



Research Article

COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF UNRIPE FRUIT, RIPE FRUIT AND BARK OF *TINDUKA* (*Diospyros Embryopteris* Pers)

Roushani Kalia^{1*}, Anjali Sanjeev²

¹Assistant Professor, Department of Dravyaguna, Shri Dhanwantry Ayurvedic College and Hospital, Chandigarh

²Assistant Professor, Department of Agada Tantra, Faculty of Indian Medical System SGT University, Gurugram India.

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ABSTRACT

Tinduka (*Diospyros embryopteris* Pers.) is a traditionally used but relatively underexplored medicinal fruit described in Ayurvedic classical texts. Different parts of the plant are attributed with distinct therapeutic applications, including conditions associated with skin disorders, allergic manifestations and other infections. The anti-microbial potential of different stages and parts of *Tinduka*, however, warrants systematic comparative evaluation. **Objective:** The present study aimed to comparatively evaluate the antimicrobial activity of unripe fruit, ripe fruit and bark of *Tinduka* (*Diospyros embryopteris* Pers.) against selected bacterial and fungal pathogens. **Methods:** Three samples of *Tinduka*- Sample A (unripe fruit), Sample B (ripe fruit) and Sample C (bark)- were subjected to extraction using ethanol, methanol and chloroform. The antimicrobial susceptibility of the extracts was evaluated against *Vibrio cholerae* (MTCC 3906), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pneumoniae* (MTCC 655), *Escherichia coli* (ATCC 25922), *Candida albicans* (MTCC 227) and *Staphylococcus aureus* (ATCC 25923) using an appropriate in-vitro antimicrobial susceptibility assay. **Results:** The extracts demonstrated variable antimicrobial activity across the tested microorganisms, with differences observed according to the plant part, stage of fruit maturity and extraction solvent. The results of this study showed that the unripe fruit extracts showed better anti-microbial action followed by the bark and then ripe fruit. Also, the alcoholic extracts (methanol and ethanol) exhibited better antimicrobial action against the selected microbes. **Conclusion:** The findings demonstrate the antimicrobial potential of different parts and maturity stages of *Tinduka*, with variation in activity according to the extraction solvent and test organism. The comparative findings provide preliminary scientific evidence supporting the traditional use of *Tinduka* and may serve as a basis for further phytochemical and pharmacological investigation.

INTRODUCTION

Tinduka is an evergreen tree, medium-sized or small with many spreading branches forming a shady crown near the ground. It is a slow growing tree whose wood is used for timber, guitar making etc^[1]. The bark is dark-grey or greenish black, exfoliating in large pieces. Young foliage reddish leaves 12-23x4.6cm., ovate-oblong to oblong, coriaceous^[2,3].

The flowers are unisexual, 4-merous, white, or cream coloured^[4]. The female flowers are solitary, drooping. Male flowers occur in peduncled cymes. Flowers buds are ovoid oblong. Fruits are 3-5cm across, or 1-2.5 in diameter, almost globose^[5]. The seeds are 4-8 in number. It is a slow growing tree. The drug finds mention in the various *Ayurvedic* classics^[6,7,8].

Tinduka is also mentioned in the *Udarda-prashmana*^[9] (part used not mentioned), in the *Basti* preparations used against *Kapha Pitta* disorders in the *Charak Samhita*^[10]. The drug is enlisted among other drugs of *Kashaya Skandha*^[11]. It is mentioned among

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the nine drugs enlisted for *Asava* preparation using its heartwood^[12].

Tinduka is described in *Sutika Paricharya*^[13], its wood being suggestive of antimicrobial and anti-septic action^[14]. *Tinduka* is found along with other drugs like *Dhataki*, leaves of *Amalaki* etc *Sidha Tailam* in a formulation named *Dhatakyadi Tailam*^[15] which is indicated in *Yonirogas*^[16] (female disorders) like *Upapluta*, *Viapluta* etc.

Sushruta Samhita mentions *Kanda Twak* of *Tinduka* for *Atidaghada Pralepana*^[17] (burns). *Tinduka* is placed in *Nygrodhadi Gana* and *Salasaradi Gana* (by the synonym *Kalaskandha*) in the *Sushruta Samhita*^[18]. There has been clear difference in the properties of *Pakva Phala* (ripe fruit). *Pakva Phala* is *Kashaya Rasa Pradhana*, *Kapha Pittahara*, *Ruksha* and *Madhura Vipaka*^[19]. The *Apakva Phala* (unripe fruit) is described as *Sangrahi* and *Vatakopana*^[20]. While describing the pharmacotherapeutics of the disease *Hastimeha*^[21] (a type of *Vataja Prameha*) a *Kwatha* preparation is indicated which contains *Tinduka*, *Kapittha*, *Shirisha*, *Palasha*, *Patha*, *Murva* and *Dusparsha* along with *Madhu* as *Anupana*^[22].

According to *Ashtang Hridayam*, the *Raga/Shadava* preparation made from *Kapitha*, *Tinduka* and *Jamun* is important dietrics for *Prameha* patient^[23]. Ayurvedic Pharmacopoeia of India mentions the drug under the basonym, *Viralal*^[24]. The stem bark is the part indicated for therapeutic uses in diarrhoea, styptic action, otorrhoea etc^[25].

Based on the disease indications in various Ayurvedic classics the unripe, ripe fruit and stem bark were tested for the antimicrobial susceptibility test against the ethanol, methanol and aqueous extract of the samples chosen for the study.

Methods: Plant Extract Preparation: The plant extract of unripe, ripe fruit and bark of *Tinduka* (*Diospyros embryopteris* Pers.) used for the study was ethanolic, methanolic and water extractive. 50 grams of the Sample A (unripe fruit of *Tinduka*), Sample B (ripe fruit of *Tinduka*) and Sample C (bark of *Tinduka*) were sent for extract preparation at Herbal Health Research Consortium, HHRC, Amritsar. The extracts obtained were in semi solid form. The following methodology was used for extraction.

Performing the Soxhlet method of ethanol, methanol and aqueous extraction

1. Dried, powdered plant material of the three samples i.e. Sample A (unripe fruit of *Tinduka*), Sample B (ripe fruit of *Tinduka*) and Sample C (bark of *Tinduka*) were used. These were crushed and powdered to provide a greater surface area. The plant material was sufficient to fill the porous

cellulose thimble. 50 grams of each sample was used.

2. The procedure started from building a rig using stands and clamps to support the extraction apparatus. Following this, the solvent (250ml of ethanol, 250ml methanol and 250 aqueous) was added to two separate round bottom flasks, which was attached to a Soxhlet extractor and condenser on an isomantle. The crushed plant material (Sample A, Sample B, Sample C) was loaded into the thimble, which was placed inside the Soxhlet extractor
3. The side arm was lagged with glass wool. The solvent was heated using the isomantle and it started to evaporate, moving through the apparatus to the condenser.
4. The condensate then dripped into the reservoir containing the thimble. Once the level of solvent reached the siphon it poured back into the flask and the cycle began again. The process ran for a total of 16 hours.

Dilution of extract

Ethanol, methanol and aqueous extracts of *Tinduka* (*Diospyros embryopteris* Pers) were diluted in 100% DMSO in the ratio 1mg/microlitre. The final dilution was used 1mg/ 1microlitre. Two dilution doses were made for each extract in an amount of 10 microlitre and 20 microlitre.

Revival of Microorganisms

The Microbial Type Culture Collection and Gene Bank (MTCC) strains of *Candida albicans* (strain no 227), *Streptococcus pneumoniae* (strain no 655) and *Vibrio cholerae* (strain no 3906) were procured in freeze dried state from CSIR-IMTEC Sector 39 A, Chandigarh. American Type Culture Collection (ATCC) strains of *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 9027) and *Escherichia coli* (ATCC 25922) were procured from Department of Microbiology, Governmental Medical College, Patiala.

Revival of the Freeze dried MTCC strains

(i) Revival of MTCC *Candida albicans* (strain no 227)

The freeze dried MTCC strain of *Candida albicans* (strain no 227) was revived as follows.

- The ampoule containing the fungi was marked near the middle of cotton wool with sharp file.
- The surface around the mark was disinfected with alcohol.
- The area marked of the ampoule was wrapped with thick cotton wool and was broken at the marked area.
- The pinpointed top of the ampoule was gently removed.

- The cotton plug was removed carefully, and 0.3 to 0.4ml of distilled water was added, and it was allowed to stand for some time before transferring into Brain Heart Infusion (BHI) medium in test tube.
- Few drops of the above suspension were streaked over the blood agar and SDA medium. The plate was incubated at 37°C for 24 hours.

- After 24 hours the growth was recorded by studying the colony characteristics and was confirmed further by gram staining, germ tube test and biochemical reactions.

The other MTCC strains were revived in the same manner as described above and were used for the study except that motility check was also done in case of all gram-negative bacteria.

Culture Characteristics

Table 1: Different media used to grow the micro-organisms.

S.No	Name of the microorganisms	Medium used
1.	<i>Streptococcus pneumoniae</i>	Blood agar ^[26]
2.	<i>Candida albicans</i>	Sabouraud dextrose agar ^[27] (SDA)
3.	<i>Vibrio cholerae</i>	Thiosulfate Citrate Bile Salts Sucrose Agar (TCBS), MacConkey Agar ^[28]

Antimicrobial disks

Preparation

The disks of the diameter 6mm approximately were punched out of Whatman paper no. 1 following which these were sterilised in the hot air oven at a temperature of 160°C for 2 hours. The disks used for the study were impregnated with plant extracts diluted in 100% DMSO.

Inoculation of plates

1. A sterile cotton swab was dipped into the bacterial suspension.
2. The excess inoculum was removed by lightly pressing the swab against the tube wall at a level above that of the liquid.
3. The agar was inoculated by streaking with the swab containing the inoculum.
4. The plate was rotated by 60° and the rubbing procedure was repeated two times. This ensured an even distribution of the inoculum.

5. The surface of the medium was allowed to dry for 3-5 minutes but not longer than 15 minutes to allow for absorption of excess moisture.

Application

1. Using sterile forceps disks were placed on the surface of the plate inoculated with different bacterial and fungal strains. (Discs were impregnated with extract of different amount of sample A, sample B and Sample C).
2. It was ensured that the disk was not moved once it had encountered the agar surface.
3. A maximum of six discs was placed in a 9-cm petri dish.
4. The plates were incubated in an inverted position at 37°C temperature for 24 hours.
5. Zone of inhibition was observed and recorded after 16 to 18 hours using a graduated ruler.

Table 2: Standards for disk diffusion method

Methods	Microorganism	Growth medium	Incubation temperature (°C)	Incubation time (h)
Disk-diffusion method	Bacteria	MHA	35±2	16-18
	Yeast	MHA+GMB	35±2	20-24

RESULTS

When 10 microlitre of extract with 1mg/1microlitre was used no significant activity was seen. At 20 microlitre following significant results were observed:

Table 3: Unripe fruit- zone of inhibition against the microorganisms at 10 microlitre extract dilution

Extract	<i>Vibrio cholerae</i>	<i>Streptococcus pneumoniae</i>	<i>Candida albicans</i>
Ethanol extract	NA	NA	NA
Methanol extract	NA	NA	NA
Aqueous extract	NA	NA	NA

Table 4: Unripe fruit- zone of inhibition against the microorganisms at 10 microlitre extract dilution

Extract	<i>Vibrio cholerae</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Ethanol extract	NA	NA	NA
Methanol extract	NA	NA	NA
Aqueous extract	NA	NA	NA

Table 5: Ripe fruit- zone of inhibition against the microorganisms at 10 microlitre extract dilution

Extract	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Candida albicans</i>
Ethanol extract	NA	NA	NA
Methanol extract	NA	NA	NA
Aqueous extract	NA	NA	NA

Table 6: Ripe fruit- zone of inhibition against the microorganisms at 10 microlitre extract dilution

Extract	<i>Vibrio cholerae</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Ethanol extract	NA	NA	NA
Methanol extract	NA	2mm	NA
Aqueous extract	NA	NA	NA

Table 7: Bark extracts- zone of inhibition against the microorganisms at 10 microlitre extract dilution

Extract	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Candida albicans</i>
Ethanol extract	NA	NA	NA
Methanol extract	NA	NA	NA
Aqueous extract	NA	NA	NA

Table 8: Bark extracts- zone of inhibition against the microorganisms at 10 microlitre extract dilution

Extract	<i>Vibrio cholerae</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Ethanol extract	NA	NA	NA
Methanol extract	NA	NA	NA
Aqueous extract	NA	NA	NA

Table 9: Unripe fruit- zone of inhibition against the microorganisms at 20 microlitre dilution

Extract	<i>Vibrio cholerae</i>	<i>Streptococcus pneumoniae</i>	<i>Candida albicans</i>
Ethanol extract	18mm	16 mm	12 mm
Methanol extract	22mm	24 mm	16 mm
Aqueous extract	Shows resistance	14 mm	8 mm

Table 10: Unripe fruit- zone of inhibition against the microorganisms at 20 microlitre dilution

Extract	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Ethanol	18 mm	16 mm	10 mm
Methanol	14 mm	10 mm	12 mm
Aqueous	6 mm	8 mm	6 mm

Table 11: Ripe fruit- zone of inhibition against the microorganisms at 20 microlitre concentrations

Extract	<i>Vibrio cholerae</i>	<i>Streptococcus pneumoniae</i>	<i>Candida albicans</i>
Ethanol extract	16mm	14 mm	14 mm
Methanol extract	14 mm	20 mm	12 mm
Aqueous extract	9 mm	8 mm	6 mm

Table 12: Ripe fruit extracts- zone of inhibition against the microorganisms 20 microlitre dilution

Extract	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Ethanol	12 mm	12 mm	16 mm
Methanol	18 mm	10 mm	12 mm
Aqueous	6 mm	6 mm	6 mm

Table 13: Bark extracts- zone of inhibition against the microorganisms at 20 microlitre dilution

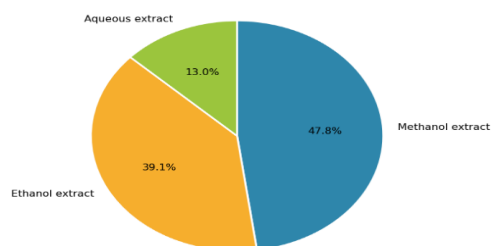
Extract	<i>Vibrio cholerae</i>	<i>Streptococcus pneumoniae</i>	<i>Candida albicans</i>
Ethanol extract	10 mm	10 mm	12 mm
Methanol extract	8 mm	12 mm	10 mm
Aqueous extract	6 mm	8 mm	6 mm

Table 14: Bark extracts- zone of inhibition against the microorganisms at 20 microlitre concentration

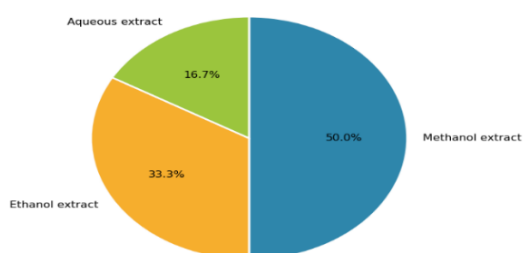
Extract	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Ethanol extract	14 mm	12 mm	12 mm
Methanol extract	20 mm	12 mm	10 mm
Aqueous extract	6 mm	6 mm	9 mm

Antimicrobial activity noted for the extracts of unripe fruit of *Tinduka (Diospyros embryopteris Pers)* against various microorganisms:

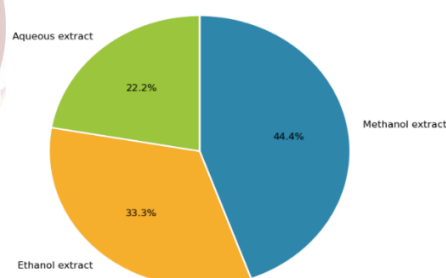
Against *Vibrio cholerae*: The organism showed maximum sensitivity towards methanol extract of unripe fruit (zone of inhibition of 22mm) followed by ethanol extract (zone of inhibition of 18mm) and the aqueous extract (zone of inhibition of 6mm)

Antibacterial Activity Against *Vibrio cholerae* (zone of inhibition)

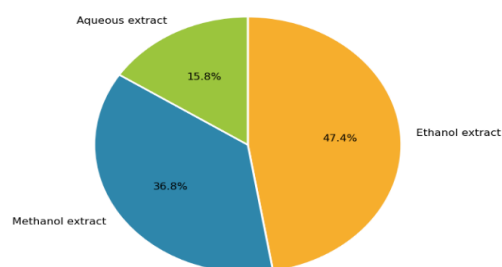
Against *Streptococcus pneumoniae*: The organism showed maximum sensitivity towards methanol extract of unripe fruit (zone of inhibition of 24mm) followed by ethanol extract (zone of inhibition of 16mm) and aqueous extract (zone of inhibition of 8mm).

Antibacterial Activity Against *Streptococcus pneumoniae* (zone of inhibition)

Against *Candida albicans*: The organism showed maximum sensitivity towards methanol extract of unripe fruit (zone of inhibition of 16mm) followed by ethanol extract (zone of inhibition of 12mm) and the aqueous extract (zone of inhibition of 8mm).

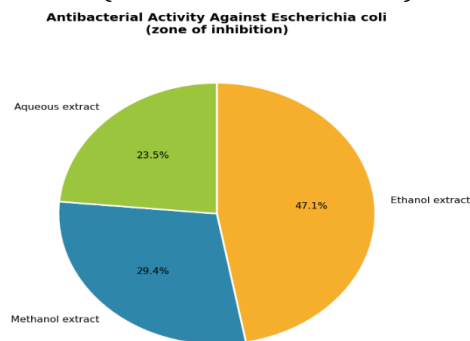
Antifungal Activity Against *Candida albicans* (zone of inhibition)

Against *Staphylococcus aureus*: The organism showed maximum sensitivity towards ethanol extract of unripe fruit (zone of inhibition 18mm) followed by methanol extract (zone of inhibition of 14mm) and aqueous extract (zone of inhibition of 6mm).

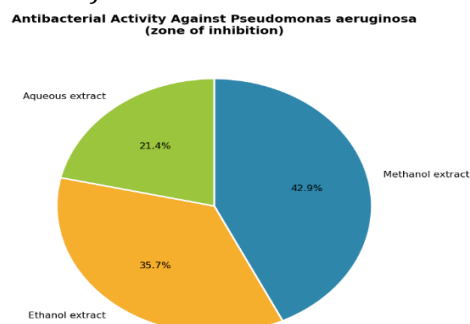
Antibacterial Activity Against *Staphylococcus aureus* (zone of inhibition)

Against *Escherichia coli*: The organism showed maximum sensitivity towards ethanol extract of unripe fruit (zone of inhibition 16mm) followed by the

methanol extract (zone of inhibition 10mm) and aqueous extract (zone of inhibition 8mm).

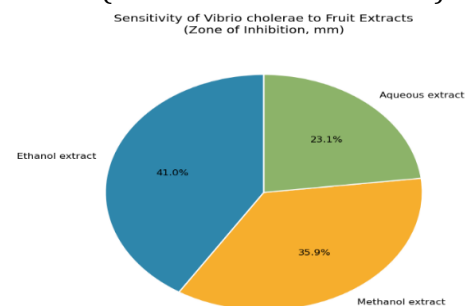


Against *Pseudomonas aeruginosa*: The organism showed maximum sensitivity towards methanol extract of unripe fruit (zone of inhibition of 12mm) followed by ethanol extract of unripe (zone of inhibition 10mm) and aqueous extract (zone of inhibition 6mm).



Antimicrobial activity noted for the extracts of ripe fruit of *Tinduka* (*Diospyros embryopteris* Pers) against various microorganisms:

Against *Vibrio cholerae*: The organism showed maximum sensitivity towards ethanol extract of ripe fruit (zone of inhibition of 16mm) followed by methanol extract (zone of inhibition of 14mm) and aqueous extract (zone of inhibition of 9mm).



1. Against *Streptococcus pneumoniae*: The organism showed maximum sensitivity towards methanol extract of ripe fruit (zone of inhibition of 20mm) followed by ethanol extract (zone of inhibition of 14mm) and aqueous extract (zone of inhibition of 8mm).

2. Against *Candida albicans*: The organism showed maximum sensitivity towards ethanol extract of

ripe fruit (zone of inhibition of 14mm) followed by methanol extract (zone of inhibition of 12mm) and aqueous extract (zone of inhibition of 6mm).

4. Against *Staphylococcus aureus*: The organism showed maximum sensitivity towards ethanol extract of ripe fruit (zone of inhibition of 18mm) followed by methanol extract (zone of inhibition of 12mm) and aqueous extract (zone of inhibition 6mm).

5. Against *Escherichia coli*: The organism showed maximum sensitivity towards ethanol extract of ripe fruit (zone of inhibition of 12mm) followed by methanol extract (zone of inhibition of 10mm) and the aqueous extract (zone of inhibition 6mm).

6. Against *Pseudomonas aeruginosa*: The organism showed maximum sensitivity towards ethanol extract of ripe fruit (zone of inhibition of 16mm) followed by methanol extract (zone of inhibition of 12mm). The organism showed no sensitivity towards the aqueous extract.

Antimicrobial activity noted for the extracts of the bark of *Tinduka* (*Diospyros embryopteris* Pers) against various microorganisms

1. Against *Vibrio cholerae*: The organism showed maximum sensitivity towards ethanol extract of the bark (zone of inhibition of 20mm) followed by methanol extract (zone of inhibition of 8mm) and the aqueous extract (zone of inhibition of 6mm)

2. Against *Streptococcus pneumoniae*: The organism showed maximum sensitivity towards ethanol extract of the bark (zone of inhibition of 16mm) followed by methanol extract (zone of inhibition of 12mm) and aqueous extract (zone of inhibition of 8mm)

3. Against *Candida albicans*: The organism showed maximum sensitivity towards ethanol extract of the bark (zone of inhibition of 12mm) followed by methanol (zone of inhibition of 10mm) and aqueous extract (zone of inhibition of 6mm)

4. Against *Staphylococcus aureus*: The organism showed maximum sensitivity towards ethanol extract of the bark (zone of inhibition 14mm) followed by the methanol extract (zone of inhibition of 20mm) and the aqueous extract of the bark (zone of inhibition of 6mm) of *Tinduka* (*Diospyros embryopteris* Pers)

5. Against *Escherichia coli*: The organism showed maximum sensitivity towards ethanol extract of the bark (zone of inhibition of 22mm) followed by methanol extract of the bark (zone of inhibition of 12mm) and the aqueous extract (zone of inhibition of 6mm)

6. Against *Pseudomonas aeruginosa*: The organism showed maximum sensitivity towards ethanol

extract of the bark maximum (zone of inhibition of 12mm) followed by methanol extract (zone of inhibition 10mm) and the aqueous extract (zone of inhibition of 9mm).

DISCUSSION

There have been severe epidemics of infectious diseases, with devastating consequences to human populations over vast geographic regions, the most recent has been the Covid-19 pandemic. The serious menace of antimicrobial resistance has raised concerns. There is constant endeavour to explore new antimicrobial agents. With the sophistication of pharmacognostical research tools phytochemicals are viewed as potent store house of antimicrobial agents that still need vigorous exploration.

Tinduka has been documented as an important antidiarrheal, antidiabetic, antiseptic, styptic in the ethnobotanical studies and Ayurvedic texts as well. It is commonly found in India and all its parts namely leaves, stem bark, heartwood, root and fruit have therapeutic uses. Due to its abundance in availability, it can be a cost-effective antimicrobial agent.

Tinduka (*Diospyros embryopteris* Pers.) has been mentioned to have therapeutic against *Yoniroga*, *Karnasrava*, *Mukhapaka*, and *Sadyovrana*. These diseases have microbial involvement in their etiology. Hence this study was an endeavour to explore the antimicrobial action of *Tinduka* extracts against microbes (*Candida albicans*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* and *Vibrio cholerae*). Pharmacognostical and physicochemical study of ripe fruit, unripe fruit and bark of *Tinduka* showed it to be of standard quality and has its natural properties.

The higher yield was obtained in more polar solvent (ethanol and methanol) than in non-polar solvent i.e., water. This indicates the polar nature of most of the phytochemicals in *Tinduka* (*Diospyros embryopteris* Pers.) plant extracts and a lower amount of non-polar compounds in *Tinduka* (*Diospyros embryopteris* Pers.). The extracts of *Tinduka* (*D. embryopteris* Pers.) exhibited different degrees of antimicrobial activity against *Vibrio cholera* and *Staphylococcus aureus*, the gram-positive bacilli, *Streptococcus pneumoniae* and *E. coli*, the gram-negative bacilli, and against the fungus *Candida albicans*.

The results of this study showed that the unripe fruit extracts showed better anti-microbial action followed by the bark and then ripe fruit. Also, the alcoholic extracts (methanol and ethanol) exhibited better antimicrobial action against the selected microbes. Therefore, alcoholic extracts of *Tinduka* (*Diospyros embryopteris* Pers.) can be stated to

have more concentration of phytoconstituents in comparison to aqueous extracts.

Hence it can be concluded from the present antimicrobial study that *Tinduka* (*Diospyros embryopteris* Pers.) has definite antimicrobial action against both gram positive and negative bacteria and fungus also. It can also be predicted that in the future drugs emerging from the phytochemicals of *Tinduka* (*Diospyros embryopteris* Pers.) will be of developing interest against microbes.

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***Address for correspondence**

Dr. Roushani Kalia

Assistant Professor

Department of Dravyaguna,

Shri Dhanwantry Ayurvedic College

and Hospital, Chandigarh.

Email: roushk99@gmail.com

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