

## FUNCTIONAL POTENTIAL OF UNCONVENTIONAL PLANT SOURCES

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### ABSTRACT

**Objective:** Plant phenols are extensively studied, thanks to their many prophylactic (anti-tumoural, ulcer, inflammatory) and therapeutic effects. Their many antioxidants may protect against diabetes, cancer, autoimmune diseases. Our objective was to evaluate antioxidant and antimicrobial activities of our samples: *Cocos nucifera* (shell), *Punica granatum* (peel), *Citrus limonum* (rind), and *Ocimum sanctum* (stem); in conjugation with phytochemical analysis.

**Methods:** The hydro-methanolic extracts of the selected plant parts were assessed for polyphenols. Antioxidant (ferric reducing power assay) and antibacterial (Kirby Bauer disc diffusion) assays were run for varying concentrations against-*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*.

**Results:** The results showed that the samples all contained polyphenols with high total phenolic activity in pomegranate and high antibacterial activity in coconut. A more comprehensive study could lead to their emphatic incorporation into mainstream medicine and pharmaceuticals-furnishing natural alternatives to their chemical counterparts.

**Conclusion:** The present study concludes that hydro-methanolic extracts of the samples contain phytochemicals in high concentrations, conferring upon them promising antioxidant and antimicrobial activity.

**Keywords:** Antibacterial potential, Antioxidant activity, Polyphenols

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### INTRODUCTION

Over the years, there has been an alarming growth in the use and consumption of synthetic antioxidants in food. These have been confirmed to cause health problems like gastrointestinal and liver issues and may even be potential carcinogens [1]. Moreover, bacteria have grown to show increased virulence against potential antibiotics creating new challenges for humans to create an efficient and target-based treatment. Several studies on plants and their parts have been investigated for their use as potential antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins and other antimicrobials including various therapeutic agents. Since such medicines are all-natural and devoid of synthetic agents, they have drawn the attention of several researchers. Ingestion of natural antioxidants could prove as an effective source of natural medicine for various bacteria to reverse mortality from degenerative disorders. The following are the plants selected for this study.

*Cocos nucifera* (Coconut) also known as 'Kalpavruksha' according to Indian scientific literature, belongs to the family Aceraceae, and is a widely consumed fruit crop in tropical countries. It is predominantly found along the coastline of the Indian subcontinent. Multiple uses for each of the fruit's components are known, all of them being biologically active in some way. Coconut shells and husks are considered to have a relatively small role to play when it comes to medicinal use. The shells are burnt in remote parts of central Maharashtra, India as a mosquito repellent. The coir of the fruit was used as a scrub in ancient times for cleaning brass, copper and aluminium utensils. Some remote villages in the country still use it to this day. Reports of husk fiber being used to treat diarrhoea, diabetes, dermatitis, kidney inflammation, and asthma, in countries such as Guatemala, Brazil, Peru and Mexico have been found earlier [2].

*Citrus limonum* (Lemon) like other citrus fruits, orange and lime have been pressed into juices so that people can get their doses of vitamin C, for generations. Lemon belongs to the family Rutaceae and has been found to be rich in phytoconstituents like vitamin C, alkaloids, flavonoids and essential oils. The peels of this fruit are usually

discarded after the pulp and juice is used. However, even the rind is somewhat of a power-packed ingredient, since peel extracts have previously shown antibacterial and antifungal properties, and have the ability to decrease occurrences of skin cancer [3, 4].

Pomegranate (*Punica granatum* L.) of the family Lythraceae, is a fruit indigenous to the Mediterranean region. However, it has been used widely in Indian folk medicine. It is already common knowledge that the seeds of the fruit (edible part) can be used to prevent and manage numerous illnesses and health conditions such as inflammation, dental plaque, dysentery, or even diabetes and cancer. However, the inedible peels, which constitute almost 60% of the total weight of the fruit, are usually thrown out as waste products. These rinds could have even more medicinal and healing properties than the fruit. They are rich sources of a host of phytochemicals, which endow them with potential antimicrobial and antioxidant activity.

The peel is said to help in reducing inflammations, diarrhoea, dysentery and bleeding. It is said to facilitate digestion and is also a good stimulant for the liver [5].

Until today, research focusing on the biochemical properties of Coconut shell, Tulsi stem, Lemon and Pomegranate rinds are comparatively limiting and are oriented to other plant parts. These plant exocarps which are usually discarded as waste, are present in abundance and could also be therapeutic in nature. These plant part extracts remain to be widely explored for their healing potential.

The following study was carried out to evaluate the antioxidant activity and total phenolic content of the hydro methanol extracts of these parts of the plant *in vitro*.

### MATERIALS AND METHODS

#### Preparation of extracts

Hydro-methanolic extracts of coconut shell, pomegranate rind, lemon rind and tulsi stem were tested against *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* to determine their antibacterial activity.

Fresh Pomegranate (*P. granatum*) fruits were bought from the local market in Pune in the month of September 2019. The stems of *O. sanctum* also known as Tulsi, were collected from several households in Pune.

The powders for Coconut (*C. nucifera*) shell and Lemon (*C. limonum*) rind were imported from a supplier in Andheri (E), Mumbai and purchased online, respectively.

The pomegranate fruits were washed well-using tap water and their peels and pulp separated from each other. The peels were cut into smaller pieces and dried in shade at approximately 25 °C for a period of 10-15 d.

Similarly, Tulsi stems were cut up into smaller pieces and dried in shade at approximately 25 °C for a week.

The dried samples were first ground to a fine powder using a mortar and pestle and later in a mechanical blender. Dried rind powders (25 g) were packed into a distillation assembly and subjected to extraction with 100 ml, 60% methanol at 60–65 °C (to prevent denaturation of the phytochemicals) for 2–4 h. Thus, hydro-methanolic extracts of all the plant samples were prepared.

The filtrate was concentrated and dried in the refrigerator for a week, following which they were stored at 4 °C in storage vials for all further experimental use. All the samples were prepared by mixing the extracts with 60% methanol. All determinations for a particular concentration of the extract were performed in duplicates, were subjected to statistical analysis and standard deviation was measured.

#### Measurement of total phenolic content (TPC)

Total phenolic content (TPC) of the samples was determined using a modified version of the Folin-Ciocalteu reagent method [5, 6]. We prepared the standard curve using different concentrations of gallic acid (stock: 1 mg/ml). Varying volumes of the plant extracts were mixed with 0.1 ml of Folin-Ciocalteu reagent (1:3 diluted), and volume was brought up to 2.9 ml with water. After 10 min at approximately 28 °C, 0.3 ml 10% sodium carbonate was added to the reaction mixture. We determined total polyphenols after letting the mixtures stand for half an hour at 37 °C. We measured the absorbance of the test samples, now blue in colour, at 765 nm with a colorimeter. The authors expressed the results as mg of gallic acid equivalent (mg GAE/g).

#### Measurement of antioxidant activity

The *in vitro* antioxidant activity of the extracts was determined using Ferric reducing power assay (FRAP). Ascorbic acid was used as a

positive control. (stock: 1 mg/ml, working standard: 100 µg/ml). We worked with different concentrations (200–1000 µg/ml) of the concentrates and the standard, obtained by dilution with distilled water. The samples were mixed with varying concentrations of 200 mmol/l phosphate buffer (pH 6.6) to bring the volume in the tube to 2 ml. 1 ml of 1% potassium ferricyanide was added to the tubes and incubated at 50 °C for 20 min in a hot air oven. We further made an addition of 1 ml (10%) of trichloroacetic. Thereafter, the reaction mixture was centrifuged at 3000 rpm for 10 min. 2 ml of the upper layer was then mixed with 2.0 ml distilled water and 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance of the reaction mixtures was measured at 670 nm, with the help of a colorimeter. The authors expressed the results as mg/g of ascorbic acid equivalent.

#### Determination of antibacterial activity

We performed antibacterial activity in both Gram-negative (*S. typhi*, *E. coli*, *P. aeruginosa*) and Gram-positive bacteria (*S. aureus*). The cultures were obtained from the Department of Microbiology, Fergusson College (Autonomous), Pune, India. The Kirby-Bauer disk diffusion method was used to determine the antibacterial efficacy of the plant extracts *in vitro* [7].

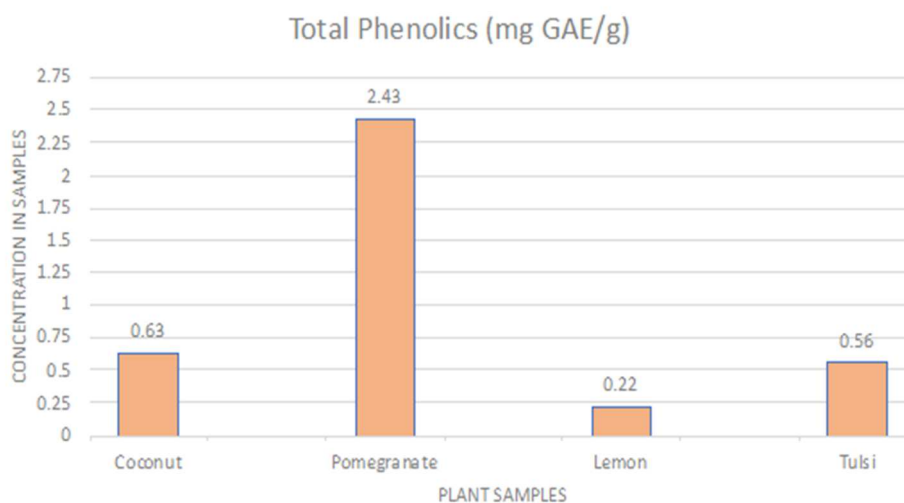
To prepare the plates on which the strains would be streaked for revival, 10 ml agar (HiMedia) was poured onto autoclaved plates. We used nutrient agar plates for *S. aureus* and *P. aeruginosa*; and MacConkey agar plates for *S. typhi* and *E. coli*.

The strains were streaked onto their respective plates and later incubated at 37 °C for 24 h. Further, single colonies were sub-cultured into a fresh nutrient broth medium (previously sterilized), and grown overnight in 10 ml broth at 37 °C Muller-Hinton agar plates were used as a bacterial medium for the final disc diffusion.

For the preparation of discs (on which samples would be made to absorb onto), sterile Whatman Paper 1 discs (5 mm in diameter) were loaded them 5 µl of varying concentrations (50 µg/5 µl, 100 µg/5 µl, 200 µg/5 µl) of sample extracts. A disc loaded with hydro-methanol was used as a control. The final step was the actual experiment, the disc diffusion. 0.1 ml of each bacterial suspension was uniformly spread in sterile conditions onto the plates. Further, the discs were placed equidistantly on the surface agar plates and allowed to incubate at 37 °C for 24 h. The efficacy of each extract was calculated by measuring the zone of inhibition caused by it.

#### RESULTS AND DISCUSSION

A primary physical analysis to determine the physicochemical properties of the plant extracts yielded table 1, as below.



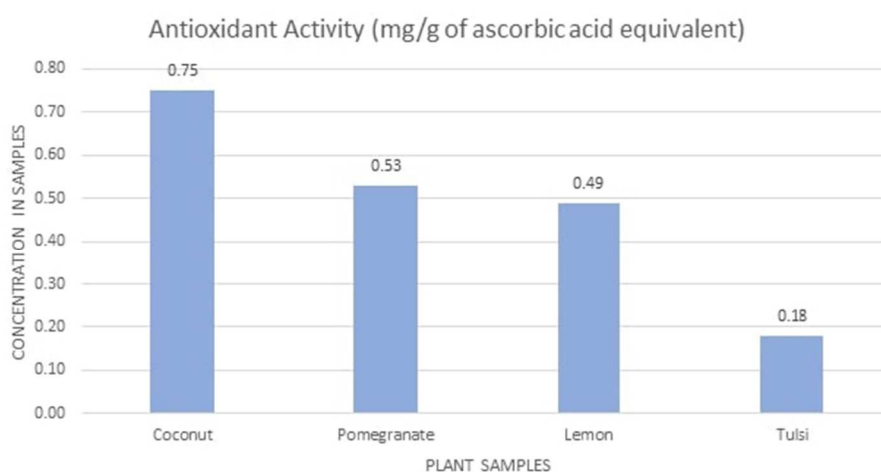
Graph 1: Total phenolics (mg GAE/g) in the four plant samples

**Table 1: Physiochemical analysis of the samples was carried out based on external primary observations**

Sample Characteristics	Pomegranate		Coconut		Lemon		Tulsi	
	Fresh peels	Extract	Fresh powder	Extract	Fresh powder	Extract	Fresh stems powder.	Extract
Colour	Maroon red	Brownish Yellow	Light brown	Brunette brown	Light brown	Light brown	Basil green	Brownish Yellow
Texture	Smooth particulate	Soft, sticky	Fine powder	Greasy, semisolid	Fine powder	Thick, flowy	Elongated granular.	Thick, sticky.
Odour	Odourless	Pungent	Odourless	Pleasant	Pleasant	Astringent	Pleasant	Strong astringent
% of extraction		36%		0.5%		20.88%		19.24%

Pomegranate extract has shown highest polyphenol content (2.43 mg/g), followed by coconut extracts (0.63 mg/g). (Graph 1) Tulsi and lemon extracts have shown comparatively fewer phenolics with readings of (0.56 mg/g) and (0.22 mg/g) respectively. The positive

results for these activities may be linked to the presence of tannins, saponins, alkaloids, and flavonoids in these extracts. The result showed higher values for coconut (0.75 mg/g) followed by pomegranate (0.53 mg/g), lemon (0.49 mg/g), and tulsi (0.18 mg/g) (Graph 2).

**Graph 2: Antioxidant activity (mg/g of ascorbic acid equivalent) in the four plant samples**

Polyphenols (for example quercetin, catechin) are secondary metabolites of plants. Secondary metabolites do not help in essential activities like growth or reproduction in a plant. Instead, they participate in UV radiation or pathogenic defence, transport of metals among other activities. When present in fruits, polyphenols may contribute to many characteristic properties such as colour, flavour, bitterness, astringency, fragrance and oxidative stability, nutritional value, and flavour. According to Pandey and Rizvi, some fruits like grapes, apples, and berries can contain as much as 200–300 mg of phenolic compounds for every 100 grams of their fresh weight [9]. The samples contain phytochemicals which absorb light and whose chromophore shows characteristic absorbance peak at 700 nm.

The antioxidant power of a substance can be determined via its reducing power. Total phenolic content is a direct indicator of antioxidant activity. Flavonoids, phenolic acids are reductants, helpful in scavenging free oxidising radicals-contributing to a sample's antioxidant properties.

The ferric reducing antioxidant power assay (FRAP) is a common colorimetric method and employs anti-oxidants as reductants in a redox reaction [9].

Phenolic acids present in Pomegranate include caffeic, chlorogenic acid, o-coumaric acid, p-coumaric acid, ferulic acid, syringic, vanillic, phlorodizin, rutin.

Our samples for pomegranate extract have shown good antioxidant activity in comparison to others used.

This is attributed to its many phenolic compounds: ellagitannins (punicalagin, punicalin, pedunculagin, gallagic acid,

ellagic acid, and gallotannins), anthocyanins (cyanidin, delphinidin and pelargonidin glycosides), flavonoids (quercetin, kaempferol and luteolin glycosides) [10].

However, ellagitannins are the most important phenolics of the fruit. Ellagic acid occurs in both, free and bound forms. Moreover, pomegranate has the highest concentration of punicalagin among commonly consumed fruits. Studies show that punicalagin has antioxidant and antimicrobial (fungi and bacteria) properties. The alpha and beta forms of punicalagin are polyphenolic, hydrolysable tannins. Punicalagin breaks down into smaller polyphenols in the human gut on account of their water solubility. According to some studies, punicalagin comprises 11–20 mg/g of the powder, while ellagic acid comprises 10–50 mg/100 g of the peel [11].

According a study conducted by S. Venkataraman and T. R. Ramanujam in 1979 on coconut shell extract, about 26.5% of total phenols were reported present in them. The experiment was carried on a small amount of sample dissolved in 95% ethanol [12].

Our present study on coconut extract showed the highest antioxidant activity—even more than pomegranate, although it has less total phenolics than pomegranate. Coconut shell is a rich source of lignin and cellulose. On comparing earlier studies of coconut extract we can estimate that an average of 29% of lignin and 25% of cellulose is present in the shell along with xylan and pentosans [13, 14]. Studies showing the antioxidant activity of lignin have been proved earlier. Lignins contain aromatic rings and functional groups. Due to presence of this chemical structure, they freeze the oxygenation reaction by stopping hydrogen donation [15]. Identification studies of phenolic compounds with antioxidant activity need to be carried out to confirm the present outcomes.

Earlier studies of TPC on tulsi leaves reported 4.33 g/100 g [16]. Another study of tulsi stems and leaves reported 2.84 g/100 g and 3.66 g/100 g respectively for phenolics [17]. Studies showing the distribution of these activities amongst the different plant parts in tulsi-leaves, inflorescence and roots have been few and far in between. As studied by Hakkim, *et al.* on tulsi, they reported the distribution of phenolic compounds like isothymusin, ursolic acid, carnosic acid, eugenol, sinapic acid and rosmarinic acid in plant parts of stem, leaves and inflorescence. The findings of these studies revealed that ursolic acid is absent in leaves, sinapic acid is absent in stems and eugenol is absent in inflorescence [18].

The genus Citrus (family Rutaceae) contain four types of flavonoids: flavonones, flavones, anthocyanins and flavonols. These are also responsible for the peel having powerful antioxidant properties. Lemon (*C. limonum*) pulp and peel have been found to be a rich source of natural occurring antioxidants. The citrus peels are rich in nutrients and contain many phytochemicals, such as  $\beta$  and  $\gamma$ -sitosterol, glycosides and volatile oils. Some polyethoxylated, phenolic compound, ascorbic acid, flavones which are otherwise very rare in other plants, confer upon lemon its special antioxidant characteristics [19].

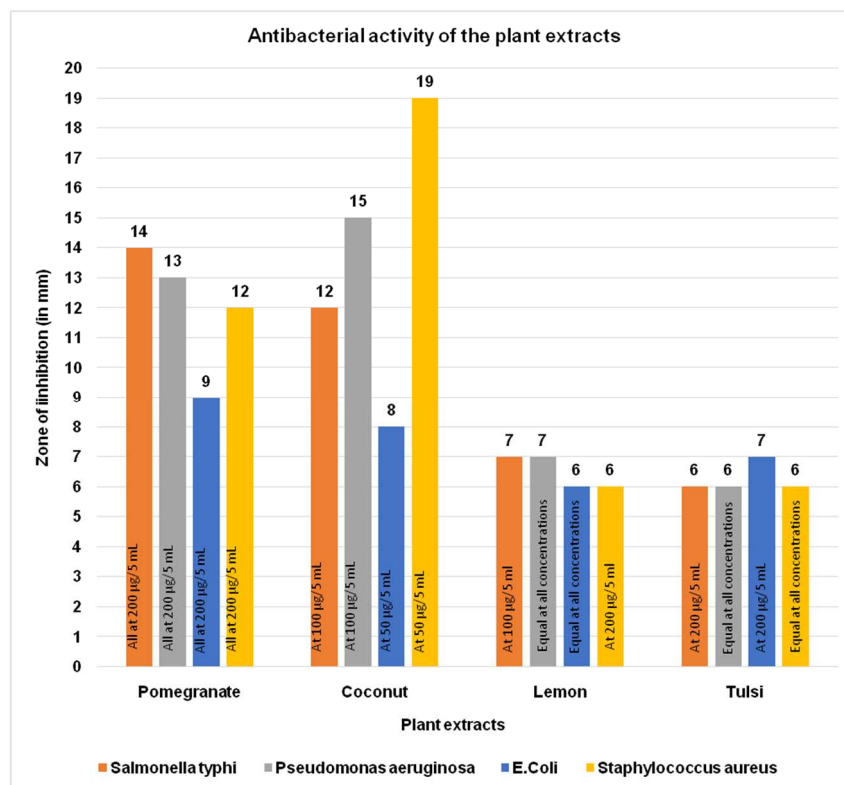
The scavenging (of free radicals) and therefore, antioxidant activity of an extract could be assigned to its phenolic content. Structurally speaking, phenols have aromatic rings with a hydroxyl group. Antioxidants work to neutralize free radicals by stopping their production chain [20, 21]. Initially, the reaction mixtures are yellow, on account of the ferricyanide complex, which when reduced, changes to its ferrous form, which is green/blue in colour.

Greater the reducing power, higher is the absorbance of  $Fe^{2+}$  at 700 nm. An increase in absorbance is noticed with an increase in concentration of reductants in sample mixture.

Extracts containing higher levels of phenolics act as agents and have the ability to inhibit reactive oxygen species (ROS) produced as a by-product of oxygen metabolism in the body or from natural conditions (like heavy metals or environmental pollution.) Oxygen free radicals play a pivotal role in the process of aging via oxidative stress. Oxidative stress is also known to cause a bevy of diseases; from cardiovascular and kidney disease to even neurological conditions.

Bacteria like *Pseudomonas* and *Staphylococcus* have grown to show increasing virulence capacity against chemical antibiotics over a sustained period of time and have made their way to the list of 'ESKAPE pathogens' [22]. *Pseudomonas*' ability to cling to life in a challenging domain is definitely credited to its skillful nutritional procurement; *S. aureus* dwells in about 20% of the global residents as a tenacious carrier qualifying to acute as well as chronic diseases [22]. Gram negative bacteria like *S. typhi* affect around 11–20 million individuals each year as reported by the World Health Organization. On the other hand, even though most of the *E. coli* strains are nontoxic, when individuals get infected by strains like Shiga toxin producing *E. coli* (STEC) antibiotics do not have a role to play [23].

Our Experiments showed that coconut shell extract significantly inhibits sturdy pathogens like *S. aureus* and *P. aeruginosa* amongst the others tested (Graph 3).



**Graph 3: Antibacterial activities of plant extracts (tested at 50, 100 and 200 µg/5 ml of extract concentrations respectively) against the four bacterial strains tested by disc diffusion assay**

Previous studies have revealed the presence of cellulose, methoxyl content, lignin, nitrogen, pentosans and uronic anhydrides in the extract [12]. Coconut shell powder shows two varieties of lignin structures. Since these gram-positive bacteria have a peptidoglycan layer they are able to link with sugar molecules of lignin, demonstrating higher activity in comparison to others used [24]. However, there have been previous studies of lignin reporting antimicrobial activity-due to the hydroxyl and methoxyl group in the

structure, but it also depends on the polarity of the solvent used [25]. Cellulose fibres, which forms the second major component of coconut shell extract are also known for showing antimicrobial activity as a functional property [26].

These phytochemicals, presence of cellulose, varied forms of lignin and apparent antioxidant activity of the shell extract may be interlinked; giving rise to the large zones of inhibitions observed.



Therefore, we can state that coconut shell (known to be used only as activated charcoal for industries) can also potentially serve as a natural antibacterial agent along with its husk.

Studies on coconut husk fibre ash earlier have revealed that, its chemical composition includes salts of sodium and potassium along with sodium oxide and sodium carbonate [27]. As it is known, sodium and potassium have a great role to play in maintaining the body's blood pressure and bone health. But unfortunately, our modern diet has more intake of sodium in comparison to potassium. Fruit exocarps like this one, which are often discarded as waste; are known to have a higher percent of potassium ions than sodium. Finding innovative ways to intake these exocarps can serve as a potential source of remedy for health disorders. Other divalent metal ions present in shell of coconut include magnesium, calcium, iron, zinc and small portions of copper [13]. Clearly, it has a good quantity of alkaline metals. Reports of coconut husk being used for treatment against diseases in South American countries have been found.

Extensive research on the whole Tulsi plant earlier has shown the presence of various activities of anticancer, antidiabetic, anti-inflammatory and antioxidant, among others [28]. Phytochemicals found in the Tulsi plant showed positive results for alkaloids, tannins, terpenoids, saponins, flavonoids, glycosides, fatty oils and carbohydrates [29]. Leaves of the plant contains anthocyanins whereas, the seed mucilage accommodates xylose and polysaccharide sugars [28].

However, it is of some importance to note that studies dealing with the antibacterial activity of tulsi stem alone are none. In a study by Singh *et al.* on the seeds of *O. sanctum. L.* showed excellent antibacterial activity against gram-positive bacteria. This was due to the fixed oils present in the seeds, primarily linoleic acid [30].

The volatile oils present in leaves contain eugenol, urosolic acid, carvacrol, and that in the seed mucilage consist of sitosterol and fatty acids [28]. Our studies show that tulsi stem (which is currently known merely to support the plant), also shows antioxidant and antibacterial capabilities. (luckily, that supports our hypothesis!) Presence of zones of inhibition can most definitely be linked to the saponins, flavonoids, triterpenoids and tannins in tulsi stem.

The lemon rind has previously shown positive results for the presence of carbohydrates, steroids, alkaloids, chlorides and flavonoids [31]. Our readings for lemon rind extract confirm the presence of antibacterial activities.

The antimicrobial activity of lemon is attributed to flavonoids such as hesperidine and naringin which are thought to enhance the activity of white blood cells and boost the body's defence system. Additionally, essential oils like monoterpenes, sesquiterpenes, alcohols, aldehydes, ketones and esters are present in lemon and its peel. Carvone and limonene are active against a wide spectrum of fungi and microbes [32]. We can suggest that the zones of inhibition executed, which are, in all probability, attributed to these phytochemicals present, in gram-positive as well as gram negative bacteria.

Pomegranate rind extract showed the highest total phenolic content of all the extracts we studied. Ellagic acid content is a factor that contributes significantly to the antimicrobial activity of the pomegranate extracts investigated [33]. Pomegranate peel powder extract has been used to treat food-borne diseases and urinary tract infections in the Indian Subcontinent for decades [34, 35] Pomegranate Peel contains polyphenols like ellagitannins, punicalagin, ellagic acid and gallic acid, which serve as natural antimicrobial agents. As a result, it has been widely exploited against *S. aureus* and the haemorrhagic *E. coli*. These compounds are able to precipitate bacterial membrane proteins and inhibit enzymes such as glycosyltransferases-bringing about cell lysis. In a study by Al-Zoreky, an *in vivo* application of an 80% methanolic extract of Pomegranate peel displayed an inhibitory effect against pathogens as: *Listeria monocytogenes*, *S. aureus*, *E. coli* and *Yersinia enterocolitica* [36, 11].

This ties in with the fact that it also showed significant antioxidant and antibacterial activities.

The extract's ability to act as a reducing agent, having amino acids, proteins, terpenoids, flavonoids, saponins, carbohydrates and phenols [5] existing in abundance help in showing maximum zones of inhibition.

Hydro-methanol acts as an excellent solvent system here since mixing water with methanol would make the solution less dense and help it diffuse better. This thus, makes it a suitable solvent for penetrating the cell's contents.

We found that hydro-methanol, by itself, shows no zone of inhibition against the bacterial pathogens used. Hence, we can deduce that our extracts have antibacterial activity in the first place. The solvent could then be used as the control for the anti-bacterial assay.

It is obvious that any kind of therapeutic activity of plant parts is on account of the phytoconstituents present in them. According to Manuranjan *et al.*, this could be attributed to the fixed oils in fruits like *C. limonum*. This just goes to show that plant parts like peels that are normally waste products, could be valuable for their potential as anti-bacteria and anti-oxidants. They could even be integrated into the beauty industry's products like soaps and lotions. It is predicted that the effectiveness of a plant extract for therapeutic use essentially depends on the extracting solvent used. The solvents used during the extraction process, confer upon the plant extracts, slightly variable pharmacological activities, depending upon their chemistry with each other. The role of the solvent is extremely important in this process since it determines how much of the metabolite will be extracted, depending upon their chemistry with each other. It has been previously shown that organic extracts are somewhat more efficacious than aqueous extracts [37].

The most important variables that determine the results and therefore reproducibility of such diffusion assays are:

1. Density of bacterial suspension.
2. Concentration of the extract/substance to be tested.
3. The time between: inoculation of bacteria and application of the extract and start of incubation [38].

Several mechanisms have been put forward to explain why metabolites such as flavonoids, tannins and phenols might confer antimicrobial activity on a particular plant part.

These mechanisms include:

- Inhibition of extracellular microbial enzymes
- Obstructing oxidative phosphorylation in pathogens or depriving them of essential growth nutrients [20]
- Inhibition in synthesis of nucleic acids and metabolisms
- Obstruction in function of cytoplasmic membrane
- Hindrance in formation of biofilm
- Alteration in degree of permeability of membrane
- Attenuation of the pathogenicity [39]

## CONCLUSION

This study proves the presence of phenols and antioxidants in hydro-methanolic extracts for the various plant parts we used. They also show potential against common disease-causing bacteria, as they all displayed at least a little activity (zone of inhibition) in both, gram-positive and gram-negative bacteria, *in vitro*. We also believe that, most of the plants and its parts are synergistic in nature. Segregating and using the plant parts (unless it's harmful) for biological activities would reduce its potency. An intact structure can be far more effective.

We hope that our study opens new avenues and encourages future researchers to dwell into the benefits of uncommon plant parts for their therapeutic abilities. However, future studies requiring long-term evaluation, *in vivo* are required.

We would urge future investigators to widen their horizons and think of unconventional plant parts as more than just having a part

to play in the plant's development. For all we know, they could do wonders for us too!

Our study however, has certain limitations. The authors were unable to conduct GC-MS assay, but are in the process of expanding their study. As a result, they had to rely on literature already present to help identify the biochemicals present in the plant extracts. Thus, they couldn't disclose the exact mechanism for their research findings. They present their hypothesis based on other scientific papers to encourage future studies.

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#### AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

#### CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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