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In-vitro antimicrobial efficacy and phytochemical analysis of *Dioscorea alata* from Bastar district of Chhattisgarh, India

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Abstract
The present research prospects deals with the assessment of antimicrobial activity and phytochemical analysis of *Dioscorea alata* (Family: Dioscoreaceae) from Bastar district of Chhattisgarh against eight pathogenic bacteria *viz*., *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435), *Escherichia coli* (MTCC 1687), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 741), *Proteus vulgaris* (MTCC 744) and two fungi *viz*., *Aspergillus niger* (MTCC 872) and *Candida albicans* (MTCC 183) procured from IMTECH, Chandigarh. Different plant parts like root, stem and leaf were extracted successively in soxhlet apparatus using four different solvents from non-polar to polar *viz*., chloroform, acetone, methanol and aqueous based on their polarity index. The antimicrobial activity was assessed by agar well diffusion method. The antibacterial potentiality of *D. alata* revealed that acetone root extract exhibited highest activity against *B. cereus*. In case of Gram-negative bacteria the extract exhibited maximum activity against *P. aeruginosa*. The acetone stem extract showed comparatively less activity. However, the acetone leaf extract exhibited comparatively less activity against Gram-positive bacteria. The evaluation of antifungal activity revealed that methanol root extract was effective against *A. niger* and *C. albicans*. The highest activity index was recorded with methanol root extract for *A. niger* and *C. albicans*. The phytochemical analysis of *D. alata* showed that percentage yield of root was highest in aqueous followed by methanol, chloroform and acetone. In stem the yield was maximum in aqueous followed by methanol, chloroform and acetone. In leaf the yield was more in chloroform followed by aqueous, methanol and acetone. Alkaloids were present in aqueous root and stem extracts. The flavonoids were present in all the extracts except chloroform. Phytosterols gave a positive reaction for all the extracts more so with methanol and acetone extracts. Tannins were detected in root and stem extracts of aqueous, methanol and acetone. Saponins were present in all the extracts except chloroform. Resins gave strong positive test in chloroform and acetone root extracts. The glycosides were feebly recorded in methanol stem, acetone and aqueous root extracts. The quantitative estimation of alkaloid, flavonoid, saponin and total phenol in root, stem and leaf of *D. alata* showed that root sample contains highest amount of saponin followed by total phenol, flavonoid and alkaloid. The content was highest in root followed by stem and leaf of *D. alata*. Therefore, in light of present context an effort to further explore the medicinal or natural products towards improving health care delivery deserves attention.

Keywords: *Dioscorea alata*, antimicrobial activity, phytochemical analysis, soxhlet

Introduction
Medicinal plants are endowed with a rich wealth of bioactive compounds that are the valuable source of drugs for curing several dreadful diseases since time immemorial. *Dioscorea* is known as yam named after the ancient Greek Physician and Botanist Dioscorides. There are over 600 species of *Dioscorea* in the world, 12 of which are used as staple medicinal food and is of great economic importance [1-2]. India holds a rich genetic diversity of *Dioscorea*; so far 26 of its species have been documented [3]. *Dioscorea alata* is an important tuber crop and is a staple food for millions of peoples in tropical and subtropical countries [4]. It is the world's leading sources for carbohydrates, low level of fats, fibers and dietary nutrients consumed [5-9]. *Dioscorea alata*, a tuberous herbaceous perennial climbing shrub reaching a length of 2- 12 meters with glabrous leaves and twining stems, generally cultivated in between forest plants [10-11]. Leaves are heart shaped and spirally arranged. Flowers are unisexual and fruits as capsules. It is highly polymorphic in their shape and colour of the tuber (Figure 1). *D. alata* L. tubers can be stored for about 3-4 months or longer depending on storage conditions. Water yam tuber varieties are identified by its flesh color as yellow (*D. alata* L.) and purple (*D. alata* L. *var. purpurea*).

The Indo-Burma region is the centre of origin of Asiatic edible yams. Among yam tubers *Dioscorea alata*, is the most widely distributed species in humid and semi-humid tropics [12]. Many species of genus *Dioscorea* are economically important worldwide *viz*., *D. alata*, *D. cayenensis* and *D. rotundata* mainly in South America, West Africa and Caribbean Central [13]. The important yam species in Nigeria are *D. alata, D. cayensis, D. bulbifera*, *D.* rotundata, *D. esculenta* and *D. dumentorium*. In Indonesia yam family such as *D. alata* L., *D. hispida*, *D. bulbilfera* L., and *D. esculenta* have productivity of 60-70 ton/ ha. Yams contains low sugar content which may be helpful in preventing diabetes and reduce blood sugar [14-16]. Diosgenin is a phytochemical that controls cholesterol metabolism and prevent colon cancer [17-18]. It is used for the commercial synthesis of corticosteroids used for anti-inflammatory, androgenic and contraceptive drugs [19]. It also shows antimicrobial activity [20], antitumor activity [21], antioxidative activity [22] and estrogenic effect [23]. Dioscorin, a storage protein, in some species of yam acts as antioxidant, immunomodulator and antihypertensive [24-25]. Thus, in light of vast potentiality of *Dioscorea alata* as therapeutics there is an urgent need to explore its bioefficacy and pharmacological potentiality with the subsequent isolation, purification and characterization of the bioactives with several sophisticated analytical instruments which will pave the path for herbal therapy in years to come.

Kingdom	Plantae
Division	Tracheophyta
Class	Liliopsida
Order	Dioscoreales
Family	Dioscoreaceae
Genus	*Dioscorea*
Species	*alata*

Fig 1: Sample collection from Bastar district of Chhattisgarh

Materials and Methods

Site of Study: Chhattisgarh is a land-locked state in the heart of India. It lies between 17º 46″-24º8″ N latitude and 80º 15″-84º24″ E longitude. The average annual rainfall in Chhattisgarh is 1405.3 mm. The state shares its boundaries with the 6 Indian states *viz*., Madhya Pradesh on the north-west,Uttar Pradesh on the north, Jharkhand on the north-east, Orissa on the south-east, Andhra Pradesh on the south and Maharastra on the south-west. The mainland of Chhattisgarh comprises of three main physiographic regions; namely, Mountains (27.50%), Plateau and pat regions (29.29%) and Plains/ river basins (43.21%). Agro-climatically the entire state falls in the category of zone-VII (Eastern Plateau and Hill zone) and can be sub divided into three sub agro-climatic zones, namely, Chhattisgarh Plains, the Northern Hills of Chhattisgarh and the Bastar plateau. The forests of the state fall under two major forest types *viz*., Tropical moist deciduous forest and Tropical dry deciduous forest. The state of Chhattisgarh is endowed with about 22 varied forest sub-type existing in the state. Total forest area of the state is 59,772 Sq. Km. Out of which, the reserved forest is 25782 Sq. Km (43.13%), protected forest is 24036 Sq. Km (40.22%) and unclassified forest is 9954 Sq. Km (16.65%). Bastar district (19.1071ºN, 81.9535ºE) is located in southern part of Chhattisgarh and has an area of 4029.98 km^2. It is surrounded by Bijapur, Dantewada, Kondagaon, Narayanpur and Sukma districts of the state. Bastar district is divided into seven Tehsils *viz*., Jagdalpur, Bastar, Bakawand, Bastanar, Darbha, Lohandiguda and Tokapal. Jagdalpur is both district and divisional headquarter of Bastar district (Figure 2). The city lies on the southern bank of river Indravati with an average elevation of 562 meters. It has a total forest area of 292130 ha which is more than 19% of the total land area of the district.

Selection of Medicinal Plant

The medicinal plants of *Dioscorea alata* (Family: Dioscoreaceae) was selected for the assessment of antimicrobial activity based on their traditional usage by the tribal community of Bastar district in curing several ailments and its ethno-medicinal importance as herbal drug.

Sample Collection and Identification

The root, stem and leaves of fresh and apparently healthy plants of *Dioscorea alata* was collected from the nursery and field area within the campus of Shaheed Gundadhur College of Agriculture and Research Station, Kumhrawand, Jagdalpur, Chhattisgarh, India after their authentication and identification at department of Agronomy and Horticulture, SGCARS, Jagdalpur, Chhattisgarh, India.

Drying and Grinding of the Sample

The collected plant samples were washed thoroughly under running tap water to remove debris and were separated into root, stem and leaf and shade dried at room temperature for about three weeks to attain a constant weight. Exposure to direct sunlight was avoided to prevent the loss of active compounds from the sample. The dried samples were mechanically grinded by pestle & mortar and finally powdered by grinding machine (Remi), packed in plastic bags and stored in airtight bottles at 4ºC for further use (Figure 3).

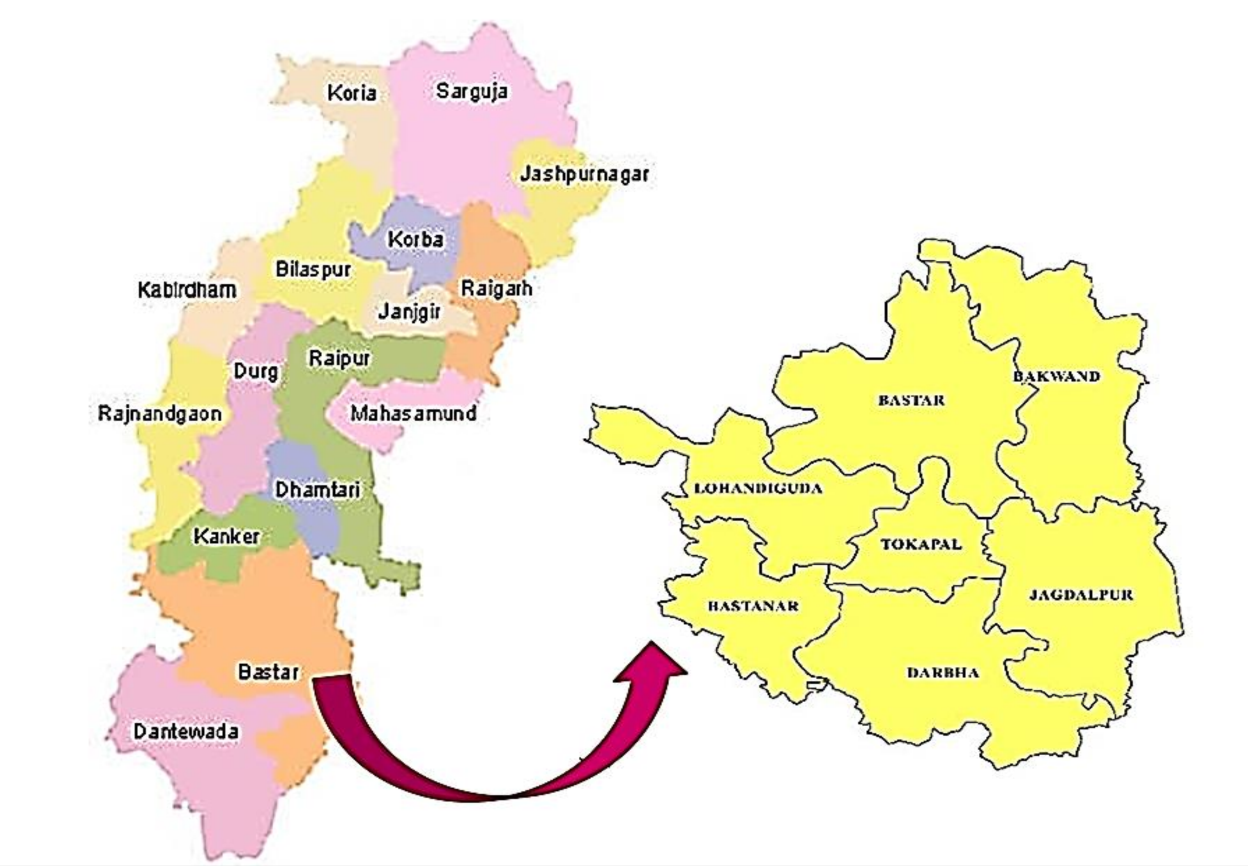

Fig 2: Map of Bastar district showing sample collection area

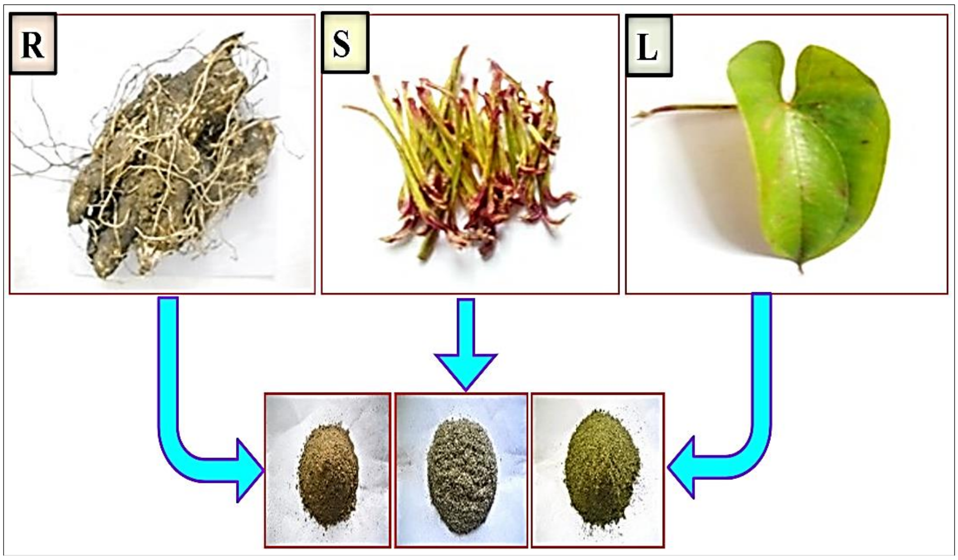

Fig 3: Dried and powdered sample of *Dioscorea alata*

Extraction Method

The extraction of phytochemicals was done through hot extraction. In soxhlet apparatus powdered material was placed in a thimble of filter paper, which was loaded into the extracting unit of the apparatus. The extracting unit was placed onto round bottom flask containing the solvent and the top was equipped with a condenser. The entire assembly was set on a heating mantle with temperature regulator. The solvent in the flask was heated and its vapour traveled up to the distillation arm and after condensation flew into the extracting unit housing the thimble (Figure 4). The powdered plant material was extracted sequentially in four different solvents *viz*., chloroform, acetone, methanol and aqueous. 15g powdered material was extracted in 150 ml of chloroform, acetone, methanol and in aqueous according to their increasing polarity index in the soxhlet apparatus (Tempo) for 8-10 hours at a temperature not exceeding the boiling point of the respective solvents. The extracted material was dried to residue and the yield was determined by gravimetric method. The sample was dissolved in 50% dimethyl sulphoxide to prepare a 10% stock solution (w/v) and stored in refrigerator at 4^{o}C in small and sterile glass tubes until use.

Fig 4: Soxhlet apparatus for extraction of phytochemicals

Determination of Percentage Yield

The percentage yield of different solvent extracts was calculated using the formula $W_2-W_1/W_0\times100$. Where, W_2 is the weight of the extract and the container, W_1 the weight of the container alone and W_0 the weight of the initial dried sample [26].

Microorganism used for Antimicrobial Activity

Antimicrobial activity was assessed against microbial cultures procured from Institute of Microbial Technology, Chandigarh, India. The bacterial strains included four Gram-positive, four Gram-negative and two fungal organisms (Table 1).

Table 1: List of bacteria and fungus MTCC cultures with their reference number

S. No.	Test Microorganisms	MTCC Reference No.
1	*Bacillus cereus*	MTCC 430
2	*Bacillus subtilis*	MTCC 441
3	*Staphylococcus aureus*	MTCC 96
4	*Staphylococcus epidermidis*	MTCC 435
5	*Escherichia coli*	MTCC 1687
6	*Klebsiella pneumoniae*	MTCC 3384
7	*Pseudomonas aeruginosa*	MTCC 741
8	*Proteus vulgaris*	MTCC 744
9	*Aspergillus niger*	MTCC 872
10	*Candida albicans*	MTCC 183

The culture details as provided by the IMTECH, Chandigarh, India included the following:

1. ***Bacillus cereus* (MTCC 430):** Growth medium: Nutrient Agar; Growth conditions: Aerobic; Temperature: 30ºC; Incubation time: 24 h; Subculture: 30 days; Special feature: Used for assay of chlortetracycline, oxytertacycline and tetracycline.
2. ***Bacillus subtilis* (MTCC 441)**: Growth medium: Nutrient Agar; Growth conditions: Aerobic; Temperature: 30ºC; Incubation time: 24 h; Subculture: 30 days; Special feature: Used for testing of several antibiotics, production of subtilin, assay of chlortetracycline, streptomycin, viomycin and penicillin in milk.
3. ***Staphylococcus aureus* (MTCC 96):** Growth medium: Nutrient Agar; Growth conditions: Aerobic; Temperature: 37ºC; Incubation time: 48 h; Subculture: 30 days; Special feature: Pathogenic, control strain for antibiotic susceptibility testing (for various antibiotics), teaching strain, coagulase test.
4. ***Staphylococcus epidermidis* (MTCC 435):** Growth medium: Nutrient Agar; Growth conditions: Aerobic; Temperature: 37ºC; Incubation time: 24 h; Subculture: 30 days.
5. ***Escherichia coli* (MTCC 1687):** Growth medium: Nutrient Agar; Growth conditions: Aerobic; Temperature: 37ºC; Incubation time: 24 h; Subculture: 30 days; Special feature: Used for assay of antimicrobial agents in aqueous metal working fluids, assay of antimicrobial preservatives, media testing, reduction of dehydroascorbic acid.
6. ***Klebsiella pneumoniae* (MTCC 3384)**: Growth medium: Nutrient Agar; Growth conditions: Aerobic; Temperature: 37ºC; Incubation time: 24 h; Subculture: 30 days.
7. ***Pseudomonas aeruginosa* (MTCC 741)*:*** Growth medium: Nutrient Agar; Growth conditions: Aerobic; Temperature: 37ºC; Incubation time: 12 h; Subculture: 30 days; Special feature: Pathogenic, used as standard control negative strain in antibiotic sensitivity testing (Stroke's method), gentamycin sensitive.
8. ***Proteus vulgaris* (MTCC 744):** Growth medium: Nutrient Agar; Growth conditions: Aerobic; Temperature: 37ºC; Incubation time: 12 h; Subculture: 30 days; Special feature: Used for Well-Felix test.
9. ***Aspergillu niger* (MTCC 872):** Growth medium: Potato Dextrose Agar; Growth conditions: Aerobic; Temperature: 30ºC; Incubation time: 48 h; Subculture: 30 days.
10. ***Candida albicans* (MTCC 183):** Growth medium: Potato Dextrose Agar; Growth conditions: Aerobic; Temperature: 30ºC; Incubation time: 48 h; Subculture: 60 days; Special feature: Used for assay of sterility testing, fungicide tests.

Activation of Freeze Dried MTCC Cultures: The ampoule containing freeze dried cultures were opened carefully by making a mark on the ampoule with a sharp file near the middle of the cotton wool and the surface around the mark was disinfected with alcohol. The marked area on the ampoule was broken by wrapping a thick cotton wool around it. The pointed top of the ampoule (Figure 5) was removed gently so as to draw the cotton plug to one end. The cotton plug was removed carefully and about 0.3 to 0.4 ml of specified medium was added to make a suspension of each culture. For fungal cultures, suspension was made in 0.4 ml sterile water and allowed it to stand for 20 minutes before transferring to the solid medium. Thereafter, few drops of the suspension were streaked on to the recommended medium in a petri plate and rest of the suspension was transferred to the 5 ml of recommended liquid medium in a test tube. Finally, all the cultures were incubated at the appropriate temperature and under defined conditions. All the remains in the original ampoule were sterilized before discarding.

Fig 5: MTCC culture vials of microorganisms

Preparation of Bacterial Inoculums: The test organisms were maintained on nutrient agar slants. An overnight grown bacterial culture was used for inoculum preparation. One loop full of overnight growth from each bacterial culture was inoculated in 25 ml nutrient broth and incubated at 37ºC for 24h in incubator. The inoculum size of each bacterial strain was standardized by adjusting the optical density of the culture broth by adding saline suspension to a turbidity corresponding to 0.08-0.13 at 620 nm using a spectrophotometer which was equivalent to 10^8 cfu/ ml [27].

Assessment of Antibacterial Activity: The antibacterial activity of the crude extracts was determined by the agar-well diffusion method [28]. 200 µl of the standardized cell suspension was spread on Muller Hinton Agar (Hi-media) plate using a sterile swab and air dried to remove the surface moisture. 6 mm diameter wells were bored into the agar plate using a sterile cork borer. The crude extract was introduced into the well at a concentration of 2 mg/ 20µl. The plates were allowed to stand at room temperature for about 1 h as a period of pre-incubation diffusion to minimize the effect of variation in time between the application of different solutions and later the plates were incubated at 37ºC for 24 h. Controls were also set up in parallel and the effects were compared with penicillin and streptomycin at a concentration of 10 µg/ 20µl. The plates were observed for the zone of inhibition after 24 h. The experiment was conducted in triplicates and the values are expressed as Mean ±SE.

Preparation of Fungal Inoculums: The test fungal organisms were maintained on potato dextrose agar slants. One loop full of each fungal culture was inoculated in 25 ml potato dextrose broth and incubated at 28-30ºC in incubator. Stock inoculum suspensions were prepared from 7 day-old cultures grown on potato dextrose agar (Hi-media) following National Committee for Clinical Laboratory Standards [29]. Stock suspensions were adjusted to optical densities that ranged from 0.09-0.11 at 530 nm using a spectrophotometer which was equivalent to 0.9×10^4 to 4.7×10^4 cfu/ ml.

Assessment of Antifungal Activity: The antifungal activity of the plant extract was evaluated by agar-well diffusion method [30]. 200 µl of the standardized cell suspension was spread on Potato dextrose agar (Hi-media) plate using a sterile swab and air dried to remove the surface moisture. Wells were bored into the agar using a sterile 6 mm diameter cork borer. The crude extract was aseptically introduced into the well at a concentration of 2 mg/ 20µl, allowed to stand at room temperature for about 1 h as a period of pre-incubation diffusion to minimize the effect of variation in time between the application of different solutions and later the plates were incubated at 28-30ºC for 48 h. Controls were also set up in parallel and effects were compared with clotrimazole as standard antifungal at a concentration of 10 µg/ 20µl. The plates were observed for the zone of inhibition after 48 h. The experiment was conducted in triplicates and the values are expressed as Mean ±SE.

Zone Size Interpretation: Test organism showing a clear zone of inhibition (ZOI) was scored as (07-10 mm) as non-inhibitory activity (Resistant), inhibition ranging (11-15 mm) was considered to be inhibitory activity (Sensitive), and greater than (16-20 mm) was considered as significant inhibitory or antimicrobial activity [31].

Activity Index: The activity index for all the five plants under study with different solvent extracts was determined using streptomycin as standard antibacterial and clotrimazole as standard antifungal agent. The activity index was expressed as zone of inhibition of test sample/ zone of inhibition with standard antimicrobials [32].

Qualitative Phytochemical Analysis: Phytochemical analysis of the extracts of root, stem and leaf of five medicinal plants was carried out following [33-34].

1. Alkaloids

a) **Mayer's Test:** 1ml of Mayer's reagent (1.36 g mercuric chloride and 5.00 g potassium iodide were dissolved in 100 ml distilled water) was added to 1ml of the extract. Whitish yellow or cream coloured precipitate indicated the presence of alkaloids.
b) **Hager's Test:** 1ml of Hager's reagent (saturated aqueous solution of picric acid) was added to 1ml of the extract. A yellow coloured precipitate indicated the presence of alkaloids.
c) **Wagner's Test:** 1 ml of Wagner's reagent (2 g iodine and 6 g potassium iodide were dissolved in 100 ml distilled water) was added to 1 ml of the extract solution. Formation of reddish brown precipitate indicated the presence of alkaloids.

2. Flavonoids

a) **Alkaline reagent Test:** Test solution was treated with 10% NaOH solution. Appearance of yellow colour which becomes colourless on addition of few drops of dilute acid indicated the presence of flavonoids.
b) **Lead acetate solution Test:** Test solution was treated with few drops of 10% lead acetate solution. Yellow precipitate indicated the presence of flavonoids.

3. Saponins

a) **Foam Test:** 1 ml of the extract was shaken vigorously with 1 ml of distilled water. Persistent foam was observed which indicated the presence of saponins.

4. Tannins

a) **Gelatin Test:** 1 ml of 1% gelatin solution was added to 1 ml of the extract. The appearance of white precipitate indicated the presence of tannins.

b) **Ferric chloride Test:** Few drops of 5% ferric chloride solution were added to 1 ml of the extract. Deep blue-black colour indicated the presence of tannins.

5. Phytosterols: 20 mg of the extract was dissolved in 5 ml of chloroform separately and was subjected to the following tests.

a) **Salkowski Test:** To 1ml of the above extract solution few drops of conc. H_2SO_4 was added. Formation of brown ring indicated the presence of phytosterols.

b) **Libermann-Burchard Test:** To 1 ml of the above prepared solution few drops of conc H_2SO_4 was added followed by few drops of acetic anhydride. A bluish green colour showed the presence of phytosterols.

6. Glycosides: 10 ml of 50% H_2SO_4 was added to 1ml of the extract. The mixture was heated in a boiling water bath for 15 min and 5 ml of Fehling A (7 g copper sulphate was dissolved in 100 ml distilled water and 2 drops of dil. sulphuric acid was added) and 5 ml of Fehling B (35 g potassium tartarate and 12 g sodium hydroxide were dissolved in 100 ml distilled water) was added to the mixture and boiled. The presence of brick red precipitate indicated the presence of glycosides.

7. Quinones: A few drops of 1% NaOH was mixed with 1 ml of the plant extract and shaken vigorously. A blue green or red colour indicated the presence of quinones.

8. Resins: 5 ml of the 1% copper acetate solution was added to 1 ml of the extract, shaken vigorously and allowed to separate. The separation of a green coloured solution indicated the presence of resins.

Quantitative Phytochemical Analysis

Alkaloids: 2.5 g of each sample was weighed into a 250 ml beaker and 100 ml of 20% acetic acid in ethanol was added, mixed and allowed to stand for 4 h. This was filtered and the extract was concentrated using a water bath to evaporate one-quarter of the original volume. The concentrated ammonia solution was added drop-wise to the extract until the precipitation was completed. The entire solution was allowed to settle. The precipitate was filtered through Whatman filter paper No. 42 and weighed as total alkaloids [35]. The value was expressed in percent.

Flavonoids: 2.5 g of sample was weighed in a 250 ml titration flask and 50 ml of the 80% aqueous methanol was added at room temperature and shaken for 4 h in an electric shaker. The entire solution was filtered through Whatman filter paper No. 42. The process was repeated. The whole filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed as total flavonoids [36]. The value was expressed in percent.

Saponins: 2.5 g of sample was weighed and dispersed in 50 ml of 20% ethanol. The suspension was heated at 55ºC in a water bath for 4 h with continuous stirring. The filtrate and the residue were re-extracted with another 50 ml of 20% ethanol. The combined extracts were reduced to 20 ml over water bath at 90ºC. The concentrate was transferred into a 250 ml separating funnel and 10 ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 15 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 5 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight as saponins [35]. The saponin content was expressed as percentage.

Total Phenols

2.5 g of each plant sample was weighed into 250 ml conical flask and 50 ml n-hexane was added twice for 4 h each, the filtrates were discarded for fat free sample preparation. 25 ml diethyl ether was added twice, heated for 15 min each, cooled up to room temperature and was filtered into a separating funnel. About 25 ml of the 10% NaOH solution was added twice and shook well each time to separate the aqueous layer from the organic layer. It was washed three times with 12.5 ml de-ionized water. The total aqueous layer was acidified up to pH 4.0 by adding 10% HCl solution and 25 ml dichloro methane twice to acidify the aqueous layer in the separating flask. Consequently, the organic layer was collected, dried and weighed as total phenols and expressed as percentage [35].

Statistical Analysis: All the results were analyzed statistically wherever required by one way-ANOVA using SPSS version 16.0 software.

Results and Discussion

The present research investigation envisages the assessment of antimicrobial activity and phytochemical analysis of *Dioscorea alata* (Family: Dioscoreaceae) from Bastar district of Chhattisgarh against eight pathogenic bacteria *viz*., *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435), *Escherichia coli* (MTCC 1687), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 741), *Proteus vulgaris* (MTCC 744) and two fungi *viz*., *Aspergillus niger* (MTCC 872) and *Candida albicans* (MTCC 183) procured from IMTECH, Chandigarh. Different plant parts like root, stem and leaf were extracted successively in soxhlet apparatus using four different solvents from non-polar to polar i:e. chloroform, acetone, methanol and aqueous based on their polarity index. An effort was made to assess which part of the plant and the solvent exhibits higher antimicrobial activity against MTCC pathogenic cultures of bacteria and fungi. The results of antimicrobial activity were compared with respect to standard antimicrobials and their activity index was determined.

The percentage yield and physical properties of root, stem and leaf extracts of *D. alata* in different solvents are presented in Figure 6. The results revealed that root extracts were almost waxy to semisolid in nature with light yellow to reddish brown in color and the percentage yield was highest in aqueous (5.00±0.08) followed by methanol (3.80±0.10), chloroform (1.25±0.08) and acetone (0.33±0.07). The stem extracts were sticky to viscous in nature with dark green to brown in color. The percentage yield was highest in case of aqueous (3.06±0.10) followed by methanol (2.73±0.10), chloroform (1.46±0.08) and acetone (0.51±0.05). The leaf extracts were sticky to waxy in nature with blackish green to dark brown in color and the percentage yield was more in chloroform (3.01±0.13) followed by aqueous (1.93±0.12), methanol (1.06±0.13) and acetone (0.58±0.11).

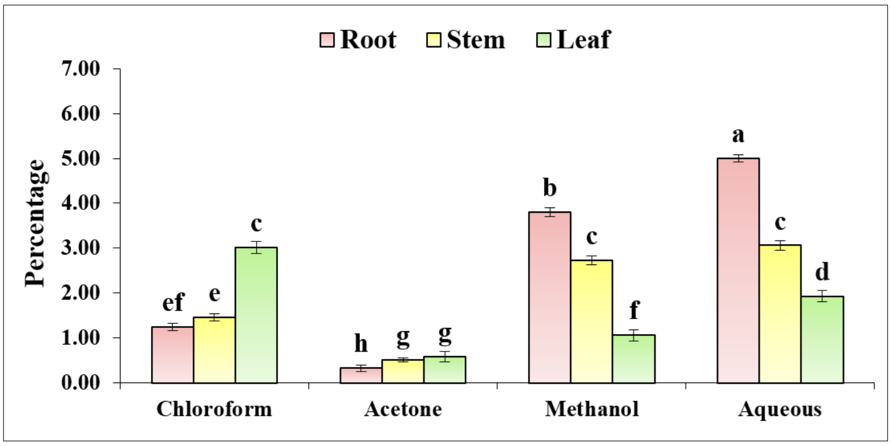

Fig 6: Yield of root, stem and leaf extracts of *D. alata* in different solvents (ANOVA Summary: $F_{11, 24} = 134.353$, $p<0.001$, Means having different alphabets, as superscripts, are statistically significant from each other at $p<0.001$) (Based on Duncan's multiple-range test)

The antibacterial activity in crude solvent extracts of root, stem and leaf of *D. alata*. was assessed against both Gram-positive and Gram-negative bacteria (Figure 7-8). The acetone root extract exhibited zone of inhibition of 10.46±0.06 mm against *B. cereus* followed by *S. aureus* (10.00±0.00 mm) and *S. epidermidis* (08.33±0.24 mm). The extract showed higher activity against *Pseudomonas aeruginosa* (14.86±0.06 mm) followed by *K. pneumoniae* (09.53±0.17 mm) and no activity against *E. coli* and *P. vulgaris* in Gram-negative bacteria. The acetone stem extract exhibited zone of inhibition against two Gram-positive bacteria *viz*., *B. cereus* (10.20±0.11 mm) & *S. aureus* (07.26±0.17 mm) and two Gram-negative bacteria *viz*., *Pseudomonas aeruginosa* (13.33±0.24 mm) & *K. pneumoniae* (09.40±0.20 mm). The acetone leaf extract exhibited comparatively less activity with zone of inhibition against two Gram-positive bacteria *viz*., *B. cereus* (08.13±0.13 mm) and *S. aureus* (07.06±0.06 mm) whereas no activity was recorded against the Gram-negative bacteria tested. However, methanol and chloroform root, stem and leaf extracts produced nearly comparable activity against all the organisms tested (Figures 7-8). The antibacterial activity of *D. alata* revealed that acetone root extract exhibited highest zone of inhibition against *B. cereus* among Gram-positive and maximum activity against *Pseudomonas aeruginosa* among Gram-negative bacteria. The acetone stem and leaf extracts showed comparatively less activity. The methanol and chloroform root, stem and leaf extract exhibited less but nearly comparable activity. The antibacterial activity in Gram-positive bacteria was found to be higher than Gram-negative bacteria in acetone root extract followed by methanol and chloroform. In good support of experimental results, several studies have also reported that Gram-positive bacteria are more sensitive to plant extracts due to their thick porous peptidoglycan cell wall which allows plant extracts to easily penetrate the cell and reach their targets, whereas Gram-negative bacteria are more resistant because their thin peptidoglycan layer is protected by an additional, complex outer membrane made up of lipopolysaccharides (LPS) with pores called porins, which acts as a significant protective barrier, preventing plant extract to penetrate and reach their intracellular targets within the cell [37-39]. The highest activity index was recorded for *P. aeruginosa* followed by *K. pneumoniae* whereas, for Gram-positive bacteria the highest activity index was recorded in case of *B. cereus*, followed by *S. aureus* and *S. epidermidis*. The evaluation of antifungal activity in *D. alata* revealed that methanol and acetone extracts showed activity against *A. niger* and *C. albicans*. However, no activity was observed in chloroform and aqueous extracts. The highest activity index was recorded with methanol root extract for *A. niger* and *C. albicans*. The decline or no antimicrobial activity in aqueous extract might be due to the excessive heating of the active constituents during the extraction process which may lead to low or no bioefficacy of the phytoconstituents [40-42].

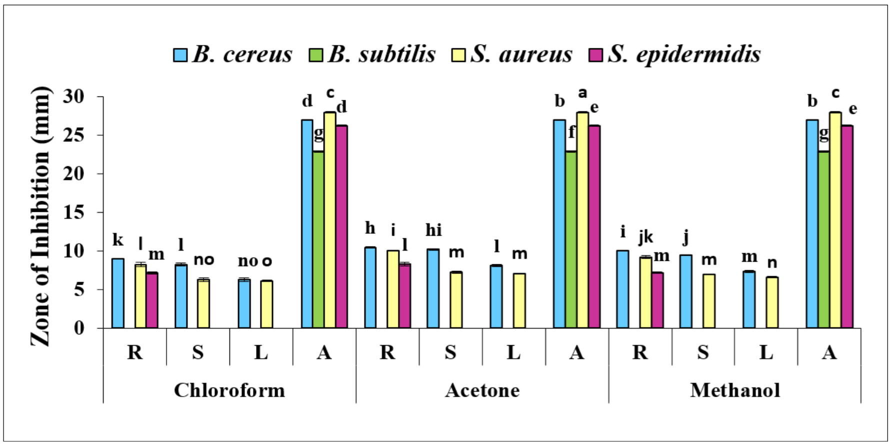

Fig 7: Antibacterial activity of root (R), stem (S) and leaf (L) extracts of *D. alata* against Gram-positive bacteria *vis-a-vis* streptomycin (A) (ANOVA Summary: $F_{47, 96} = 6706.00$, $p<0.001$, Means having different alphabets, as superscripts, are statistically significant from each other at $p<0.001$) (Based on Duncan's multiple-range test)

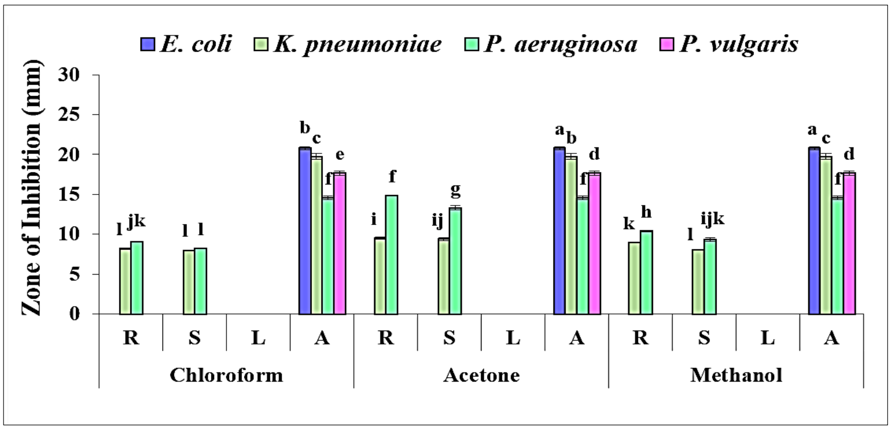

Fig 8: Antibacterial activity of root (R), stem (S) and leaf (L) extracts of *D. alata* against Gram-negative bacteria *vis-à-vis* streptomycin (A) (ANOVA Summary: $F_{47, 96} = 3914.00$, $p<0.001$, Means having different alphabets, as superscripts, are statistically significant from each other at $p<0.001$) (Based on Duncan's multiple-range test)

The activity index of root, stem and leaf extracts of *D. alata* with respect to streptomycin is presented in Figure 9. The highest activity index of 0.99 was recorded in case of Gram-negative bacteria *P. aeruginosa* followed by *K. pneumoniae* (0.50) whereas, for Gram-positive bacteria the highest activity index was recorded in case of *B. cereus* (0.40) followed by *S. aureus* (0.37) and *S. epidermidis* (0.32). The least activity index was recorded in case of chloroform leaf extract in case of *B. cereus* (0.22) and *S. aureus* (0.23).

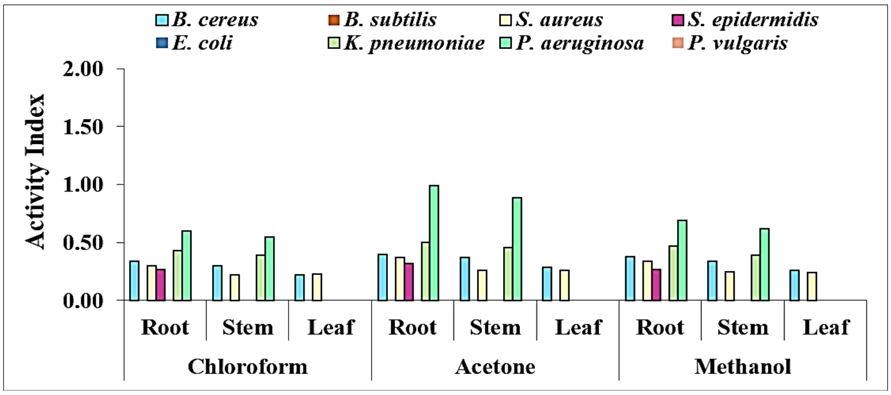

Fig 9: Activity index of root, stem and leaf extracts of *D. alata* with reference to streptomycin

The antifungal activity in crude extract of root, stem and leaf extracts of *D. alata* was assessed against two fungal cultures *viz*., *A. niger* and *C. albicans*. The study revealed that the methanol root extract showed zone of inhibition of 08.33±0.24 mm followed by acetone (08.13±0.13 mm) against *A. niger*. In case of *C. albicans* the inhibition was found to be 08.46±0.06 mm in methanol root extract followed by 07.20±0.11 mm in acetone root extract. The least antifungal activity was observed in case of acetone leaf extract for *A. niger* (07.00±0.00 mm) and for *C. albicans* (06.66±0.13 mm) The activity with clotrimazole is presented in Figure 10.

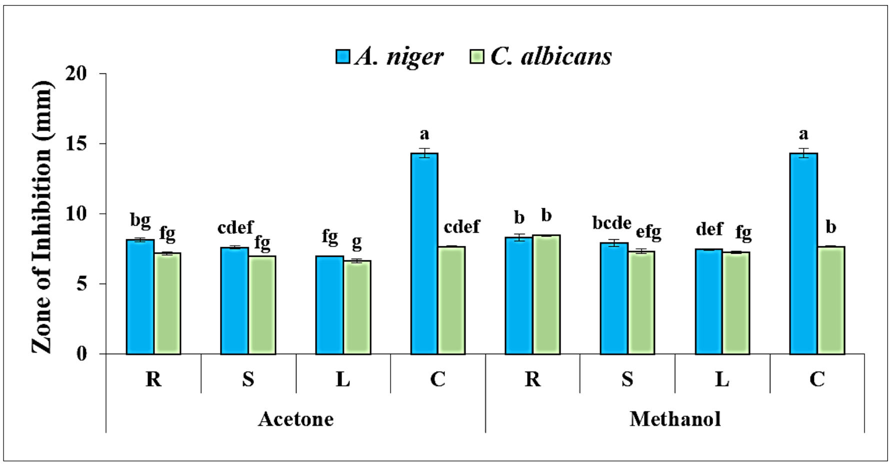

Fig 10: Antifungal activity of root (R), stem (S) and leaf (L) extracts of *D. alata* compared with clotrimazole (C) (ANOVA Summary: $F_{23, 48} = 143.379$, $p<0.001$, Means having different alphabets, as superscripts, are statistically significant from each other at $p<0.001$) (Based on Duncan's multiple-range test)

The activity index of root, stem and leaf extracts of *D. alata* L. with respect to clotrimazole was assessed and the highest activity index of 0.60 was recorded in case of methanol root extract for *A. niger* and 1.11 for *C. albicans*. The least activity index was recorded for *A. niger* (0.50) and *C. albicans* (0.82) in case of acetone leaf extracts (Figure 11).

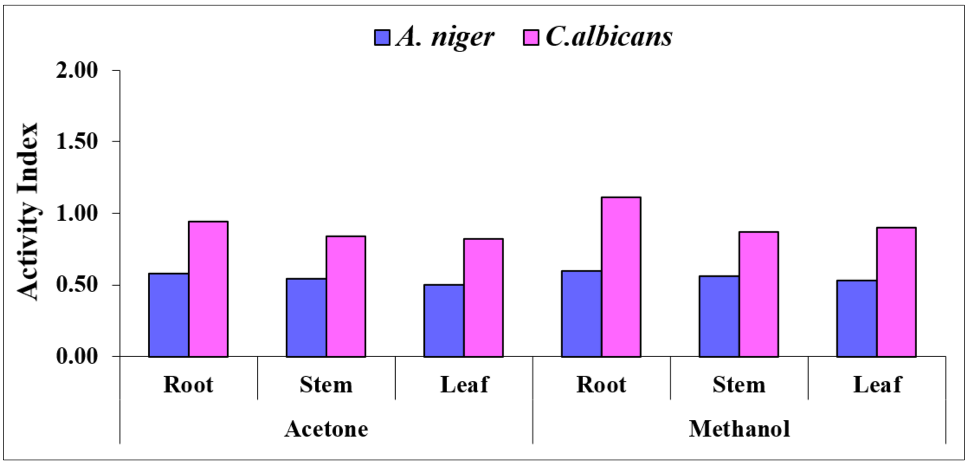

Fig 11: Activity index of the extracts of *D. alata* with reference to clotrimazole

The results of phytochemical analysis of *D. alata* are present in Table 2. The alkaloids were recorded in case of aqueous root and stem extracts and were found to be absent in methanol, acetone and chloroform extracts. The flavonoids were found to be present in all the extracts under study except for chloroform extracts. The phytosterols gave a positive reaction against all the extracts more so with methanol and acetone extracts. Tannins were detected in case of root and stem extracts of aqueous, methanol and acetone and were absent in chloroform extracts. Saponins were found to be present in all the extracts except chloroform. They were recorded more in methanol and acetone root extracts. Although quinines were not detected in all the extract tested but resins gave strong positive test in chloroform extracts and positive in acetone root extract. The glycosides were feebly recorded in case of methanol stem, acetone and aqueous root extracts. The qualitative phytochemical analysis of *D. alata* extract revealed that methanol root extract was moderately positive for phytosterol and saponins; positive for flavonoids and tannins. Similar results showing the presence of more phytocompounds in the methanol root extract of *Dioscorea sp.* conferring antimicrobial activity [43]. Acetone root extract showed strong positive reaction for saponins; positive reaction for flavonoid, tannins, resins and glycosides. The stem and leaf extracts followed similar trend. The chloroform extract showed moderate to strong positive reaction for resins and phytosterols. Reports of methanol, acetone, ethyl acetate extracts conferring antimicrobial activity are many and describes the nature of antibacterial and antifungal compounds. Accumulation of these substances in root and stem results in higher activity in these plant parts.

Table 2: Phytochemical analysis of root, stem and leaf extracts of *D. alata*

Tests	Aqueous			Methanol			Acetone			Chloroform		
	R	S	L	R	S	L	R	S	L	R	S	L
Alkaloids												
Mayer's	+	+	-	-	-	-	-	-	-	-	-	-
Wagner's	+	+	-	-	-	-	-	-	-	-	-	-
Hager's	+	+	-	-	-	-	-	-	-	-	-	-
Flavonoids												
Alkaline reagent	+	+	+	+	+	+	+	+	+	-	-	-
Lead acetate	+	+	+	+	+	+	+	+	+	-	-	-
Phytosterols												
Salkowski	+	+	+	++	++	++	++	++	++	+	+	+
Libermann-Burchard	+	+	+	++	++	++	++	++	++	+	+	+
Tannins												
Ferric chloride	+	+	-	+	+	-	+	+	-	-	-	-
Gelatin	+	+	-	+	+	-	+	+	-	-	-	-
Saponins												
Foam test	+	+	+	++	+	+	+++	++	+	-	-	-
Quinones	-	-	-	-	-	-	-	-	-	-	-	-
Resins	-	-	-	-	-	-	+	-	-	++	+++	+++
Glycosides	+	-	-	-	+	-	+	-	-	-	-	-

+++ Strongly positive; ++ moderately positive; + positive; - negative; R- Root; S- Stem; L- Leaf

The quantitative estimation of alkaloid, flavonoid, saponin and total phenol in root stem and leaf samples of *D. alata* is presented in Figure 12. The root sample of plant contains highest amount of saponin (0.692±0.022%) followed by total phenol (0.413±0.019%), flavonoid (0.350±0.020%) and alkaloid (0.267±0.027%). The root sample contains higher amount of saponin (0.692±0.022%) followed by stem (0.513±0.021%) and leaf (0.348±0.024%). Total phenol was found to be highest in root (0.413±0.019%) followed by stem (0.314±0.021%) and leaf (0.169±0.014%). Flavonoid was more in root (0.350±0.020%) followed by stem (0.245±0.028%) and leaf (0.179±0.025%). Alkaloids (0.267±0.027%) were found to be more in root sample followed by stem (0.210±0.013%) and leaf (0.145±0.015%). In *D. alata*, the quantitative profile revealed that root contains highest amount of saponin followed by total phenol, flavonoid and alkaloid. The stem and leaf followed in same order. Higher amount of saponin were reported in *Tribulus terrestris* in north India with regional variations in the content of phytoconstituents [44]. Steroids have been documented to possess antimicrobial properties; the steroids specifically associate with membrane lipid and exert its action by causing leakages from liposomes conferring antimicrobial activity [45].

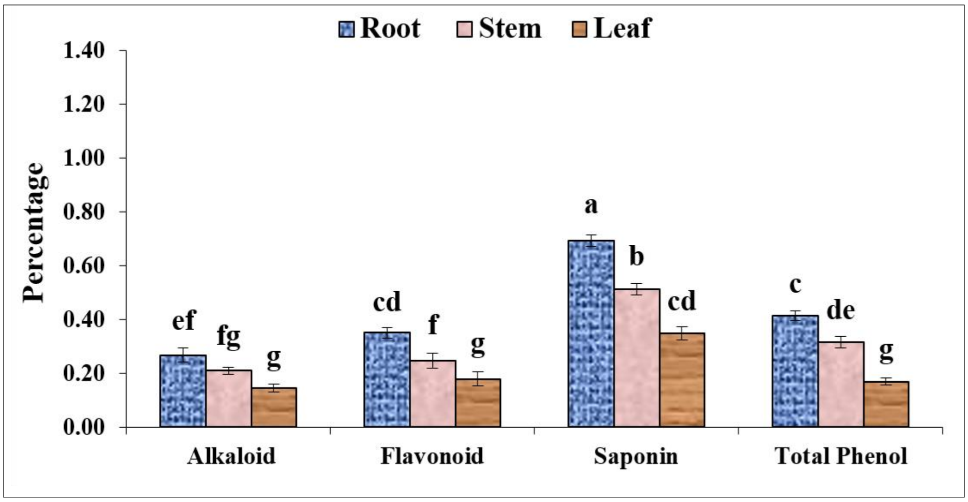

Fig 12: Alkaloids, flavonoids, saponins and total phenols in root, stem and leaf of *D. alata* (ANOVA Summary: $F_{11, 24} = 54.29$, $p<0.001$, Means having different alphabets, as superscripts, are statistically significant from each other at $p<0.001$) (Based on Duncan's multiple-range test)

Conclusion

The present deals with the assessment of antimicrobial activity and phytochemical analysis of *Dioscorea alata* (Family: Dioscoreaceae) from Bastar district of Chhattisgarh against eight pathogenic bacteria two fungi procured from IMTECH, Chandigarh.

The antibacterial potentiality of *D. alata* revealed that acetone root extract exhibited highest activity against *B. cereus*. In case of Gram-negative bacteria the extract exhibited maximum activity against *P. aeruginosa*. The evaluation of antifungal activity revealed that methanol root extract was effective against *A. niger* and *C. albicans*. The highest activity index was recorded with methanol root extract for *A. niger* and *C. albicans*. The phytochemical analysis of *D. alata* showed that percentage yield of root was highest in aqueous followed by methanol, chloroform and acetone. In stem the yield was maximum in aqueous followed by methanol, chloroform and acetone. In leaf the yield was more in chloroform followed by aqueous, methanol and acetone. Alkaloids were present in aqueous root and stem extracts. The flavonoids were present in all the extracts except chloroform. Phytosterols gave a positive reaction for all the extracts more so with methanol and acetone extracts. Tannins were detected in root and stem extracts of aqueous, methanol and acetone. Saponins were present in all the extracts except chloroform. Resins gave strong positive test in chloroform and acetone root extracts. The glycosides were feebly recorded in methanol stem, acetone and aqueous root extracts. The quantitative estimation of alkaloid, flavonoid, saponin and total phenol in root, stem and leaf of *D. alata* showed that root sample contains highest amount of saponin followed by total phenol, flavonoid and alkaloid. The content was highest in root followed by stem and leaf of *D. alata*. Thus, there is an urgent need of extensive research in the area of medicinal plants with the aim of exploring their ethno-medicinal use and subsequently the isolation and characterization of compounds which will contribute for the better, safer and cost-effective novel drug development.

Conflict of Interest

The authors declared no conflicts of interest.

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