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Antimicrobial Potential of *Balanites Aegyptiaca* (L.) Del, *Stevia Rebaudiana* (Bert.) Bertoni, *Tylophora Indica* (Burm.f.) Merrill, and *Cassia Sophera* (Linn.)

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Abstract: *Background:* Despite the medicinal importance of *Balanites aegyptiaca* (L.) Del, *Stevia rebaudiana* (Bert.) Bertoni, *Tylophora indica* (Burm.f.) Merrill and *Cassia sophera* (Linn.), the antimicrobial potential of these plants has not been explored extensively, especially those of *Cassia sophera*.

Methods: Both aqueous and alcoholic extracts of various parts of respective plants, and their *in vitro* raised calli were tested for antimicrobial activity by agar well diffusion method against a range of gram-positive and gram-negative bacteria and several fungal species. The calli of *Balanites aegyptiaca* and *Tylophora indica* were only tested as calli of *Stevia rebaudiana* and *Cassia sophera* could not be generated due to failure of regeneration in our laboratory. The extracts were also tested against genetically characterized bacterial isolates harboring *bla* (antibiotics resistance) genes. Minimal inhibitory concentrations (MICs) of the extracts were determined by broth microdilution method.

Results: The extracts from all the four plants showed significant antibacterial activity against gram-negative bacteria, including, *Salmonella typhi*, *Salmonella paratyphi* A, *Escherichia coli*, *Klebsiella* species, *Proteus vulgaris*, *Citrobacter* spp., *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Vibrio cholerae*. Among all the tested plants, only *B. aegyptiaca* showed significant antibacterial activity against the tested gram-negative bacteria harboring *bla* genes including, *Escherichia coli* (*bla* $_{ampC}$), *Klebsiella* spp. (*bla* $_{CTX-M}$), *Klebsiella* spp. (*bla* $_{SHV}$), *Escherichia coli* (*bla* $_{SHV+CTX-M}$) and *Citrobacter* spp. (*bla* $_{ampC+SHV}$). Extracts of *B. aegyptiaca* and *S. rebaudiana* also showed activity against gram-positive bacteria whereas the extracts from *Tylophora indica* and *Cassia sophera* did not. Extracts of *Balanites aegyptiaca*, *Tylophora indica*, and *Stevia rebaudiana* demonstrated potential antifungal activity whereas extracts of *Cassia sophera* din not demonstrate the antifungal activity.

Conclusions: This is among the premier reports describing the antimicrobial potential, especially through calli extracts, of *Tylophora indica* (Burm.f.) Merrill and *Balanites aegyptiaca* (L.) Del and in particular against gram-negative bacteria harboring *bla* genes. This is also a premier report looking extensively for the antimicrobial potential in *Cassia sophera*.

Keywords: *Balanites aegyptiaca*, *Tylophora indica*, *Stevia rebaudiana*, *Cassia sophera*, antimicrobial activity, *in vitro*, callus, minimal inhibitory concentration.

INTRODUCTION

Balanites aegyptiaca (L.) Del (Zygophyllaceae or Simaroubaceae) (Fig. **1A**), known as 'desert date' in English and 'Hingoli' in Hindi [1], is a savanna tree that grows in Sahel-Savanna regions and drier parts of middle-belt zones of Nigeria, Ghana and Ivory Coast, and is cultivated as a fruit tree in semisavanna parts of the continent. This plant is a small evergreen thorny tree found in drier parts of India [1]. Various parts of the *Balanites* tree have been used in folk medicine in many regions of Africa and Asia [2, 3]. Literature has revealed antifeedant, antidiabetic, molluscicide, anthelminthic, and contraceptive activities in various *Balanites* extracts [4-7]. The bark, unripe fruits, and leaves of this plant are reported to have anthelminthic, antifertility, purgative and antidysenteric properties [8-10]. Aqueous suspensions of dried fruits of this plant are being used as abortifacient by local healers [1]. The root has been indicated for the treatment of malaria, herpes zoster, and venereal diseases [11].

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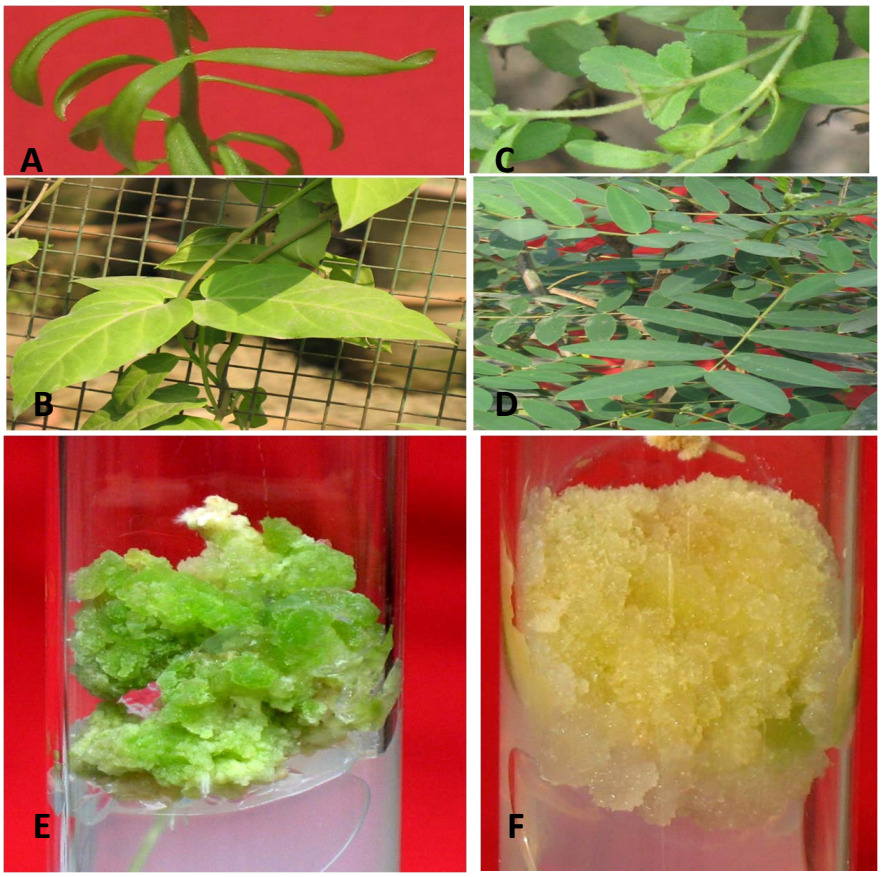

Fig. (1). Photographs showing the plants *Balanites aegyptiaca* (L.) Del (**A**), *Tylophora indica* (Burm.f.) Merrill (**B**), *Stevia rebaudiana* (Bert.) Bertoni (**C**), *Cassia sophera* (Linn.) (**D**), and calli of *Tylophora indica* (Burm.f.) Merrill (**E**) and *Balanites aegyptiaca* (L.) Del (**F**).

Tylophora indica (Burm.f.) Merrill (Asclepiadaceae) (Fig. **1B**), also known as 'Indian ipecac' in English, and 'Jangali pikvan' in Hindi, is a dark copper-coloured delicate creeper found growing wild in the plains of India and other sub-tropical regions of the world [12]. It is found in Eastern, North-East and Central India, Bengal and, parts of South India and also harbor in Ceylon, Malay island and Borneo [10]. It is traditionally used as a folk remedy in certain regions of India for the treatment of bronchial asthma, inflammation, bronchitis, allergies, rheumatism and dermatitis [13-15]. The leaf extract is reported to possess anti-tumor activity [16].

Stevia rebaudiana (Bert.) Bertoni (Fig. **1C)** belonging to the family Asteraceae or Compositae [17], is commonly known as Honey leaf, Candy leaf, Sweet leaf [18], and sweet herb [19]. It is a perennial and endemic, medicinal shrub. The distribution range of this taxon extends from the southwest United States to northern Argentina, through Mexico, the Andes and the Brazilian highlands. *S. rebaudiana* is originally a South American wild plant [20, 21]. In ancient Indian traditional Ayurvedic system of medicine, *Stevia rebaudiana* has a long history of use by tribal people [22]. It has several medicinal uses such as vasodilator cardiotonic, anesthetic and anti-inflammatory [18]. It is well known for its application in the treatment of many diseases like diabetes, candidiasis, hypertension and weight loss in various traditional systems of medicine [23].

Cassia sophera Linn. (Caesalpiniaceae), commonly known as 'Senna sophera', 'Kasondi' and 'Kasa marda' [24, 25], is a medicinal plant of Bangladesh and Indian subcontinent, which is widely used as a folk medicine for the treatment of many diseases [26]. *Cassia sophera* is a shrubby herb, grown in gardens as a hedge plant. The plant grows as diffuse undershrubs, up to 3 m tall. Its Leaves are 10-15 cm long, bearing leaflets 6-10 pairs, oblong-lanceolate, glaucous. The flowers are yellow in colour, borne in terminal and axillary racemes (Fig. **1D**). The plant is found throughout India, in all plain districts, and in most tropical countries (Pantropical). It is common in waste lands, on roadsides and in the forests. *Cassia sophera* is an important drug of Islamic System of Medicine (Unani Medicine). It is described in Unani literature to be repulsive of morbid humors (especially phlegm), resolvent, blood purifier, carminative, purgative, digestive, diaphoretic [24, 26]. In ethnobotanical literature, it is mentioned to be effective in the treatment of pityriasis, psoriasis, asthma, acute bronchitis, cough, diabetes and convulsions of children [10, 24, 26, 27]. The Root bark of this plant is used for preparation of the medicines. It has been used by ancient Indian physicians for its efficacy in respiratory disorders. It has also been recommended in common cold and asthma. It has also been reported to be extensively used in Homeopathic preparations for ailments like osteoarthritis, asthma and allergic rhinitis.

Although there are preliminary reports on antimicrobial activities of *Balanites aegyptiaca*, *Tylophora indica* and *Stevia rebaudiana* [28-31], there is paucity of scientific reports validating the antimicrobial activity of *Cassia sophera*. Moreover, *Balanites aegyptiaca* and *Tylophora indica* are reported as endangered plant species in India [32,

33]. Therefore, an attempt was made to optimize the *in vitro* callus induction from various explants of all the plants, including the endangered species mentioned above, and then to find out the antimicrobial activity from the extracts of the calli thus obtained. The study was designed to find out the antimicrobial activity of the alcoholic and aqueous extracts of various parts (leaf, fruit and stem) of the parent plants and from their *in vitro* raised calli with special reference to extracts from *Cassia sophera*.

MATERIALS AND METHODS

Plant Parts Used

The fruit pulp of a 15 years old parent plant of *Balanites aegyptiaca* was provided by Dr. Santosh Kumar, Tissue culture Laboratory, Department of Botany, Gujarat University, Ahmadabad (Gujarat). For *in vitro* cultivation of the same plant, seed materials initially procured from Dr. Santosh Kumar, Ahmadabad, were grown in Botanical garden, Department of Botany, A.M.U., Aligarh, and plant materials were taken from a 5 years old plant.

Fresh leaves and nodal segments (from a 6 years old plant), stem (from a 6 months old plant), and leaves (from a 6 months old plant) were collected for *Tylophora indica*, *Cassia sophera* and *Stevia rebaudiana*, respectively, from the plants grown in Botanical garden, Department of Botany, A.M.U., Aligarh.

All the plants were identified and deposited in the herbarium of Department of Botany (Herbarium/Account Numbers 1709-1711, respectively), A.M.U., Aligarh, Uttar Pradesh, India.

In Vitro Culture of Explants for Callus Induction

Collected explants were subjected to *in vitro* cultivation for callus induction. The young explants (leaf, stem and seed) were isolated from the plant and washed under running tap water for 30 min to remove any adherent particles and then washed thoroughly in sterile double-distilled water (DDW). Subsequently, these explants were kept in 1% (w/v) Bavistin (Carbendazim Powder, BASF India Limited), a broad spectrum fungicide for 15 min (25 min in case of seeds), followed by thorough washing with 5% (v/v) Teepol (Qualigens Fine Chemicals, India), a liquid detergent for 15 min by continuous shaking (method according to Shahid *et al*. [34, 35], with slight modifications). Further, the treated explants were washed in sterilized DDW 3-4 times under an aseptic condition, to remove the chemical inhibitors. The explants were then treated with 70% (v/v) ethanol for 30-40 sec, and followed by a rapid washing with sterile DDW and then surface sterilized by emersion in a freshly prepared aqueous solution of 0.1% (w/v) $HgCl_2$ (Mercuric Chloride) (Qualigens Fine Chemicals, India) [36], for 2-3 min (for 4 min in case of seeds) under Laminar flow. Finally, the explants were washed 5-6 times with sterile DDW with intervals of 5 min to remove all traces of sterilants (mercury ions).

The sterilized explants were inoculated aseptically in culture tubes (25×150 mm, Borosil) containing culture medium i.e. Murashige and Skoog's (MS) medium [37] containing BA [6-Benzyladenine] (5µM) or MS with 2,4-D [2,4-Dichlorophenoxy acetic acid] (2.5µM) or MS with TDZ [Thidiazuron] (7.5µM) and MS with BA (1µM) and TDZ (5µM); depending upon the plant used. The pH of the medium was adjusted at 5.8±0.2 with the help of 0.1N NaOH or 0.1N HCl. The MS-medium tubes were agarified with 1% Agar (HiMedia Lab. Ltd., India) before autoclaving at 15lb pressure per square inch, 121°C temperature for 15 min and incubated at 25 ± 2°C with relative humidity of 55 ± 5% and exposed for 16 h photocycle of 2,500 Lux intensity [38].

Plant Extracts Tested for Antimicrobial Activity

Both aqueous and alcoholic extracts of each plant were tested for antimicrobial i.e. antibacterial and antifungal activity. The extracts were derived according to the method described previously by Shahid *et al*. [34, 35, 38].

Aqueous Extracts

Fresh plant parts i.e. leaf, stem, fruit and flower head (15 g each) both from parent plant and *in vitro* raised plants were taken and surface sterilized in 70% ethyl alcohol for 1 min and then washed three times in sterilized DDW at an interval of 5 min. As the calli were grown in sterile conditions they were not subjected to surface sterilization. The respective calli of the explants were aseptically removed from culture tubes and all plant materials, including calli were grounded with a sterile pestle and mortar in 150 ml sterilized DDW. The homogenized tissues were centrifuged at 5000 rpm for 15 min, and the supernatant was taken as the aqueous extract.

Alcoholic Extracts

To prepare alcoholic extracts, fresh plant materials i.e. leaf, stem, fruit and flower head (15 g each) were homogenized in 150 ml of absolute alcohol (100% ethanol) and centrifuged as above. The supernatant was taken as the alcoholic extract.

The extracts were immediately used for experimentation.

Bacterial Strains Used

Standard and Clinical bacterial strains (stocked in the department of Microbiology, Jawaharlal Nehru Medical College & Hospital, AMU, Aligarh) of various Gram-positive and Gram-negative bacteria were used for detection of antibacterial activity. The bacterial strains used were *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus* species, *Salmonella paratyphi* A, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Shigella dysenteriae* type 1, *Klebsiella* species, *Proteus vulgaris*, *Citrobacter* species, *Salmonella typhi*, *Salmonella typhimurium*, and *Vibrio cholerae*. The bacterial strains harboring *bla* genes were *Escherichia coli* (*bla* $_{ampC}$), *Klebsiella* species (*bla* $_{CTX-M}$), *Klebsiella* species (*bla* $_{SHV}$), *Escherichia coli* (*bla* $_{TEM}$), *Escherichia coli* (*bla* $_{SHV+CTX-M}$), and *Citrobacter* species (*bla* $_{ampC+SHV}$).

Fungal Strains Used

Standard and Clinical fungal strains (stocked in the department of Microbiology, Jawaharlal Nehru Medical College & Hospital, AMU, Aligarh) were used for the detection of antifungal activity. The fungi were grown at 25°C and maintained on Sabouraud Dextrose agar (SDA) slants. The fungal strains used were *Candida albicans*, *Candida*

krusei (ATCC 6258), *Candida parapsilosis* (ATCC 22019), *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* (ATCC 204305), *Alternaria* species, *Penicillium* species, *Rhizopus* species, *Mucor* species, and *Fusarium* species.

Antibacterial Susceptibility Testing

Antibacterial activity was determined using agar well diffusion method as described previously by Shahid *et al*. [35]. Muellar-Hinton Agar (M 173; HiMedia, India) was used for determining antibacterial susceptibility testing. For fastidious organisms such as *Streptococci*, blood agar was used composed of 5% defibrinated sheep blood. Plant extracts in a volume of 20µl (aqueous as well as alcoholic extracts) were used for determining the antibacterial activity. Sterile double distilled water and ethanol were used to serve as blank controls in the aqueous and alcoholic plates respectively. The plates were kept upright for 5-10 min until the solution diffused into the medium. The results of activity were measured as zone of inhibition recorded in mm. Each experiment was performed in triplicate.

Determination of Minimal Inhibitory Concentrations (MICs) for Bacterial Species

Broth micro-dilution testing was performed as described previously by Shahid *et al*. [35]. Briefly, doubling dilution of the extracts was prepared using RPMI-1640 (HiMedia Lab. Ltd., India) broth supplemented with 0.3g l L-glutamine (HiMedia Lab Ltd., India), 0.165M MOPS buffer (HiMedia Lab. Ltd. India) (35.54 g/l) and 0.01% DMSO (Qualigens Fine Chemicals, India). Extracts were diluted in pure (100%) DMSO, and further diluted 1:50 in RPMI-1640 medium, and each resulting solution were used for a doubling dilution series. Microtitre plates were prepared containing 20µl of undiluted extracts in the first well, followed by doubling dilutions of extracts from second well. 20µl of the standardized inoculums of each bacterial species was added to the respective dilution wells, including the first well. The final concentrations of the extracts ranged from 1×10^{-1}µg/ml (in first well) to 7.81×10^{-4}µg/ml (in the last dilution well). For each test there was a sterility control-well containing only RPMI-1640 (40µl), two sets of growth control-wells (one containing RPMI-1640 broth plus the bacterial growth and the other containing RPMI-1640 broth plus the bacterial growth plus DMSO, each 40µl in volume), and an extract control (40µl). The microtitre plates were incubated at 37°C for 24 hours, with their upper surface covered by sterile sealers. The lowest concentration that did not show any visible growth was considered the MIC of that extract for the tested bacterial species and was expressed in µg/ml. All the MIC experimentations were performed in duplicate.

Antifungal Susceptibility Testing

Antifungal testing was performed on Sabouraud Dextrose Agar (SDA) plates using agar well diffusion method as described previously [38]. An inoculum size of 2×10^5 yeast cells or fungal spores was used to inoculate the SDA plates. Well of 5mm diameter was made in each plate using a sterile borer. Plant extracts in a volume of 20µl (aqueous as well as alcoholic extracts) were used and the plates were incubated at 25°C in a BOD incubator for 24-72 hrs. Sterile double distilled water and ethanol were used to serve as blank controls in the aqueous and alcoholic plates respectively. The results of the activity were measured as zone of inhibition (in mm). Each experiment was performed in triplicate.

Minimal Inhibitory Concentration (MIC) Determination for Fungal Species

Broth micro-dilution method was used to determine the MICs according to the method described previously [35]. The final concentrations of the extracts ranged from 1×10^{-1}µg/ml (in first well) to 7.81×10^{-4}µg/ml (in last dilution well). For each test there was a sterility control well containing only RPMI-1640 (40µl), two sets of growth control wells (one containing RPMI-1640 broth plus the fungal growth and the other containing RPMI-1640 broth plus the fungal growth plus DMSO, each 40µl in volume), and an extract control (40µl). The microtitre plates were incubated at 25°C for 24 to 96 hours, with their upper surface covered by sterile sealers. The lowest concentration that did not show any visible growth was considered the MIC of that extract for the tested fungal species and was expressed in µg/ml. All the MIC experimentations were performed in duplicate.

Statistical Analysis

Data were statistically analyzed by using one way analysis of variance (ANOVA) followed by Tukey's multiple analysis test used to compare the antimicrobial (antibacterial and antifungal) effect of the different explants extracts (SPSS Software, Chicago, III, version 10). *P* values were calculated by one-sample T-test and $P < 0.05$ was taken as statistically significant.

RESULTS AND DISCUSSION

***In Vitro* Callus Induction**

For *Tylophora indica***,** when the leaf explants were cultivated in callus induction medium comprising of MS (Murashige and Skoog's medium) + 2, 4-D (5µM), callusing was initiated from the cut ends of the explants after 25 days of inoculation. Callus was yellow in colour and friable in nature. 4g fresh weight of callus (dry weight of 367 mg) was induced after 5 weeks of culture. The leaf callus thus obtained was used for evaluation of the antimicrobial effect (Fig. **1E**). And for *Balanites aegyptiaca*, when the seed explants were cultivated in callus induction medium comprising of MS + 2,4-D [2,4-Dichlorophenoxy acetic acid] (2.5µM), surface callusing started on pieces of seeds during third week of incubation. Callus growth was moderate at this concentration of the medium. The callus was yellow and friable in nature. As optimal response was noted on MS + 2,4-D (2.5µM), 5 weeks old callus (seed callus) was used for evaluation of the antimicrobial effect [Fig. **1F**].

The calli of *Tylophora indica* and *Balanites aegyptiaca* were only tested as calli of *Cassia sophera* and *Stevia rebaudiana* could not be induced due to failure of *in vitro* regeneration in the culture medium in our laboratory.

Antibacterial Activity

Of the aqueous extracts tested, only extracts of *B. aegyptiaca* and *S. rebaudiana* showed antibacterial activity against limited gram-positive bacterial species; notably

aqueous fruit extract of plant *B. aegyptiaca*, showed significant ($P<0.05$) antibacterial activity against *Streptococcus pyogenes*, *Streptococcus agalactiae* and *Bacillus* species, and aqueous leaf extract of plant *S. rebaudiana* showed significant antibacterial only against *Staphylococcus epidermidis* (Table **1**)(See Supplementary data). Aqueous extract of only *B. aegyptiaca* showed significant activity against the tested gram-negative bacteria, notably against *Salmonella paratyphi* A, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Citrobacter* spp. and *Vibrio cholera* (Table **2**) (See Supplementary data). Aqueous fruit extract of *B. aegyptiaca* also showed activity against gram-negative bacteria harboring *bla* genes (Table **3**) (See Supplementary data). None of the other aqueous extracts showed any activity against the tested gram-negative bacteria or those harboring *bla* genes. Alcoholic extracts of all the four plants showed significant antibacterial activity Tables **1-3**) (See Supplementary data). In various other studies, on different plant species, the same fact has been reported where they found ethanolic extracts to be more effective than the aqueous extract in inhibiting the bacterial growth [39, 40]. The alcoholic fruit extract of *B. aegyptiaca* showed the best antibacterial activity amongst the tested extracts. It showed activity against *Salmonella paratyphi A*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Proteus vulgaris*, *Citrobacter* spp., *Salmonella typhi*, *Salmonella typhimurium* and *Vibrio cholera* (Table **2**). This is similar to a study done by Parekh and Chanda [41] who showed slight activity of whole plant extract of *Balanites aegyptiaca* against *Klebsiella pneumoniae* and *Salmonella typhimurium*. Also, in similar studies done by Doughari *et al*. [28] and Maregesi *et al*. [42], significant antibacterial activity of extracts of *Balanites aegyptiaca* were demonstrated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Salmonella typhimurium*. Alcoholic fruit extract of *B. aegyptiaca* also showed good activity against the gram-negative bacteria harboring *bla* genes notably against *Escherichia coli* (*bla* $_{ampC}$), *Klebsiella pneumoniae*. (*bla* $_{CTX-M}$), *Klebsiella pneumoniae*. (*bla* $_{SHV}$), *Escherichia coli* (*bla* $_{SHV + CTX-M}$) and *Citrobacter* spp. (*bla* $_{ampC + SHV}$) (Table **3**) (See Supplementary data). The alcoholic leaf extract of *S. rebaudiana* showed significant activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcos faecalis* and *Bacillus* spp. amongst the gram-positive bacteria (Table **1**) (See Supplementary data); whereas, it also showed activity against *Escherichia coli* and *Klebsiella* spp. amongst the tested gram-negative bacterial species (Table **2**) (See Supplementary data). Similar study done by Ghosh *et al*. [23] also showed good activity of leaf extract of this plant against *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis*. Studies done by Jayaraman *et al*. [18], Ghosh *et al*. [23] Tadhani and Subhash [43] and Debnath [44] also showed good activity of the leaf extract of plant *Stevia rebaudiana* against *Escherichia coli,* similar to our study. However, they also reported good activity of this plant against *Pseudomonas aeruginosa* and *Proteus vulgaris* which is in contrast to the findings in our study. The disparity could be different bacterial strains tested having different sensitivity pattern. The alcoholic stem extract of *Cassia sophera* showed antibacterial activity only against *Salmonella typhi* (Table **2**) (See Supplementary data). Among the calli tested, the seed callus of *B. aegyptiaca* did not show any activity, whereas, the leaf callus of *T. indica* showed good activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Table **1**) (See Supplementary data). These activities were not found in leaf extract derived from parent plant of *T. indica*. Since this was the first report showing the activity of calli, the results could not be compared with other studies. The MICs of aqueous fruit extract of *B. aegyptiaca* against gram-positive bacteria ranged from 12.5×10^{-3} to 37.5×10^{-3} µg/ml, whereas, the MICs against gram-negative bacteria ranged from 9.38×10^{-3} to 37.5×10^{-3}µg/ml. The MICs of alcoholic fruit extract of *B. aegyptiaca* against gram-positive bacteria ranged from 9.38×10^{-3} to 25.0×10^{-3} µg/ml and MICs against Gram-negative bacteria ranged from 4.68×10^{-3} to 37.5×10^{-3}µg/ml. The MICs of alcoholic leaf extract of *T. indica* against the tested gram-negative bacteria ranged from 9.38×10^{-3} to 25.0×10^{-3}µg/ml. The MICs of alcoholic leaf extract of *S. rebaudiana* against gram-positive bacteria ranged from 9.38×10^{-3} to 25.0×10^{-3} µg/ml, whereas, MICs against gram-negative bacteria ranged from 6.25×10^{-3} to 9.38×10^{-3} µg/ml.

Antifungal Activity

The detailed results of antifungal activities of the aqueous and alcoholic extracts of parent plants and their respective calli are shown in (Table **4**) (See Supplementary data). The aqueous extract of only *B. aegyptiaca* showed antifungal activity and that too only against *Candida parapsilosis*, whereas, none of the other aqueous extracts showed any antifungal activity against the tested fungal species. Among the alcoholic extracts tested best antifungal activity was shown by alcoholic leaf extract of *T. indica*, notably against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Penicillium* spp. This is similar to the study done by Reddy [45] who showed significant antifungal activity of alcoholic leaf extract of *Tylophora indica* against *Aspergillus niger*, *Aspergillus fumigatus* and *Fusarium* species. However, in our study there was no activity detected against *Fusarium* species. The alcoholic fruit extract of *B. aegyptiaca* showed good antifungal activity against *Candida albicans* and *Candida parapsilosis*. This is similar to the studies done by Runyoro *et al*. [46] and Maregesi *et al*. [42] who tested the extracts of *Balanites aegyptiaca* and demonstrated high antifungal activity against *Candida albicans*. However they did not test *Candida parapsilosis* and *Penicillium* species against which good antifungal activity has been shown in our study. The alcoholic stem extract of *Cassia sophera* showed no antifungal activity against the tested fungal species. Among the calli tested, the seed callus of *B. aegyptiaca* did not show any activity whereas, the leaf callus of *T. indica* showed good activity against *Candida parapsilosis* (Table **4**) (See Supplementary data). This activity was not found in leaf extract derived from parent plant of *T. indica*. Since this was the first report showing the activity of calli the results could not be compared with other studies. The MICs of alcoholic leaf extract of *T. indica* against the tested *Aspergillus* species ranged from 6.25×10^{-3} to 18.7×10^{-3}µg/ml and MICs against *Penicillium* spp. was found to be 9.38×10^{-3} µg/ml. The MICs of alcoholic fruit extract of *B. aegyptiaca* against the tested *Candida* species ranged from 4.68×10^{-3} to 12.5×10^{-3}µg/ml.

CONCLUSION

To conclude, aqueous fruit extract of *B. aegyptiaca* and its alcoholic extract both showed good antibacterial activity. The alcoholic extracts of all the four plants showed significant antibacterial and antifungal activity. The alcoholic fruit extract of *B. aegyptiaca* also showed good activity against gram-negative bacteria harboring *bla* genes. The *in vitro* raised leaf callus of *T. indica* showed good antibacterial and antifungal activity, which was not shown by the extract of parent plant. Based on this preliminary study, it is thus suggested that the alcoholic extracts of these plants, including from their respective calli, may be used in phytotherapy as an antibacterial and antifungal agent. As the extracts from calli gave good results, *in vitro* cultivation of the explants may be used to obtain phytotherapeutic compounds, especially, at places where the plants cannot be grown because of adverse atmospheric conditions. Through *in vitro* cultivation it would also be possible to preserve and conserve these important endangered plant species. Moreover, the bioactive compounds responsible for the antibacterial effects could further be enhanced in the culture conditions by nutritional and hormonal manipulations in the cultivation medium. Further experiments with a wider range of microorganisms are required to analyze in greater detail the antibacterial and antifungal activities of these plants.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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