A sustainable synthesis, characterization of modified waste onion peels and its exploration in various applications

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Abstract. The modification of waste to a bio-based material that is economical, environmentally sustainable, and extremely effective is crucial for organic conversions. For bio waste, that doesn't decompose easily and have bad impact on environment such as onion peels, a suitable method is introduced which focuses on utilizing onion peels, a commonly discarded agricultural waste, as a valuable resource for antibacterial studies and bio sensing of heavy metals. A comparative study to analyse these properties were done using onion peel extract and saponified onion peels. The saponified material was obtained by treating onion peels (Allium cepa) with sodium hydroxide in the presence of calcium carbonate. The popularity of saponification reaction and saponified material is rising because of their special qualities and uses as disinfectants, surfactants, antifungal agents, in drugs for target based delivery etc. The saponified onion peels was analyzed by FTIR (Fourier transform infrared spectroscopy), and XRD (X-ray diffraction), EDX (energy-dispersive X-ray), SEM (scanning electron microscopy). Furthermore, against *Bacillus subtilis (Gram positive)*, antibacterial activity of onion extract and saponified onion peels were studied. In comparative study onion peel extract shows better inhibition zone then saponified onion peels. Bio sensing of heavy metals were done by using these materials, onion peel extract shows visual colour change with Nickel chloride ($NiCl_2$), Cobalt chloride ($CoCl_2$), Potassium bromide (KBr), Manganese sulphate ($MnSO_4$), Mercury chloride ($HgCl_2$) and Copper sulphate ($CuSO_4$) metal salts.

Keywords. Waste onion peels, Saponification, Green Synthesis, Antibacterial Action.

1 Introduction

With increase in population, the demand of products provided by nature also increases but there is a limited supply of product. As consumption is on raise, the waste produced have also increased, one such example is onion peels from kitchen waste. Onion, an essential ingredient in cuisines all over the world, has drawn interest for its unique flavour as well as

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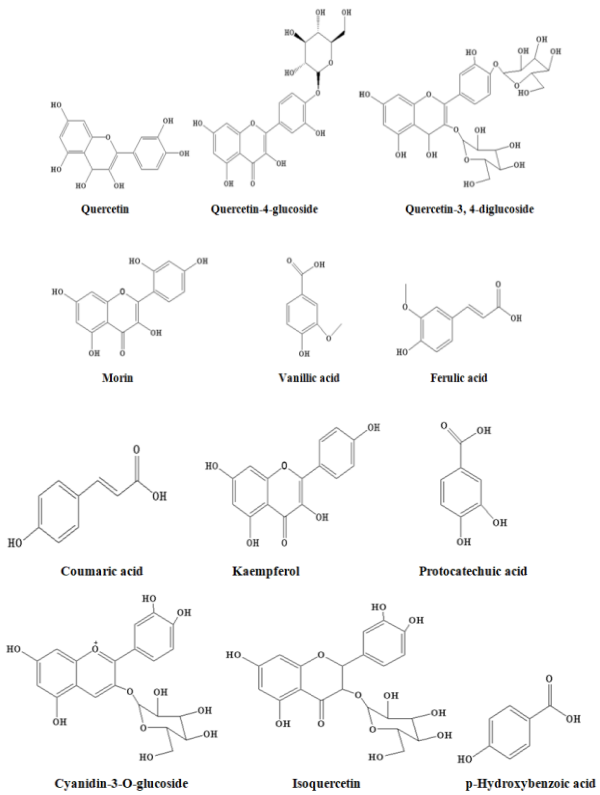
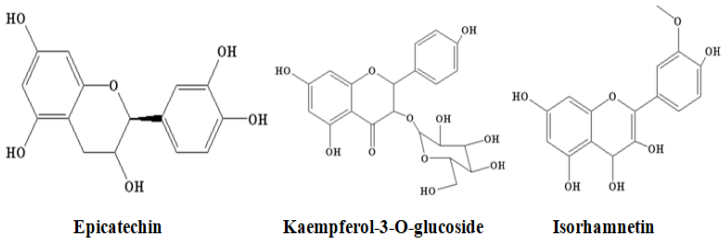
its biological health advantages. Apart from its culinary use, onion peels possess an abundance of bioactive substances that have captured the attention of both scientists and health enthusiasts. Due to high utilization of onions in day to day life produces large amount of onion waste. Globally 93.23 million tons (MT) onion is produced among which Iran (2.35MT), USA (3.03MT), Egypt (3.12MT), India (19.42MT) and China (23.91 MT) are top five producers according to Atlas Big. According to studies, the European nations are able to generate close to 0.6 million tons of onion waste yearly out of all producers [1]. Decomposition of this waste is not that so easy even it cannot be used as fertilizer because of strong aroma, and possess negative effects on the environment if improperly disposed off [2]. For such bio wastes, the need for sustainable waste reduction strategies has become increasingly important. Onion peels, the outer layers of onion, have various uses and benefits[3][4][5]. It contains phenolics[6][2][7], flavonoids[7][2][6], flavanols[2][5], anthocyanins[8][9][7], tannins[9][5], vanillic acid[10][5], and ferulic acid[10][5] (quercetin, quercetins-4-glucoside, isoquercetin, coumaric acid, epicatechin, kaempferol, protocatechuic acid, isorhamnetin, cyaniding-3-*O*- glucoside, *p*-hydroxybenzoic acid, kaempferol-3-*O*-glucoside, cyaniding-3-malonylgiucoside, isorhamnetin-3,4-diglucoside, quercetins-3,7,4'-triglucoside)[5] as shown in figure.1. Onion skin has the high amount of phenolics and flavonoids when compared to edible onion flesh/bulb[6][11]. They have rich amount of flavonoids (19.45, 25.9, 43.1 quercetin equivalent (QE)/g), flavanols (19.2, 15.29, 7.89 mg/g) phenolics (19.7, 30.5, 52.7 mg gallic acid equivalent (GAE)/g) on the other hand inner scales (9.4 mg GAE/g, 7.0mg QE/g, 6.19 mg/g) and onion (17.3 mg GAE/g, 10.3 mg QE/g, 8.84 mg//g), respectively, based on dry matter.



(a)



(b)

Fig. 1. (a)&(b) Structures of various important bioactive compounds in onion peels[5].

According to the recent study, onion wastes also have dietary fibre content (169-1750 mg/g dm (dry matter)), protein content (8.3-15.6% dm), ash content (4.4- 8.6% dm), minerals (such as potassium, calcium, magnesium, selenium, iron, manganese, zinc) and carbohydrates[2][12]. As it have lot of bioactive components they have been studied for their potential health benefits, including anticancer activity[13], antimicrobial activity[14][15], antiobesity activity[16][4], neuroprotective activity[17], cardioprotective or antiplatelet activity[4], anti diabetic activity[18][9], erectile dysfunction[19][20] in different forms.

In its actual form onion wastes are not of any use but by doing some treatment and modification it can be used for various applications. Various ways for modification:

The OPE (Onion Peels Extract) consists of total flavonoids, phenols and volatile organic compounds (VOCs) with ester being majority. These compounds are responsible for the bioactivity of the OPE. When OPE is treated with any metal salt then enhanced bioactivity was observed[21][14][5][20][3]. In recent years different nanoparticles of onion peels are studied for different purposes. Nanoparticles formed by combining onion peels with metals have gained significant attention in recent years. These hybrid nanoparticles offer unique properties and have a wide range of potential applications[22][23][14][21]. By harnessing the natural compounds present in onion peels and incorporating metals, these nanoparticles exhibit enhanced properties such as improved catalytic activity, increased stability, and unique optical or magnetic properties. These characteristics make them highly versatile and suitable for various fields, including catalysis, sensing, drug delivery, antimicrobial coatings, water purification and environmental remediation[21][14][23][15]. But in these methods many solvents are required and also the amount of product formed is very small as compared to amount of starting material so in this work crude onion peels were used directly in formation of products.

In this paper onion peel extract and modified onion peels were studied for different applications. Onion peels were modified into saponified onion peels. Saponification is beneficial chemical process that has attracted a lot of attention because of its various uses in the pharmaceutical, cosmetic, and sustainable material synthesis sectors[24][25][26]. It is a chemical technique used to produce soap from fatty materials, and used to extract chemicals from natural sources. Numerous bioactive substances, such as flavonoids phenolics, and other antioxidants, have been found in onion peels and have drawn interest due to their possible medical and industrial uses[24]. The process of saponification which releases the beneficial chemicals found in onion peel requires using a strong basic, like potassium or sodium hydroxide, to dissolve ester/acid bonds present in bioactive compounds.

2 Experimental

2.1 Reagents

Thermo Fisher Scientific India (Pvt) Ltd. provided the calcium carbonate and sodium hydroxide, onion peels (Alluium cepa) were collected from nearby marketplaces. Saturated solution of calcium carbonate and concentrated solution of sodium hydroxide were prepared using distilled water. Every piece of glass wares were cleaned properly with distilled water, it was dried at 337K in oven.

2.2 Greener method for synthesis

2.2.1 Synthesis of saponified onion peels

Onion peels were collected from local vegetable market. Washed repeatedly with distilled water to remove unwanted soluble contaminants and dried in oven at 343K until completely dehydrated. Grind properly to convert into powder. From here named as raw onion peels abbreviated as ROP.

About 100ml saturated solution of calcium carbonate (CaCO3) was added in 20g of ROP. Observed pH of the mixture was 10pH. The mixture's pH was brought to around 12.0 by adding little amount of concentrated sodium hydroxide (NaOH) solution. Kept the mixture stirring for 30 hours at a speed of 420 rpm at temperature of 293K in order to finish saponification. Following equilibration, the obtained material was washed continuously with distilled water to attained neutral pH (using Buchner funnel). Resultant was dried in oven at 343K. Here the final product was obtained named as saponified onion peels (figure 2).

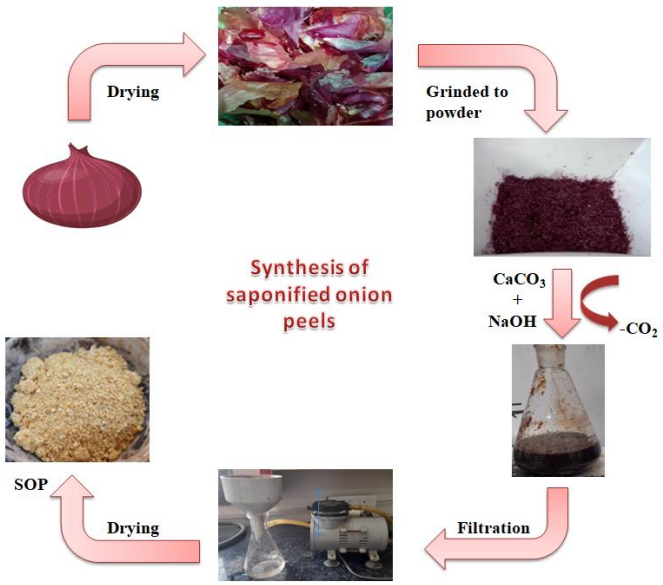


Fig. 2: Synthesis of Saponified Onion peels.

2.2.2 Extract Preparation

10 grams of washed and dried raw onion peels were taken in 500 mL of distilled water. Mixture was heated for 4 hours at high temperature. Color of the mixture turns brown. After cooling filteration was done and 250 mL of extract was obtained**.**

2.3 Phytochemical Screening of raw onion peels

To analyse the presence of polyphenols, tannins, and flavonoids in onion peels, extract was used for different tests.

2.3.1 Test for polyphenols:

$FeCl_3$ + OPE ⟶ Brown- red solution (positive)

1% $FeCl_3$ solution was drop by drop added in a test tube containing 2mL Onion peel extract. Presence of polyphenols was indicated by immediate colour change of OPE to brown red (figure.3 (A)).

2.3.2 Test for Tannins:

Onion peel extract + H_2O + $FeCl_3$ ⟶ Greenish Brown solution (positive)

In a test tube, 3 mL of Onion peel extract, 5mL of warm water, and 3 drops of 0.1% FeCl3 solution were added drop by drop. OPE's colour instantly changed to a greenish brown, indicates that tannins were present in Onion peel extract (figure.3 (B)).

2.3.3 Test for Flavonoids:

NaOH + OPE ⟶ Yellowish solution (positive)

2N NaOH solution was drop by drop added to a test tube containing 3mL of OPE. Flavonoid's existence in OPE was confirmed by instant color change to yellow (figure.3 (C)).

2.3.4 Litmus test for hydroxyl group and carboxylic acid:

Blue litmus paper becomes red when introduced with OPE. This indicates the presence of carboxylic acid and hydroxyl group.

2.3.5 Test for confirmation of carboxylic acid:

$NaHCO_3$ + OPE ⟶ Brisk effervescence (evolution of CO_2)

2mL of onion peel extract was treated with 5mL $NaHCO_3$ solution. Brisk effervescence was observed, caused by evolution of CO_2. It confirms the presence of carboxylic acid.

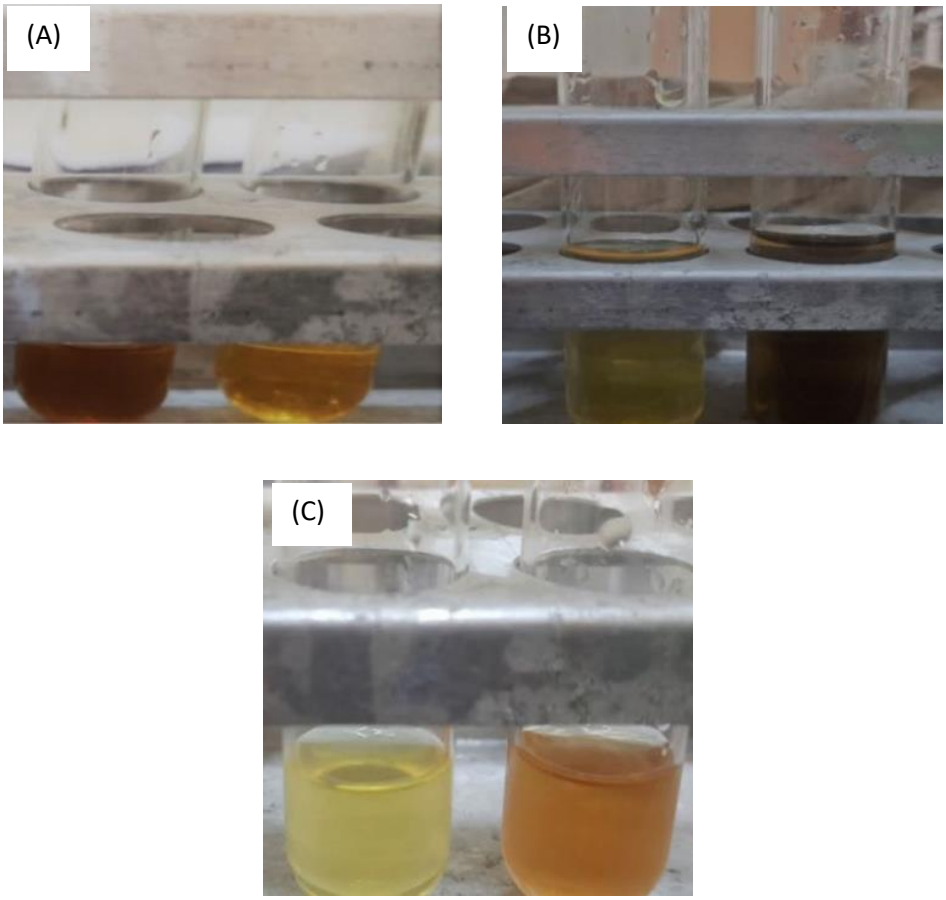


Fig. 3 : (A) Positive test for polyphenols. (B) Positive test for tannins. (C) Positive test for flavonoids.

3 Characterization

For FTIR analysis a Perkin Elmer Infrared Spectrophotometer was used to determine functional groups and changes in saponified onion peels, which was found in range 4000 to 400cm^{-1}. The material's surface morphological investigations were conducted by use of Scanning electron microscopy, or SEM technique. Information about crystals was analysed using an X- ray diffractometers (XRD) designed by the Bruker D8 Venture. Elemental composition was evaluated by using energy dispersive X-ray (EDX) spectrometer.

3.1. FT-IR data

IR analysis was carried out to identify different groups, which are present in raw onion peels and saponified onion peels. The IR spectra show peak at around 1732cm^{-1} is stretching vibration for C=O of Carboxyl group in acids which disappears after saponification and two new peaks at 1640cm^{-1} and 1380cm^{-1} appeared, indicates characteristic peaks of metal carboxylate (calcium carboxylate). In ROP spectra broad band at around 3282cm^{-1} is stretching vibration of –OH group of flavonoids, flavanols, phenolics, tannis, vanillic acid, ferulic acid, and anthocynanins due to coordinated hydroxyl group this peak becomes broad enough in saponified onion peels. Due to Presence of C-H stretching

vibration of an alkyl group the peak at around 2915cm^{-1} is observed (figure.4).

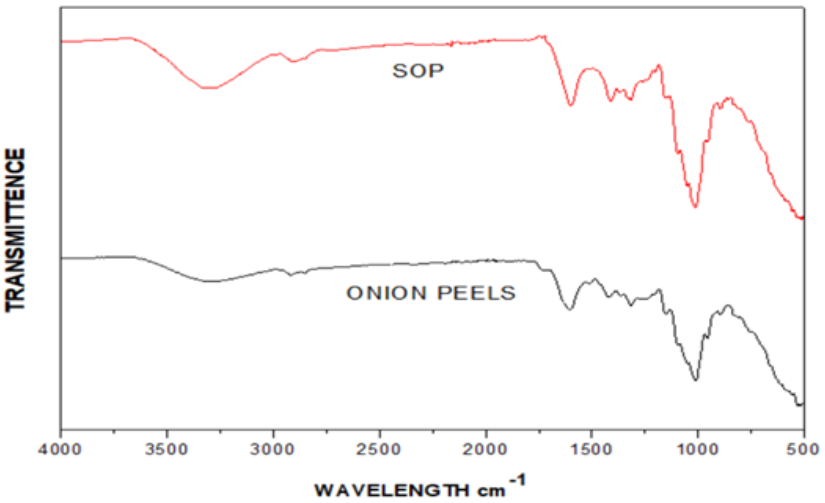


Fig. 4. FTIR Spectrum of onion peels and saponified onion peels.

3.2 SEM data

Through SEM scanning electron microscopy magnified image of the size, shape, crystallography, composition, and other chemical and physical properties of specimen. To see surface structure and assess surface variations, SEM material sciences and surface sciences are magnified many times. SEM Offers the interaction of electron beam (reflected electrons) with material. The characterization of surface of obtained material was done using SEM with an accelerating voltage of 15.00 kV, magnification X 700 and count rate 251.00 CPS. The structure observed for saponified onion peel was spiral and fibrous (as shown in figure.6) not reported yet in literature. In general, onion peels shows linear and uniform structure but from SEM data we observed disturbance in structure which indicate modification in structure takes place (figure.5).

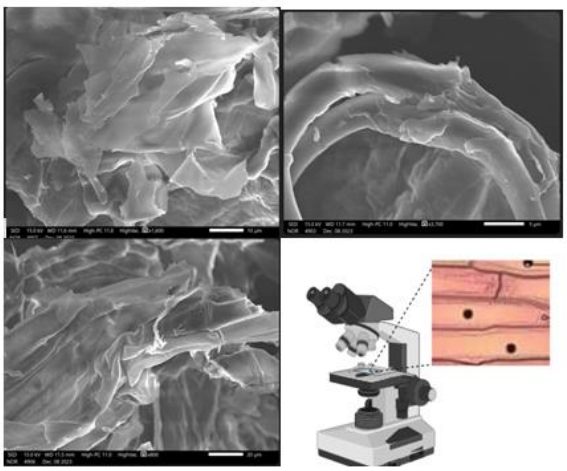
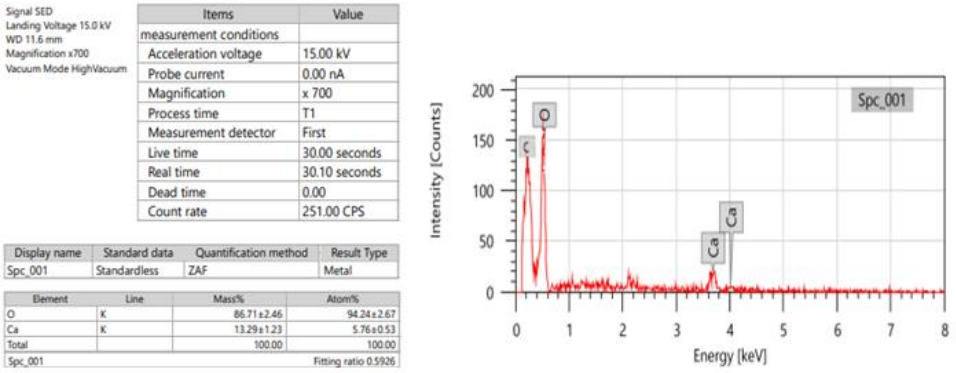


Fig. 5. SEM data analysis, disturbed structure of SOP and original layered structure of onion peels.

3.3 EDX data

Additionally, elemental composition is evaluated using an EDX, or energy dispersive X-ray spectrometer and the SEM. To get a localised chemical fingerprint, when a solid sample is exposed to a focused electron beam, EDX examines the X- ray spectrum to give results. From EDX data high intensity peaks were observed for carbon (at 140 count), for oxygen (at 180 count), for calcium (at 20 count) (as shown in figure.6).



Signal SED
Landing Voltage 15.0 kV
WD 11.6 mm
Magnification x700
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	15.00 kV
Probe current	0.00 nA
Magnification	x 700
Process time	T1
Measurement detector	First
Live time	30.00 seconds
Real time	30.10 seconds
Dead time	0.00
Count rate	251.00 CPS

Display name	Standard data	Quantification method	Result Type
Spc_001	Standardless	ZAF	Metal

Element	Line	Mass%	Atom%
O	K	86.71±2.46	94.24±2.67
Ca	K	13.29±1.23	5.76±0.53
Total		100.00	100.00
Spc_001			Fitting ratio 0.5926

Fig. 6: EDX data analysis.

3.4 XRD data

X- ray diffraction method was used to analyse structural information of saponified onion peels. Well defined sharp diffraction peaks at 22.8º, 32.35º were observed for saponified onion material rich in calcium and oxygen. Crystallinity index shows that material is 60% crystalline and 40% amorphous (shown in figure. 7).

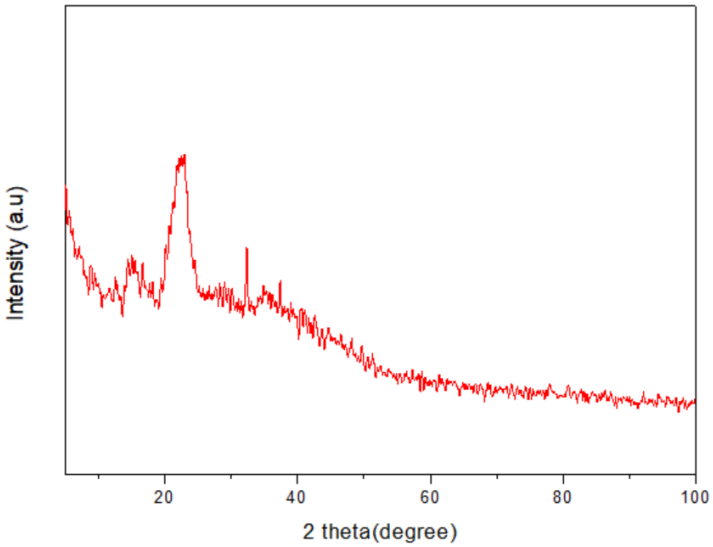


Fig. 7. XRD data analysis.

4 Discussion

From previous studies, onion contains acids (vanillic acid, ferulic acid, coumaric acid, etc.) and phenolic groups in quercetins, flavanols flavonoids and their derivatives. So when it reacts with calcium carbonate then evolution of carbon dioxide is observed and the wall of the container becomes hot. From here it is predicted that Calcium from calcium carbonate binds with two phenolic groups, two acidic groups, and one acidic – one phenolic group as calcium is divalent in nature. And reaction takes place (as shown in figure.8).

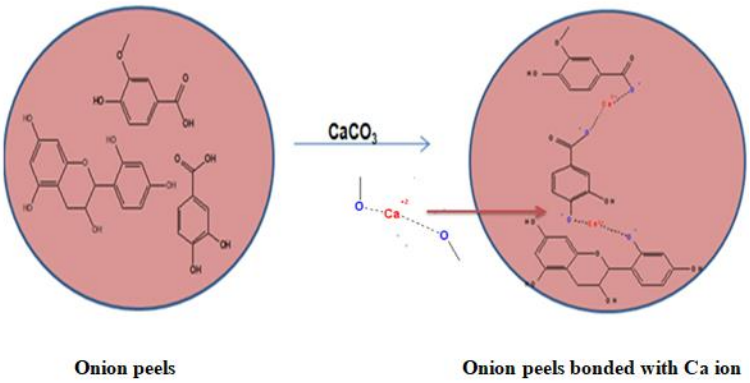


Fig. 8: Binding of Components of Onion Peels with Calcium Carbonate.

5 Application:

From literature Onion peel SWE (subcritical water extract) showed better antimicrobial activity compared to the control (methanol) against various pathogenic bacterial strains of Staphylococcus aureus (KCCM 32395, KCCM 40510, and KCCM 11335) indicated a decrease of 0.7-1.1 log CFU/mL in cell growth. But it was less efficient when compare to quercetins (used as reference); this might be because SWE extracts had lower quercetins content[27]. In Onion peels quercetins distrupts cytoplasmic membrane processes, energy metabolism, and nucleic acid synthesis, which all contribute to its antibacterial properties. SWE at concentration 110ºC at 0.6mg/mL shows bactericidal effects against Bacillus cereus (KCCM 11341 and KCCM 40935). Also SWE (160ºC) had a bacteriostatic effect at concentration of 1.2 mg/mL because it contains antimicrobial components such as quercetins and quercetins oxidation products. By disrupturing the cell wall, resulting in cell lysis, and allowing antimicrobial chemicals to enter the cell while leaking cell membranes, these compounds demonstrated antioxidant capability. Thus, a reaction with bacterial DNA eventually results in cell death[28]. The antibacterial activities of ethanolic and freeze – dried extracts against Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 11229 were measured using minimal inhibitory concentration (MICs) in comparison with quercetin. MICs for pure quercetin was (170-680), for ethanolic extract of onion skin powder (10.6-21.3) against bacteria. This clearly indicates that the onion skin powder ethanolic extract have significant antibacterial action against pure quercetins may have been facilitated by the presence of other phenolic bioactive components[29]. Gold nanoparticles (OP-AuNPs) were created using an aqueous extract of dried onion peel, and their potential biological uses were examined. The antimicrobial potential of Bacillus cereus ATCC 13061, Escherichia coil ATCC 43890, Listeria monocytogenes ATCC 19115, Staphylococcus aureus ATCC 49444, and Salmonella typhimurium ATCC 43174 were the foodborne pathogenic bacteria against which OP-AuNPs and antibiotics (kanamycin and

rifampicin) were identified using the disc diffusion method. OP-AuNP exhibited potent inhibitory effects on proteasomes as well as synergistic antibacterial and anticandidal action[14]. Antimicrobial activity of silver nanoparticles against Staphylococcus aureus (gram- positive) and Salmonella typhimurium (gram negative) was also studied. From comparative study it was noticed that nanoparticles shows excellent antimicrobial property this may happens because of their small size which made their entry inside cell easier. Comparative study is summarized in table 1.

Table 1: Comparative Study of antimicrobial property.

S.No.	Extraction Solvent	Bioactive Compound	Type of Cell line	Key Points	References
1.	Subcritical water extraction (SWE), Ethanolic, Hot water extraction	Phenolic group	B.Cereus KCCM 11341 and KCCM 40935	SWE had antibacterial properties and gives better results than ethanolic and water extract. Extracts were resistant for B. Cereus KCCM11341 and effective against KCCM40935. SWE extract at 0.6 and 1.2 mg/mL exhibited bactericidal and bacteriostatic activities, respectively, SWE at 1.2 mg/mL inhibited B.cereus KCCM 11341 from growing for B. Cereus KCCM 40935 cell death time was 60 minutes.	[28]
2.	SWE extract	Phenolic compounds	Staphylococcus aureus KCCM 1133, KCCM 40501, KCCM 32395	Among all the strains, S. Aureus KCCM 32395 was the most affected by onion peel extract. Less activity is observed when quercetin acts as standard, But reduction in cell number by 0.7-1.1 log CFU/mL compared with the control were noticed.	[27]
3.	Silver nanoparticles from onion peels	Phenolic groups	Staphylococcus aureus (gram positive) Salmonella	Against staphylococcus aureus (gram positive), at 8mm	[30]

			typhimurium (gram negative).	and for salmonella typhimurium (gram negative) at 9mm inhibition zones were observed.	
4.	Nanoparticles (OP-AuNPs)	Phenolic groups	*Escherichia coli* ATCC 43890, *Listeria monocytogenes* ATCC 19115, *Salmonella typhimurium* ATCC 43174, *Staphylococcus aureus* ATCC 49444, *Bacillus cereus* ATCC 13061.	With inhibition zone diameter of 10.66-19.95 mm OP-AuNP andkanamycin mixture shows good effect against all pathogens. But OP-AuNP and rifampicin mixture only stops the development of S.aureus and E.coli with inhibition zone 22.49mm and 9.99mm respectively.	[14]
5.	Nanoparicles of silver metal using aqueous extract of onion peel		Gram-negative *(Vibrio cholera and Escherichia coli, Salmonella sp. organism,)* Gram-positive *(Corynebacterium sp., Bacillus sp., Staphylococcus aureus)*	In dose-dependent manner (25-100 µg/mL) onion peel-based silver nanoparticles shows very good antibacterial efficacy against all tested pathogens. Inhibition zones against Staphylococcus aureus, Salmonella sp., Vibrio cholera, Corynebacterium sp., Escherichia coli and Bacillus sp. ranged from 13-19mm, 13-17.5mm, 14.6-18mm, 14-17mm, 14.5-19.3 mm and 14-17mm.	[31]

5.1 Antibacterial Activity

Onion peel extract and saponified onion peels were explored for antibacterial activity using well diffusion technique against the *Bacillus subtilis (Gram positive)* bacteria by zone inhibition test. In order to verify this activity nutrient agar (NA) solution was first prepared by adding 2.8g of nutrient agar in 100mL of DW (distilled water). The mixture, micropipettes and petri dishes were then autoclaved at 121ºC for 20 minutes. After this 25mL NA solution was poured in a petri dish in laminar flow. Till the NA solution solidified wells were prepared and samples were introduced inside the wells. In centre *A* represents *Gentamicin* used as standard inhibitor. *1* represents onion extract, *2* represents

SOP and *3* represents raw onion peels (ROP). It was observed that onion peel extract clearly displayed a good inhibitory zone of 11mm, saponified onion peels shows inhibition zone of 3mm and standard gentamicin inhibit bacterial growth with inhibition zone of 30 mm against *Bacillus subtilis*. Further, more studies are going on in the lab. In this study it was observed that onion extract shows better antibacterial activity against *Bacillus subtilis* (figure.9).

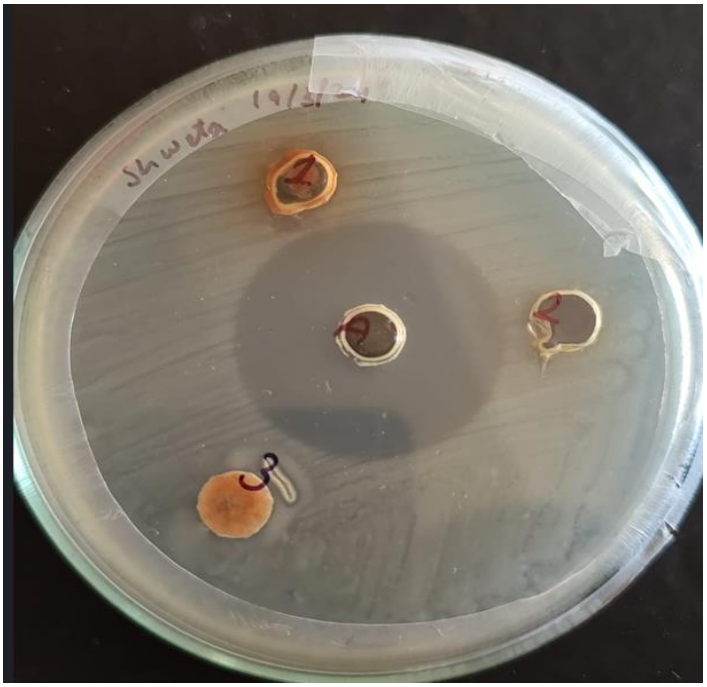


Fig. 9: Antibacterial activity against. Bacillus subtilis (gram-positive bacteria). A is standard gentamicin, 1 is onion peel extract, 2 is saponified onion peel, 3 is raw onion peels**.**

5.2 Biosensor:

Onion peel extract and saponified onion peels were studied for bio sensing of heavy metals. Nickel chloride ($NiCl_2$), Cobalt chloride ($CoCl_2$), Potassium bromide (KBr), Manganese sulphate ($MnSO_4$), Mercury chloride ($HgCl_2$) and Copper sulphate ($CuSO_4$) were chosen for bio sensing. 0.1M solution of these metal salts was prepared in 10mL of distilled water. 1mL of onion peel extract was added in 2mL of $NiCl_2$, $CoCl_2$, KBr, $MnSO_4$, $HgCl_2$, and $CuSO_4$ metal salts solutions. Visual colour change was observed for onion peel extract (as shown in fig 5.2). For saponified onion peels firstly solution of SOP was prepared by dissolving 2g of SOP in 20mL of distilled water then 1mL of saponified onion peel solution was added in 2mL of $NiCl_2$, $CoCl_2$, KBr, $MnSO_4$, $HgCl_2$, and $CuSO_4$ metal salts solutions. But there was no change in colour was observed (as shown in figure.10). From this observations it was analysed that onion peel extract can be used as biosensor for $NiCl_2$, $CoCl_2$, KBr, $MnSO_4$, $HgCl_2$, and $CuSO_4$ metals.

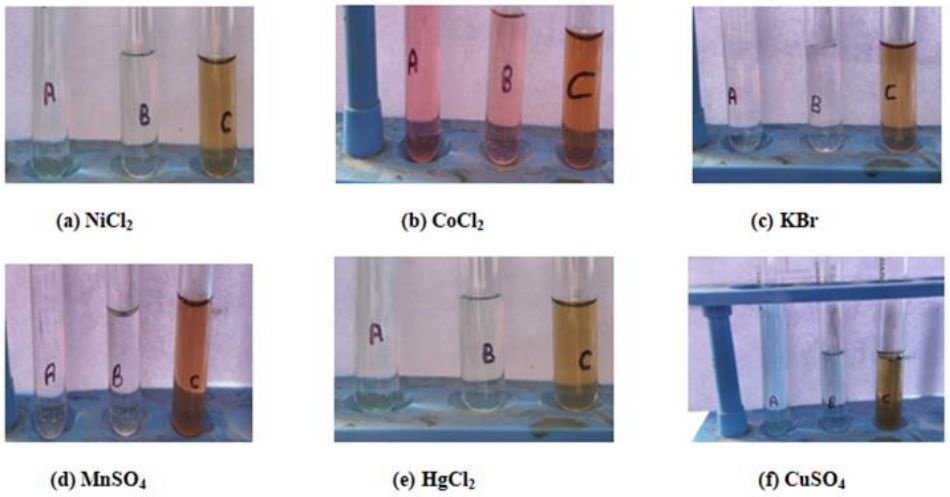


Fig. 10: As biosensors for Nickel chloride (NiCl2), Cobalt chloride (CoCl2), Potassium bromide (KBr), Manganese sulphate (MnSO4), Mercury chloride (HgCl2) and Copper sulphate (CuSO4). (A) denotes standard metal salt solution, (B) denotes metal salt solution with SOP, (C) denotes metal salt solution with onion peel extract.

6 Future scopes

Modified Onion peels, obtained by modification of outer layers of onion have shown promising as a natural chemical source with a wide range of possible uses. Because these compounds have special qualities, further study in this field has great potential in a wide range of fields. Numerous areas have great prospects to explore through the process of saponification, isolation, and characterisation of bioactive chemicals from onion peels. Future applications for modified onion peels cover a broad range of fields of study. Few future research scopes that could be explored are as *organic surfactants and emulsifiers, antifungal properties, biodegradable packaging materials, anti- inflammatory and antioxidant properties, treatment of waste water.*

7 Conclusion

In summary, there is great potential for using onion peel extract as a natural antimicrobial agent against *Bacillus subtilis*, a Gram- positive bacterium. Our study has shown that the onion peels extract produces bioactive chemicals that can impede the development and spread of *Bacillus subtilis*, providing a good substitute for artificial antibacterial agents. Apart from this, onion peel extract also shows bio sensing application for heavy metals. It can be further studied for different purposes.

The results highlight how crucial it is to investigate natural sources for their antimicrobial capabilities, for particularly in the fight against antibiotic resistance and the problems that bacterial infections bring to public health. The possibility for developing new antimicrobial agents derived from easily accessible, affordable, and eco-friendly source is suggested by the efficacy of onion peel extract and saponified onion peel against *Bacillus subtilis*.

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