Comparative Studies on Juice and Agro Waste from *Benincasa hispida*, with Emphasis on Phytochemicals, Antioxidant, and Antibacterial Abilities.

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ABSTRACT

Medicinal herbs include significant amounts of useful phytochemicals and minerals. Phytochemicals play a significant part in reducing the incidence of chronic diseases, according to studies conducted in recent decades. *Benincasa hispida*, a member of Cucurbitaceae (major food family) is being used throughout the Asia for its nutritive values and delicious cuisines. To compare the utility of the Agrowaste (peel, pulp) and juice from *B. hispida* fruit, all parts were extracted in separate portions using three distinct solvents viz. methanol, chloroform, and hexane. Extracts of *B. hispida* fruit was quantitatively analyzed for total phenolic content present in the peel, pulp and juice. The well diffusion technique was used to test prepared extracts against specific types of pathogenic bacteria, including *Escherichia coli* and *Staphylococcus aureus*. Antioxidant activity was applied to extracts by DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate), the ascorbic acid (88.94%) demonstrated less scavenging in the model then methanolic juice extract (92.52%). Juice of *B. hispida* had shown prominent activity against GNB (*Escherichia coli*) and GPB (*Staphylococcus aureus*).

Keywords: *Benincasa hispida, Total phenolic contents, Antioxidant activity, Antibacterial activity*.

INTRODUCTION

Ancient Indian civilization have made use of thousands of herbs and spices to treat various diseases and approximately 8000 herbal medicines are currently mentioned in Ayurveda, the Indian traditional system of medicine. Herbal plants and their various components such as fruits, leaves, flowers, bark, roots, seeds, and heartwood are regularly utilized to be a productive resource in curing numerous ailments since they contain medicinal compounds that are employed for the cure of a number of illnesses (Kanimozhi and Anitha, 2021). Reactive oxygen species [ROS], commonly referred to as active oxygen species in certain situations, occur across numerous distinct varieties of reactive oxygen. These involve free radicals such as super oxide ions (O^{2-}) and non-free radical species as hydrogen peroxide (H_2O_2) (Yildirim, 2001). Reactive oxygen species induce serious illnesses with names like asthma, cancer, inflammation, ischemia, arthritis, and neurological disorders such as mongolism, premature aging and Parkinson's. Antioxidants can scavenge free radicals and avoiding the harm they cause. They can minimize oxidants by eliminating free radicals prior to enter the cell, preventing carbs, lipids, enzymes, proteins, and DNA (Ahmed *et al.*, 2013; Fang *et al*., 2002).

Butylated hydroxyl toluene (BHT), an artificial antioxidant molecule used in food preparation, has been linked to adverse effects (Akinmoladun *et al*., 2007). Antioxidant-rich plant extracts are employed as antioxidant scavengers because they cause less adverse effects.

The major family of food plants known as Cucurbitaceae (Cucurbit) includes some of the most structurally varied groupings of food plants. Fruits from the cucumber family species are prized for their health-promoting and therapeutic properties. The leftover section of Cucurbit fruits, particularly the peel (which is generally discarded as agricultural waste), might be employed in a various of food usage, notably preservation, oil extraction, and feed for livestock, leading to reduced waste dumping and value addition. The Cucurbitaceae family includes a variety of gourds, such as gourd melon, Chinese watermelon, white and tallow gourd (Ghebretinsae *et al.*, 2007).

B. hispida is a popular vegetable in several nations due to its delicious taste and common ingredient in Asian cuisine due to its low calorie count, high water and fiber content, and other nutritional benefits (Chaitali *et al*., 2022; *Zaini et al*., 2011). It is rich in important minerals Ca, Mg, Cu, Se, Zn, and Fe, but low in protein and fat compared to other gourds. *B. hispida fruit* is use as to produce such as jam, ketchup, beverages,

cakes, and ice cream. Immature fruit, free of seeds and fiber, can be stir-fried, braised, or steamed, utilized in spicy dishes and making famous sweet of north India(Asia) called petha (Gupta *et al.*, 2021).

Table 1. The major constituents of fresh *B. hisipida* fruits

Plant Part	**Compound**	**Reference**
Peel	*β*-Carotene, ascorbic acid	(Nagarajaiah and Prakash, 2015)
Seeds	Lupeol	(Une and Doshi, 2014)
Stem	*β*-sitosterol, α-amyrin, quercetin	(Battu et al., 2007)
Fruit	Tryptophan	(Girdhar et al., 2010)
Peel	Galactose, glucose, xylose, sorbose	(Chidan Kumar et al., 2012)
Seeds	Linoleic acid, linolenic	(Mandana et al., 2012)
Seeds	Palmitic acid, oleic acid, stearic acid	(Rayees et al., 2013)
Seeds	*β*-Sitosterol	(Doshi et al., 2015)
Fruit	*β*-Carotene	(Roy and Ghosh, 2008)

The nutritious content of 100 g of *B. hispida* includes 13 calories and contains a less than 1g protein, 3g carbohydrates, and less than 1g fiber. It contains 14% of the recommended intake for vitamin C, 8% for riboflavin, and 6% for zinc. As per Indian traditional medicinal system, *Benincasa hispida*, (Kushmanda in Ayurveda), is commonly employed to manage a variety of disorders likes; epilepsy, urinary infection, diabetes mellitus, cough, fever, heart problems, liver disorders and pitta-vitiated (Pitta is an Ayurvedic body type that is said to be based on fire and water) diseases (Islam *et al.*, 2021).

Nevertheless, recent research has demonstrated that the *B. hispida fruit* peel exhibit potent pharmacological activity (Gu *et al.*, 2013). The peel can be used as a medium for microorganisms that produce industrially relevant products through fermentation. Peel can also be utilized as a primary packing material after it has been isolated and extracted from wax (Mitra *et al.*, 2017). The *B. hispida* pulp and seed have been found to have numerous benefits, including antioxidant activity and the ability to inhibit angiotensin converting enzyme (ACE) (Huang et al., 2005). Seeds from *B. hispida* are appropriate for industrial uses due to their high oil content and advantageous qualities including odorless, nice color, and appearance. Seeds of *B. hispida* carries chemotherapeutic properties and can be used to treat syphilis, cardiovascular diseases, and as an antioxidant (Qadrie *et al.*, 2009; Lee *et al.*, 2005; Choi and Kim, 2003). Antioxidants prevent lipids or other molecules from oxidizing, while antimicrobials inhibit the growth of bacteria, mold, and yeast. Polyphenolic molecules have capacity to scavenge free radicals, in addition to their antioxidant and antibacterial capabilities, suggests potential applications in food and pharmaceuticals. In India, *B. hispida* is used commercially for preparation of Petha (sweets) mainly in Agra, City of Tajmahal (UNISCO world heritage site). An article published in a magazine named Down To Earth, quotes a study about the impact of agrowaste generated in the commercial preparation of Petha from *B. hispida* on the environment (https://www.downtoearth.org.in/waste/bitter-sweet-petha-production-in-agra-leaves-behind-waste-and pollution-80564). So, there is an urgent need to address the problem of agrowaste. Keeping this in view, current studies compare the utility of agrowaste (pulp and peel) with Juice as antioxidant and antibacterial of *B. hispida*.

MATERIAL AND METHODS-

B. hispida fresh fruits were picked in March 2023 from vegetable market. Moreover, was authenticated by Dr. Ashok Kumar Sharma, Ex Scientist G, Plant Tissue ulture, CSIR-National Botanical Research Institute, Lucknow. Ascorbic acid, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), hexane, Chloroform, Methanol, Acetone were bought from Sigma Aldrich chemicals, India.

Extraction of B. hispida fruits-

Cold extraction method was employed to prepare the extract (Anandh *et al.*, 2014).The fresh *B. hispida* fruit(2.5kg) was cleaned thoroughly with distilled water to remove foreign particles. For processing fruit was peeled off to remove and collect the peel. Peeled fruit was finely chopped and juice extracted by cold pressing. Juice and remaining fruit residue along with seeds were collected separately. The peel and pulp residue left after squeezing the juice are generally discarded as waste products and contribute to agrowaste is taken for further processing.

Extracts of peel, pulp and juice of B. hispida fruit- Formation of hexane extract from the peel, juice and pulp of *B. hispida* through cold extraction method. Peel (250g) and pulp residue (1000g) were stirred separately with hexane in the ratio 1:3 thrice by using a mechanical stirrer. The extracts were filtered through vacuum filtration. Hexane extract collected further dried over thin film vacuum evaporator. Juice was subjected to lypholization and dried juice was stirred with hexane thrice for about 4 hours and filtered. Filtrate hexane extract was dried and residual extract left afterwards was used for further processing. The marc of peel and pulp left after extraction with hexane was extracted thrice with chloroform and methanol to obtain chloroform and methanol extracts successively. In case of juice, residue left after extraction with hexane was partitioned with chloroform and methanol to obtain chloroform and methanol fraction respectively.

Phytochemical investigations of the B. hispida for different solvent extracts- The prepared extracts and fractions of *B. hispida fruit* were used for preliminary phytochemical screening with the methodologies reported earlier *Viz,* amino acids was investigated by Ninhydrin's test; sterols by Liebermann-Burchard's and Salkowski reactions; glycosides by Shinoda tests and alkaline, flavonoids by Dragendorff's, Mayer's, Hager's, and alkaloids by Wagner's tests; and $Pb(CH_3COO)_2$, dilute iodine, $FeCl_3$, potassium dichromate test for phenolics, triterpenoids and tannins, Fehling solution test for carbohydrates (Oshadie *et al.*, 2017).

Total phenolic content determination- Folin-Ciocalteu method was used to assess phenolic content of *B. hispida fruit* extracts of peel, pulp and juice (Yu et al., 2002). The 0.5mL extract, 2 mL of Na_2CO_3, was taken and after 2 min 0.1ml of Folin-Ciocalteu reagent was added and left for rest at ambient temperature for 25-30 minutes before measuring absorbance at 675 nm toward a control. A curve for calibration was created utilizing different concentrations of gallic acid as control and data was represented as mg/g of sample.

Antioxidant property of B. hispida different extract- The antioxidant property of extracts and fractions of *B. hispida* peel, juice, and pulp was determined in vitro using the free radical scavenging DPPH assay described by Van Beek and coworkers (Benhammou et al., 2008).

Free radical scavenging DPPH assay- To investigate the antioxidant potential of *B. hispida* extracts, a modified version of Van Beek and coworker's approach was applied (Koleva *et al.*, 2002). 1ml of 0.3mM DPPH sol, 3ml fruit extract were added and kept in absence of sunlight at ambient temperature for 25-30 minutes. Absorbance was recorded at 517 nm. A tube with 3ml of the DPPH methanol solution and 1ml of methanol was employed to be negative control. Ascorbic acid was employed to be reference. The antioxidant's ability to scavenge free radicals was used to assess the percentage of staining of DPPH in methanol solution. The fraction of antioxidant activity was estimated by a particular formula (Valko *et al.*, 2007).

% Antioxidant activity = (Absorbance of control – Absorbance of sample / Absorbance of control) × 100

Antimicrobial evaluation- The antimicrobial potential of extracts and fractions produced was evaluated using the Mueller well diffusion technique (Anita *et al.*, 2023; Thukkaram *et al.*, 2014). 200µl of inoculums were injected onto the Mueller-Hinton Agar plate. Agar was punctured to form 6 mm wells. Distilled water was used as a negative control, while antibiotics (Ampicillin and Gentamycin) served as a positive control. To test for microbes, 40 µl of synthesized pulp extract (Hexane, Chloroform, Methanol extracts), 40 µl of peel extract (Hexane, Chloroform, Methanol extracts), 40 µl of juice extract (Hexane, Chloroform, Methanol extracts), Antibiotics (40 µl) were applied to wells of a plate made of nutrient agar with *Escherichia coli* and *Staphylococcus aureus*.

RESULTS

Phytochemical screening of B. hispida fruit extracts - Phytochemicals, which are contained in vegetables, seeds, grains, fruits, and related plant foods, not only supply basic nutrition but also have positive health effects which reduces the chances of significant persistent illnesses. To determine the active ingredients in the *B. hispida fruit* extract, a preliminary phytochemical screening was carried out in the current study. The results showed the presence of pholobotannins, phenols, amino acids, sterols, saponins, carbohydrates and triterpenoids (Fig 1).

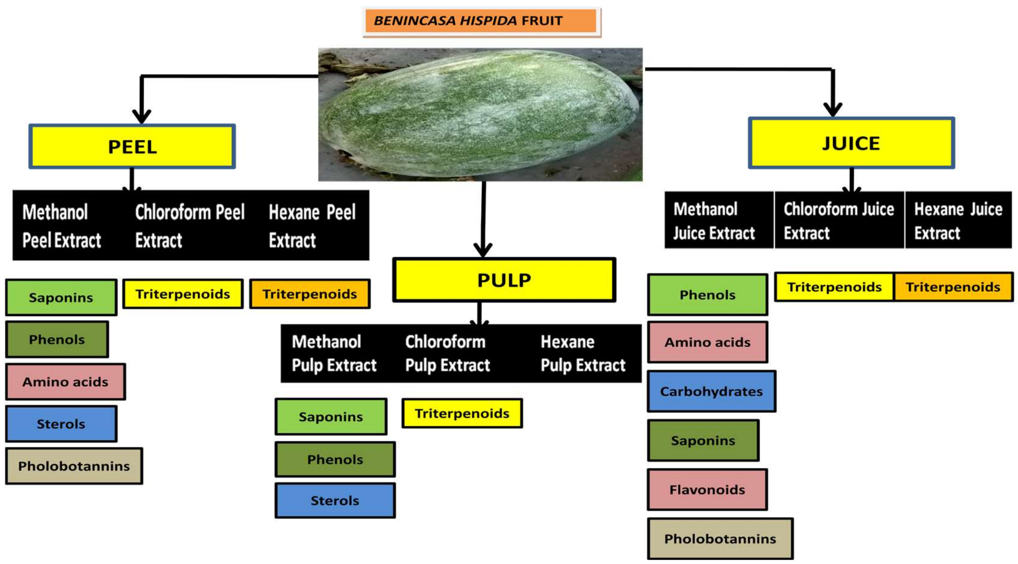

Fig 1. Presence of different phytochemicals present in peel, pulp, juice extracts of *B. hispida* fruit.

DPPH scavenging activity in B. hispida fruit extracts- The radical-scavenging capacity of extracts from plants serves as one of the most common methods for assessing their antioxidant activity. DPPH, a known stable free radical, was used to test the extracts' ability to scavenge radicals. When a nitrogen-centered free radical termed 1, 1-diphenyl-2-picrylhydrazyl is reduced; its hue changes from violet to yellow. The extent of discoloration reflects the extracts' ability to scavenge. Used DPPH radical scavenging to demonstrate the antioxidant activity of methanol extracts of peel, pulp, and juice. The extract of methanol of *B. hispida fruit* peel, pulp, and juice showed a comparable dose-dependent reduction in DPPH activity (Fig 2a, 2b and 2c). Juice extract's scavenging activity maximum at around 92.52% at a concentration of 100 µg/ml. Antioxidants capacity to donate hydrogen is suggested to be the reason for their impact on DPPH (Baumann et al., 1980).

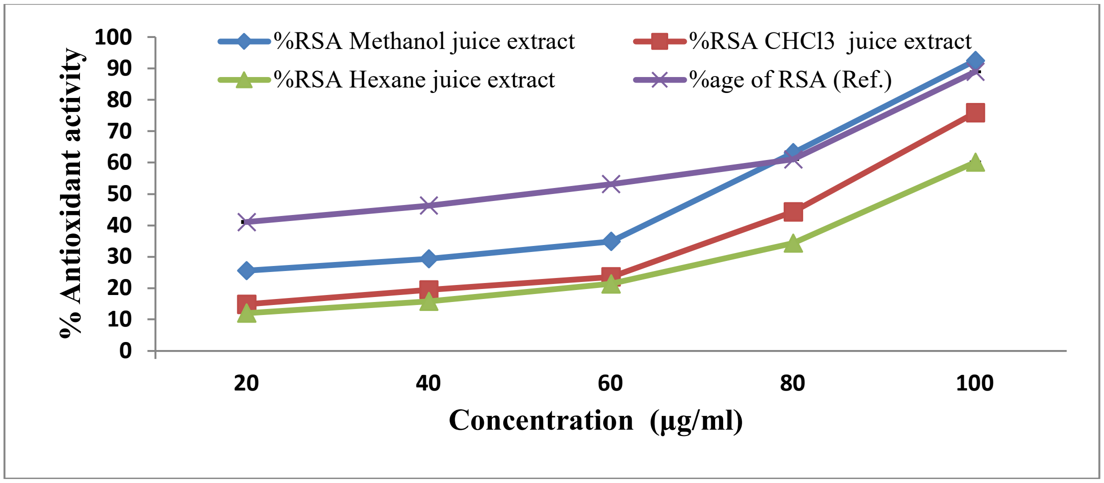

Fig 2a. Free radical scavenging activity (%) of *B. hispida* Juice extract.

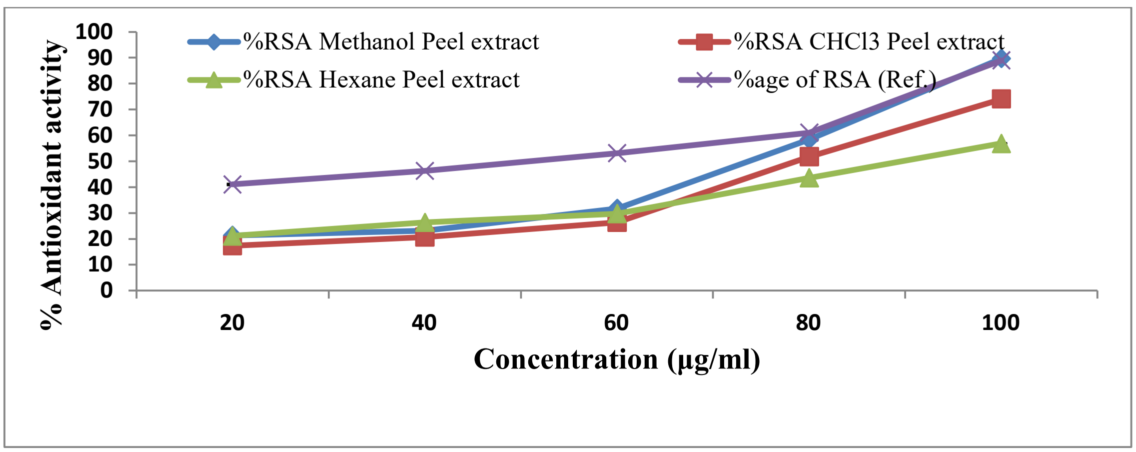

Fig 2b. Free radical scavenging activity (%) of *B. hispida* Peel.

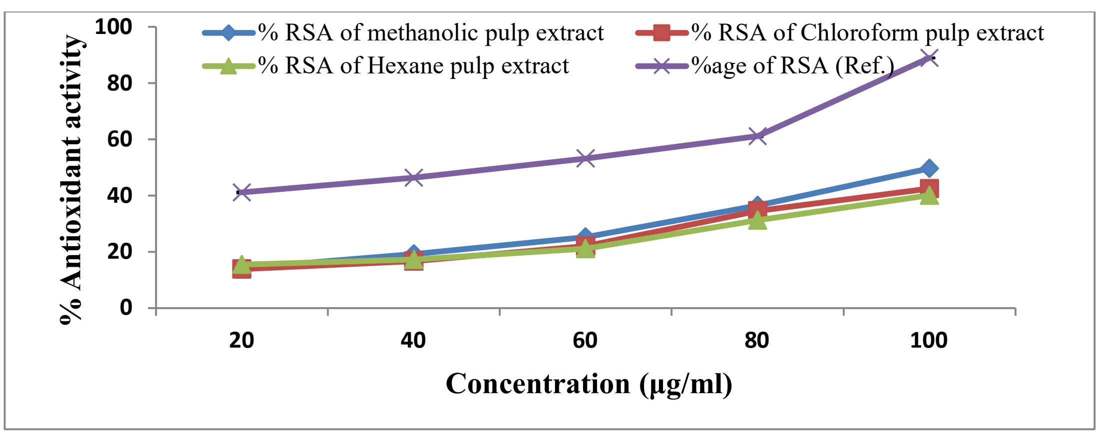

Fig 2c. Free radical scavenging activity (%) of *B. hispida* Pulp.

3.3 Total Phenolic Content- Hence total phenolic content of methanol extract of juice shows 90.57±0.98 mg/g, chloroform extract of juice has 60.18±1.63 mg/g and hexane extract of juice shows 22.56±1.87mg/g. Table 2 summarizes the phenolic content found among different extracts of *B. hispida* fruit.

Table 2. Total phenolic Content of *B. hispida* juice extracts.

S.No	Parameter	Concentration mg/g of extract		
		Methanol extract	Chloroform extract	Hexane extract
1	Total Phenolic content (Gallic acid/gm of *B. hispida* juice extract)	90.57±0.98	60.18±1.63	22.56±1.87
2	Total Phenolic content (Gallic acid/gm of *B. hispida* peel extract)	68.47±0.88	56.68±1.34	16.26±1.47
3	Total Phenolic content (Gallic acid/gm of *B. hispida* pulp extract)	34.42±1.74	17.61±0.69	8.34±1.28

Antibacterial potential in B. hispida fruit extract- Agar well diffusion was the technique employed to ascertain the antibacterial properties of several fruit extracts from *Benincasa hispida*. Methanolic extract was shown to be more efficient against GNB than GPB in the graphs (Fig 3 and 4). This variation could be attributed to changes in the chemical structure of the cell walls of both bacterial species. The strong peptidoglycan coating on the cell wall of the GPB increases its resilience and, consequently, its resistance to the penetration of juice containing methanolic extract from *Benincasa hispida*. The peptidoglycan layer that forms the inside of the GNB cell wall is quite thin.

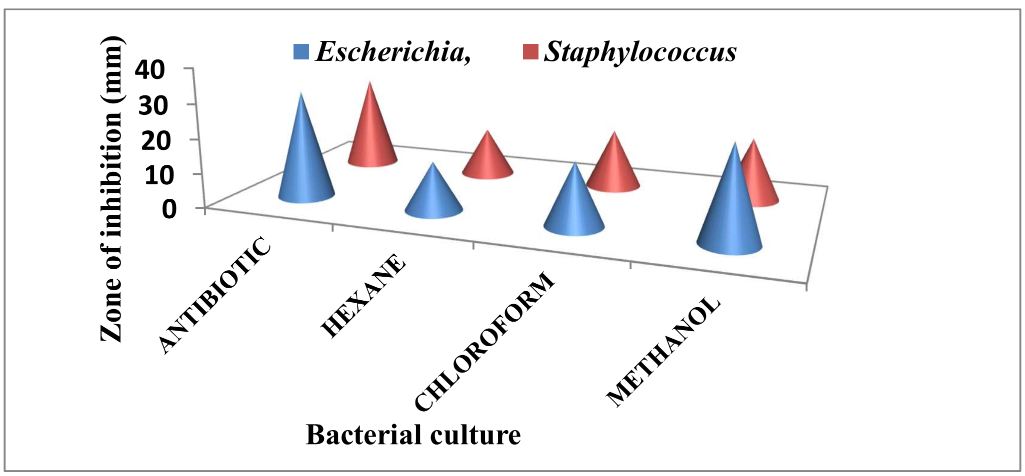

Fig 3. Antibacterial activity of peel of *B. hispida* (methanol, chloroform, hexane extracts) against gram positive and gram-negative bacterial strains.

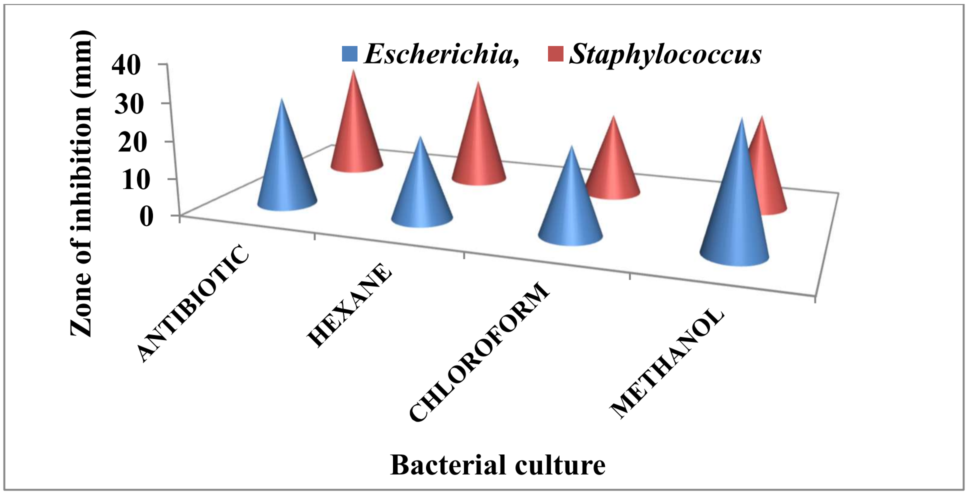

Fig 4. Antibacterial activity of juice of *B. hispida* (methanol, chloroform, hexane extracts) against gram positiveand gram negative bacterial strains.

Plant extracts can be utilized to break down bacteria's protective layers due to their enormous surface area, which accounts for their heightened chemical and biological activity. Active oxygen species formed by the juice of *B. hispida* methanolic extract harm the bacterial cell wall by connecting across the bacteria cell membrane and allowing plant extracts inside the cell (Anjali *et al.*, 2023).

DISCUSSION

B. hispida fruit is widely used for the production of variety of candies and delicacies called petha. Petha industry produces 700 to 800 tons of petha every day and generate lots of agrowaste. *B. hispida fruit* is not only commercially used, it also possesses therapeutic and nutritive values. *B. hispida* juice is an excellent detoxifier, this drink effectively absorbs toxins, pathogens, and pollutants from the body throughout the day (Aggarwal *et al.*, 2023). *B. hispida fruit* exhibits phytochemical screening of several extracts, resulting in high levels of triterpenoids, amino acids, carbohydrates, saponins, flavonoids, phenols, and Pholobotannins and these phytoconstituents are responsible for the antioxidant and antibacterial activity (Elleuch *et al.*, 2011). The free radical scavenging activity using DPPH assay was used to compare the different extracts of *B. hispida* fruit. The increasing in concentration of plant components leads to an increase in radical scavenging activity %age. The higher the hydrogen donor capacity, the greater the antioxidant activity. As a result Phenolic, other bimoleculars found in plants are more abundant in the polar extracts than in other extracts (Ghanimi *et al.*, 2022).Agar well diffusion technique was employed to ascertain the antibacterial properties of different fruit extracts from *Benincasa hispida*. These extracts have been shown to have excellent antibacterial activity against both GPB (*Staphylococcus aureus*) and GNB (*Escherichia coli*). Methanolic extract was shown to be more efficient against GNB than GPB (Epand and Epand, 2009).

CONCLUSION

Petha from *B. hispida* is thought to be the purest type of sweet in India. Its exceptionally nutritious nature has led to its widespread serving throughout world. Due to its large-scale production disposal of its waste has become a challenge. The current study indicates that the agrowaste (Peel and pulp) possesses a noteworthy quantity of phytochemicals; hence, its potential as an antioxidant and antibacterial agent was investigated and Biomass waste exhibited noteworthy biological activity in results. Although hexane extracts of peel and pulp does not show good biological activities but chloroform and methanol extract have potency against the bacteria and free radicals. While the juice extracts show phenolic content ranged between 22.56±1.87 and 90.57±0.98 mg/gm which is quite high and contributes to the antioxidant activity. Methanolic juice extracts had higher phenol concentration (90.57±0.98) as compared to chloroform and hexane extract. The findings demonstrate that all extracts of juice, peel, and pulp included several active phyto-components that inhibit the growth of particular pathogenic bacteria GNB (*Escherichia coli*) and GPB (*Staphylococcus aureus*). Although Pulp has not shown any significant zone of inhibition, which indicated that pulp of devoid of antibacterial activity yet it has good free radical scavenging potential. On the other hand, the results indicate that juice showed greater antibacterial values rather than peel extract**.** Methanolic extract of juice found to exhibit more efficiency towards GNB (*Escherichia coli*) than GPB (*Staphylococcus aureus*). Therefore, it is concluded that the juice of *B. hispida* has more potent antibacterial and antioxidant activities. Moreover, the peel, and pulp (agrowaste) of methanolic extract has shown antioxidant activity. At the same time high fiber content presents in the edible, harmless pulp it can have applications like uses as natural laxative. Due to the pharmacological importance of agrowaste of petha (peel and pulp) and high fiber content present in the pulp there is a wide scope for future studies. It will prove a milestone in curbing pollution caused due to agrowaste and it can also be employed in drug designing/pharmaceutical industry due to its therapeutic value in the coming years.

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