10% ethanolic sodium hydroxide and filtered into a 100-ml conical flask. Ten milliliters of the filtrate was pipetted, and the color was developed by the addition of 10 ml 1% potassium dichromate; the absorbance was measured at 360 nm [34].

**Determination of alkaloids**

The alkaloid content was determined [35]. For this, 5 g of the sample was weighed and taken into a 250-ml beaker containing 200 ml of 10% acetic acid in ethanol and allowed to stand for 4 h. This preparation was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The precipitate was collected and washed with dilute ammonium hydroxide followed by filtration. The residue was dried and weighed.

**Determination of total phenols**

Total phenol was determined [36]. 200 mg of sample was crushed in 10% methanol and centrifuged for 20 min at 5000 rpm. 1 ml supernatant was taken and 1 ml Folin Ciocalteu reagent was added followed by the incubation for 3 min at room temperature. Then, 1 ml of saturated 20 % Na2CO3 was added and kept in a water bath for 1 min. The absorbance was measured at 725 nm. Gallic acid was used as a standard.

**Determination of flavonoids**

The flavonoid content was estimated [37]. 0.5 ml of test sample solution in methanol (5 mg/100 ml) was mixed with 2 ml of distilled water and 150 μl of 5% sodium nitrate. After 6 min, 150 μl of 10% aluminum chloride and 2 ml of 1 M sodium hydroxide was added and left at room temperature for 15 min. The absorbance of the mixtures was measured at 510 nm. Catechin was used as a standard.

**Determination of saponin**

Saponin was determined [38]. Twenty grams of tuber samples were put into conical flasks containing 100 ml of 20% aqueous ethanol and heated at 55 °C for 4 h. The mixture was then filtered and re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml in a water bath. The concentrate was transferred into a 250-ml separating funnel containing 20 ml of diethyl ether and shaken vigorously. The aqueous layer was recovered and further purification was done in 60 ml of n-butanol. The preparation was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath followed by the evaporation of the samples. The residue was dried and weighed.

**Determination of tannin**

The tannin content was estimated [39]. Five hundred milligrams of powdered sample was dissolved in 50 ml of distilled water and shaken for about 1 h in a mechanical shaker. This was filtered through cheese clothes into a 50-ml volumetric flask and made up to the mark. Then, 5 ml of the filtered was pipetted into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 760 nm within 10 min. Tannic acid was used as a standard.

**DPPH radical scavenging activity**

The free radical scavenging activities of methanol extract of all the samples were evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method [40]. Different concentrations of methanol extracts (30, 60, 120, 240, 480, 600, 720, 840, 960 μg/ml of the sample) were mixed with 300 μl DPPH (0.02 mM). The absorbance was measured at 517 nm using a UV-VIS double beam spectrophotometer (Dynamica, DB-20 and SL. No. - 6622065) after 30 min of incubation at dark. Ascorbic acid was used as the reference sample. Scavenging of DPPH was calculated by using the formula:

\[
\text{DPPH scavenging activity (\% of inhibition)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

where \(A_0\) is the absorbance of the control reaction and \(A_1\) is the absorbance of the sample.

**Evaluation of antimicrobial activity**

The antibacterial potentiality of the tubers of male and female tubers was determined by the agar well diffusion method. Streptomycin and dimethyl sulfoxide (DMSO) were used as positive and negative controls for antibacterial study. The results were recorded by using a ruler with a sliding caliper [41], and the inhibition zone was expressed in millimeters. The anti-fungal activity of the compounds was determined by the agar well diffusion method.

**Data analysis**

Each of the analysis was performed in triplicate and expressed as mean ± SD. Antioxidant activity was determined and inhibition concentration (IC50) values were
calculated using the linear regression curve in Microsoft Excel 2007. All the statistical analysis was done by using Microsoft Excel 2007.

**Results**

**Nutritional aspects**

**Moisture content**
Female tubers of most of the *Dioscorea* species show higher moisture content (MC) compared to male ones. Maximum and minimum MC was recorded in female and male tubers of *D. alata*. The MC varied significantly among the tubers of the female and male plant of *D. alata* ($P < 0.01$) and *D. oppositifolia* and *D. pubera* ($P < 0.05$). No significant difference was observed in the male and female tubers of *D. hamiltonii* and *D. wallichii*.

**Total protein**
The total protein (TP) content of the tubers ranged between 3.15 ± 0.05 and 13.25 ± 0.22 mg/gm fresh weight. Maximum and minimum TP content was recorded in the tuber of the male plant of *D. pubera* and *D. oppositifolia*, respectively. The total protein content differs significantly among the tuber of male and female plant of *D. oppositifolia* and *D. pubera* ($P < 0.001$), *D. alata* ($P < 0.01$), *D. hamiltonii* ($P < 0.05$).

**Total carbohydrate**
Maximum and minimum total carbohydrate (TC) content was observed in female tubers of *D. hamiltonii* and male tubers of *D. wallichii*, respectively. Significant differences were observed in the TC content of male and female tuber of *D. alata*, *D. oppositifolia*, *D. pubera* ($P < 0.05$), and *D. wallichii* ($P < 0.01$).

**Total soluble sugar**
Maximum and minimum total soluble sugar (TSS) content was recorded in female tubers of *D. glabra* and *D. oppositifolia*, respectively. No significant differences existed between the male and female tuber of *D. hamiltonii*, *D. pubera*, *D. oppositifolia*, and *D. glabra* while only *D. alata* showed significant difference ($P < 0.01$).

**Total free amino acid**
Total free amino acids (TFA) do not differ significantly among the male and female Dioscorea tubers. Only the male and female tubers of *D. wallichii* showed a significant ($P < 0.05$) difference. Maximum and minimum TFA was recorded in the tubers of the female plant of *D. hamiltonii* and *D. pubera*, respectively.

**Total crude fiber**
The total crude fiber (TCF) content significantly differed ($P < 0.05$) among the tuber of male and female plant of *D. hamiltonii* and *D. wallichii*. However, no significant variation was observed in the male and female plant tuber of *D. alata*, *D. oppositifolia*, and *D. pubera*. The maximum amount of TCF was recorded in the female tuber of *D. alata* and minimum in the male tuber of *D. glabra*.

**Total fat**
The total fat (TF) content varied significantly among the male and female tuber of *D. oppositifolia* ($P < 0.01$) and *D. wallichii* ($P < 0.05$). No significant variation was observed in the tuber of male and female plant of *D. alata*, *D. pubera*, and *D. hamiltonii*. Maximum and minimum TF was recorded in female tuber of *D. alata* and male tuber of *D. hamiltonii*, respectively.

**Vitamins**
Among the studied *Dioscorea* species, the maximum ascorbic acid (Aa) content was observed in the female tuber of *D. oppositifolia* while minimum in male tuber of *D. wallichii*. No significant variation was observed among the male and female tuber of *Dioscorea*. Riboflavin (Rf) content in the tuber of male and female *Dioscorea* species showed no significant difference except *D. oppositifolia* ($P < 0.05$). Maximum and minimum Rf content was recorded in female tuber of *D. alata* and male tuber of *D. oppositifolia*, respectively. In tuber thiamine (Th) content, *D. pubera* and *D. wallichii* showed significant variation ($P < 0.05$) between their male and female plants. Female tuber of *D. alata* showed the maximum Th content while male tuber of *D. pubera* showed the least.

**Anti-nutritional aspects**

**Total alkaloid**
Total alkaloid (TA) content in both male and female tubers of *D. alata* and *D. pubera* was significant ($P < 0.05$), whereas no significant difference was observed between the male and female tubers of other species. Maximum and minimum TA content was recorded from the tubers of female *D. hamiltonii* and *D. oppositifolia*, respectively.

**Total phenol**
The total phenol (TPH) content of male and female tubers of two species viz. *D. wallichii* and *D. oppositifolia* showed highly significant ($P < 0.001$) variation. Maximum phenol content was recorded in *D. oppositifolia* female tuber while the female plant of *D. wallichii* showed the least.

**Total flavonoid**
The total flavonoid (TF) contents of tubers of male and female plant of *D. alata*, *D. hamiltonii* ($P < 0.05$), and *D. wallichii* ($P < 0.01$) varied significantly. The maximum
TF was recorded in female tuber of *D. oppositifolia*, whereas the least was recorded in the male tuber of male and female *D. hamiltonii*, *D. wallichii*, and *D. pubera* plants reflected no significant difference.

**Total tannin**

Maximum total tannin (TT) content recorded in the male tuber of *D. oppositifolia* and the least was observed in the male tuber of *D. hamiltonii*. TT in the tuber of male and female exhibited significant differences except *D. pubera* and *D. wallichii*. TT differs significantly in the tubers of male and female plants of *D. oppositifolia*, *D. wallichii*, *D. alata*, and *D. hamiltonii* (*P < 0.001*).

**Total saponin**

Tubers of male and female plants of *D. alata* showed a significant (*P < 0.05*) difference in total saponin (TS) content. Maximum and minimum TS were recorded from tubers of female *D. hamiltonii* and male tuber of *D. oppositifolia*, respectively.

**DPPH radical scavenging activity**

The methanolic extracts of tubers male and female *Dioscorea* plants possessed potent DPPH radical scavenging activity in terms of percentage of inhibition. Antioxidant activity varies significantly among the male and female counterpart. No significant difference was observed in the tubers of male and female *D. alata*.

**Antimicrobial activity**

Antimicrobial activity of methanol extracts of male and female tuber of five *Dioscorea* species were screened against four pathogenic bacteria and two pathogenic fungi. The antimicrobial activity was determined in terms of the inhibition zone around the respective microbial colonies. Maximum microbial activity against all the pathogenic bacteria and fungi was recorded in the methanolic extracts of both male and female tubers of *D. pubera*. Male and female tubers *D. oppositifolia* showed proficient activity against the selected fungal strains and bacterium *Klebsiella pneumoniae*. *D. hamiltonii* male and female tubers exhibited noticeable antibacterial activity against *Streptococcus pneumoniae*. The female tubers of *D. wallichii* showed strong activity than their male counterpart.

**Discussion**

This present study has been carried out to evaluate the sex-specific variation of nutrients and antioxidant aspects along with their antioxidant and antimicrobial potentiality of *Dioscorea* tubers (Tables 2, 3, 4, 5 and 6). A total of five edible species were selected for this present study out of which all are dioecious, i.e., male and female plants are developed separately. The tubers of superior crop yam [42] assist nutrients three times more than the most important food crops like cassava and sweet potato [43]. A considerable amount of research work has been carried out throughout the world but none of them are emphasizing the sex-specific evaluation of phytochemical constituents and biological efficacy of *Dioscorea* spp. The results obtained from the study revealed that significant differences existed between the most of the male and female tubers in terms of nutrient, vitamins,

### Table 2 Nutritional status of different sexes of *Dioscorea* species found in Tripura

| Name of the plant  | Proximate composition | Moisture content (mg/gm) | Total protein (mg/gm) | Carbohydrate (mg/gm) | Soluble sugar (mg/gm) | Free amino acid (mg/gm) | Crude fiber (%) | Total fat (%) |
|--------------------|------------------------|--------------------------|-----------------------|-----------------------|-----------------------|------------------------|----------------|--------------|
|                    | M F                    | M F                      | M F                   | M F                   | M F                   | M F                   | M F            | M F          |
| Dioscorea alata    | 58.21 ± 77.97 ± 4.22 ± 5.93 ± 0.30 0.22 | 211.92 ± 20.91 ± 0.44 0.92 | 180.25 ± 21.12 ± 6.54 | 18.64 ± 0.98 | 1.68 ± 1.72 ± 0.14 0.12 | 3.00 ± 2.74 ± 0.14 0.16 | 0.14 0.12 | 1.66 ± 1.97 ± 0.16 0.14 |
| Dioscorea hamiltonii| 66.72 ± 71.44 ± 3.34 ± 4.21 ± 0.42 0.32 | 254.95 ± 23.63 ± 0.31 0.42 | 20.81 ± 13.37 ± 0.22 0.34 | 1.64 ± 1.87 ± 0.13 0.14 | 1.82 ± 2.23 ± 0.18 0.14 | 3.16 ± 0.12 0.14 | 0.14 0.16 | 1.58 ± 1.24 ± 0.14 0.16 |
| Dioscorea oppositifolia | 66.94 ± 71.18 ± 3.19 ± 5.73 ± 0.14 0.17 | 173.86 ± 14.02 ± 0.22 0.34 | 13.37 ± 2.24 ± 0.09 0.12 | 1.18 ± 1.28 ± 0.18 0.16 | 0.23 ± 3.04 ± 0.23 0.14 | 0.14 0.16 | 0.14 0.16 | 1.58 ± 1.24 ± 0.14 0.16 |
| Dioscorea pubera    | 66.43 ± 75.72 ± 13.21 ± 9.67 ± 0.22 0.22 | 158.01 ± 23.54 ± 0.24 0.19 | 28.20 ± 13.58 ± 0.94 0.15 | 1.19 ± 1.28 ± 0.13 0.23 | 0.14 ± 0.12 ± 0.18 0.14 | 0.14 0.16 | 0.14 0.16 | 1.58 ± 1.24 ± 0.14 0.16 |
| Dioscorea wallichii | 68.62 ± 70.55 ± 7.04 ± 9.84 ± 0.32 0.30 | 136.73 ± 14.92 ± 0.15 0.24 | 5.11 ± 3.24 ± 0.15 0.19 | 1.57 ± 1.58 ± 0.10 0.14 | 0.19 ± 0.14 ± 0.12 0.14 | 0.14 0.16 | 0.14 0.16 | 1.58 ± 1.24 ± 0.14 0.16 |

M male, F female, significant level 5%

*Not significant

< 0.001

< 0.01

< 0.05
antinutrient, antioxidant, and antimicrobial efficacy. The possible reasons for these variations may be attributable to different factors such as genetic, climate, and environmental conditions [44–47]. In line with the earlier findings [48, 49], experimental results showed that a significant and positive correlation exists between the phenolic contents and antioxidant potentiality. The high antioxidant potentiality of Dioscorea tubers may be one of the key determinants of their inclusion in traditional folkloric medicine. Mittelstrass et al. [50] proclaimed that male and female plants differ in their metabotypes which may be attributable to herbivore preferences for gender [51–54] which in turn are correlated secondary metabolite contents. Higher amounts of phenols and antioxidants are connected with the defense strategies of plants [55, 56]. The pinpointing findings of this present study are the sex-specific study of the nutrient, antinutrient, antioxidant, and antimicrobial activity which is lacking in earlier findings. The methanolic extracts of the female tubers have proficient antibacterial potentiality which is in congruence with the earlier finding [57]. Among the tested bacterial strains, gram-positive bacterium (Streptococcus pneumoniae) is more susceptible compared to the gram-negative bacteria which reaffirmed the earlier findings [58–60]. Moreover, among all the selected Dioscorea species, most of the tubers of female plants are superior compared to the male counterparts although they are residing at the same climatic conditions or ecosystems which were the re-confirmation of earlier findings although their studied samples are different [17–19]. To consign, a specific reason for the findings of this present study is may be due to the different physiological and reproductive adaptive responses of both the sexes irrespective of their occurrences in the same microclimate.

| Name of the plants      | Ascorbic acid | Thiamine | Riboflavin |
|-------------------------|--------------|----------|------------|
|                         | M            | F        |            |
| Dioscorea alata         | 13.49 ± 3.64 | 18.26 ± 3.17 | 1.14 ± 0.16 |
|                         | 1.26 ± 0.11  | 1.75 ± 0.26 | 1.87 ± 0.26 |
| Dioscorea hamiltonii    | 10.31 ± 2.75 | 12.7 ± 3.64 | 1.15 ± 0.09 |
|                         | 1.03 ± 0.16  | 0.82 ± 0.07 | 0.98 ± 0.12 |
| Dioscorea oppositifolia | 19.84 ± 3.63 | 26.19 ± 3.28 | 0.94 ± 0.14 |
|                         | 1.14 ± 0.16  | 0.78 ± 0.11 | 1.14 ± 0.08 |
| Dioscorea pubera        | 14.29 ± 2.38 | 15.88 ± 1.37 | 0.85 ± 0.07 |
|                         | 0.99 ± 0.11  | 1.02 ± 0.08 | 0.94 ± 0.14 |
| Dioscorea wallichii     | 9.52 ± 2.38  | 12.7 ± 1.4  | 1.25 ± 0.13 |
|                         | 1.11 ± 0.12  | 1.18 ± 0.1  | 1.13 ± 0.24 |

M male, F female, significant level 5%
*< 0.001
b< 0.01
c< 0.05

Table 4 Anti-nutritional status of male and female tubers of Dioscorea species found in Tripura

| Name of the plant     | Antinutrient status |
|-----------------------|---------------------|
|                       | Alkaloid (%)        |
|                       | Phenol (mg/gm)      |
|                       | Flavonoid (mg/gm)   |
|                       | Tannin (mg/gm)      |
|                       | Saponin (%)         |
|                       | M       | F       | M       | F       | M       | F       | M       | F       | M       | F       |
| Dioscorea alata       | 0.65 ± 0.08        | 1.0 ± 0.07      | 12.21 ± 0.82   | 17.53 ± 1.30   | 14.80 ± 0.69   | 9.17 ± 0.3  | 3.33 ± 0.12 | 5.48 ± 0.18 | 0.48 ± 0.06 | 0.93 ± 0.17 |
| Dioscorea hamiltonii  | 1.05 ± 0.07        | 1.21 ± 0.11     | 41.40 ± 2.94   | 50.70 ± 2.49   | 25.67 ± 0.93   | 36.67 ± 0.99 | 10.08 ± 0.14 | 6.25 ± 0.14   | 0.95 ± 0.14 | 1.16 ± 0.18 |
| Dioscorea oppositifolia | 0.27 ± 0.03       | 0.41 ± 0.04     | 11.03 ± 0.60   | 13.65 ± 0.36   | 7.21 ± 0.99   | 15.03 ± 1.08 | 0.22 ± 0.1    | 3.04 ± 0.06    | 0.45 ± 0.09 | 0.81 ± 0.15 |
| Dioscorea pubera      | 0.35 ± 0.05        | 0.33 ± 0.05     | 31.76 ± 0.21   | 21.83 ± 2.25   | 19.68 ± 1     | 22.17 ± 0.24 | 1.45 ± 0.16  | 1.05 ± 0.22   | 0.88 ± 0.23 | 0.92 ± 0.17 |
| Dioscorea wallichii   | 0.31 ± 0.23        | 0.52 ± 0.06     | 10.73 ± 0.25   | 9.73 ± 0.28    | 20.6 ± 0.6    | 26.00 ± 2.14  | 1.28 ± 0.19  | 2.11 ± 0.21   | 0.91 ± 0.09 | 1.07 ± 0.13 |

M male, F female, significant level 5%
*Not significant
a< 0.001
b< 0.01
c< 0.05
**Conclusion**

This study is a precursive effort to estimate the sex-specific variations in the phytochemical constituents and biological activities of *Dioscorea* tubers. Further researches are needed to be carried out by incorporating modern scientific tools to underpin the actual physiological mechanism associated with these variations which may be helpful in the early delimitation of sexes. This study also ensures that female *Dioscorea* tubers are the reservoir of biological compounds compared to the male counterpart which may be able to draw the attention of nutraceutical industries leading to the discovery of noble drugs.

**Table 5** Antimicrobial activity of male and female tubers of *Dioscorea* species found in Tripura

| Name of the plants            | Antimicrobial activity ( inhibition zone in mm) | Fungal strain                                      |
|-------------------------------|-------------------------------------------------|---------------------------------------------------|
|                               | Sex     | Klebsiella pneumoniae (MTCC-3384) | Streptococcus pneumoniae (MTCC-655) | Escherichia coli (MTCC-443) | Shigella dysenteriae (MTCC-227) | Candida albicans (MTCC-227) | Candida tropicalis (Agartala medical college) |
|-------------------------------|---------|---------------------------------|--------------------------------------|-----------------------------|-------------------------------|---------------------------|-----------------------------------------------|
| **Dioscorea alata**            | M       | –                               | –                                    | –                           | –                             | –                         | 7.8 ± 0.20                                     |
|                               | F       | –                               | 8.76 ± 0.25                          | –                           | –                             | 7.66 ± 0.35                | –                                             |
| **Dioscorea hamiltonii**       | M       | –                               | 11.80 ± 1.05                         | –                           | –                             | –                         | 6.93 ± 0.15, 7.23 ± 0.32                        |
|                               | F       | –                               | 12.13 ± 0.75                         | –                           | 8.70 ± 0.36                  | –                         | –                                             |
| **Dioscorea oppositifolia**    | M       | 10.70 ± 0.36                     | 9.36 ± 0.55                          | 8.23 ± 0.25                 | –                             | 7.83 ± 0.15                | 7.53 ± 0.50                                   |
|                               | F       | 9.46 ± 0.50                      | –                                    | 11.0 ± 0.40                 | –                             | 7.96 ± 0.15                | 9.83 ± 0.15                                   |
| **Dioscorea pubera**           | M       | 9.43 ± 0.51                      | 11.76 ± 0.68                         | 10.53 ± 0.50                | 10.86 ± 0.23                 | 8.56 ± 0.40                | 6.83 ± 0.20                                   |
|                               | F       | 10.56 ± 0.40                     | 11.76 ± 0.68                         | 9.8 ± 0.2                   | 8.60 ± 0.52                  | 10.80 ± 0.20               | 8.93 ± 0.20                                   |
| **Dioscorea wallichii**        | M       | 8.60 ± 0.40                      | –                                    | –                           | 7.83 ± 0.20                  | –                         | 8.80 ± 0.20                                   |
|                               | F       | 9.30 ± 0.26                      | 8.76 ± 0.20                          | 8.86 ± 0.32                 | 9.96 ± 0.15                  | 9.83 ± 0.28                | 4.23 ± 0.25                                   |

M male, F female

**Table 6** Inhibitory concentration of male and female tubers of *Dioscorea* species found in Tripura

| Plant name               | Sex  | IC<sub>50</sub> (μg/ml) |
|--------------------------|------|-------------------------|
| Dioscorea alata          | Male | 232.58                  |
| Dioscorea alata          | Female | 284.71                |
| Dioscorea hamiltonii     | Male | 648.86                  |
| Dioscorea hamiltonii     | Female | 340.50                |
| Dioscorea oppositifolia  | Male | 25.17                   |
| Dioscorea oppositifolia  | Female | 18.98                 |
| Dioscorea pubera         | Male | 313.58                  |
| Dioscorea pubera         | Female | 209.02                |
| Dioscorea wallichii      | Male | 234.85                  |
| Dioscorea wallichii      | Female | 267.50                |
| Ascorbic acid (standard) |      | 8.03                    |

**Abbreviations**

m asl: Height above sea level; SD: Standard deviation; MC: Moisture content; TP: Total protein; TC: Total carbohydrate; TSS: Total soluble sugar; TFA: Total free amino acids; TF: Total fat; TCF: Total crude fiber; Aa: Ascorbic acid; Th: Thiamine; Rf: Riboflavin; TA: Total alkaloid; TPH: Total phenol; TF: Total flavonoid; TT: Total tannin; TS: Total saponin

**Acknowledgements**

The authors are thankful to the Head of the Department of Forestry and Biodiversity for providing the laboratory facilities to carry out the work. The authors are thankful to Dr. Bipin Kumar Sarma for providing the microbial strains to study the antimicrobial activity.

**Authors’ contributions**

CP and BD designed the manuscript. CP, AD, and SG carried out the experimental work. CP and KC contributed to the analysis of the data. CP, KC, and AB wrote the manuscript. All authors read and approved the final manuscript.

**Funding**

None

**Availability of data and materials**

All data and materials are available upon request.

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Competing interests**

The authors declare that they have no competing interests.

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