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GC-MS Profiling, Antioxidants and Antimicrobial Activity of Prickly Pear (Opuntiaficus-indica) Pulp Extract

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ABSTRACT

The objective of this study was to evaluate phytochemical screening, antioxidants and antimicrobial activity of prickly pear pulp extract. Phytochemical screening was performed on the methanolic extract of the sample followed by gas chromatography mass spectrometry (GC-MS).The antioxidant activity was determined by measuring total phenolic content (TPC), ferric reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The antibacterial activity was determined using paper disc method against two bacteria namely *Staphylococcus aureus* and *Escherichia coli*. Total of 36 compounds belonging to phenolics, anhydrides, aldehydes, fatty acids and hydrocarbons were identified in the extracts. The highest content of total phenol and antioxidant activity (FRAP and DPPH) were found in methanol extract228.50 ± 3.67. mg GAE/100g DW, 118.63 ±3.86 mg TE/100g DW and 92.81% respectively. The maximum zone of inhibition observed was 14.45 ± 0.67 mm against *Staphylococcus aureus* at methanol extract. It was concluded that fractions solvent plays important roles on the bioactive compound of prickly pear pulp extract and it can be used to control infectious diseases and prevent oxidative damage.

Key words: Prickly pear pulp, Fraction, Antioxidants, Antibacterial activity, GC-MS.

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INTRODUCTION

Opuntiaficus-indica commonly called prickly pear belongs to the *Cactaceae* family, native to Central America, is today cultivated in Europe, the Middle East and northern Africa. The fruit of the prickly pear is healthy because it contains bioactive compounds such as polyphenols, flavonoids and pigments. These pigments have proved useful effects in the redox-regulated pathways implicated in cellular inflammation and growth, and no poisonous effects have been observed in humans.[1-3] Other studies reported the presence of carotenoids and ascorbic acid.[1-4] The fruit of the prickly pear has shown that it is rich in phenolic compounds with more antioxidants activity effects than vitamins. Numerous phenolic compounds were identified in prickly pear pulp extract essentially represented by catechin, rutin, ferulic acid andgallic acid.[5-6] Antioxidants are compounds capable of delaying, retarding or preventing auto oxidation. The importance of the natural antioxidant constituents in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers and consumers as the end of the future is moving toward functional food with specific health effects.[7-9] The beneficial health-related effects of certain phenolic compounds and their potential antioxidant properties, especially when these compounds are present in large quantities in foods, are of importance to consumers. Aromatic metabolites produced from plant secondary metabolism, phenolic compounds exhibit a common configuration with an aromatic ring including a minimum of one hydroxyl group, which affords the body protection against oxidative stress due to the neutralising effect on reactive species.[10] Therefore, our study aimed to find out the suitable solvent in obtaining the extract of prickly pear pulp possessing the highest phytochemicals, antioxidants and antibacterial activity.

METHODS

Samples collection and extraction and fractionation

Prickly pear pulps were collected from farms at Nasiriyah during season 2021. Samples were transfer on the same date to University of Thi-Qar, Biology laboratory. The fruits were selected with ripening stages of prickly pear. The pulps of prickly pear were cleaned and cut into small pieces, and then oven dried at 60 ^{0}C for 48 h and then stored at 4^{0}C until use. The methanolic extract of prickly pear pulp extract was prepared by maceration using 80 % methanol following the procedure describe by[11] before submitted to the fractionation phase. Fractionation was completed by gradient elution of 100 ml of acetone, ethanol and methanol respectively, stirred 24 h and evaporated at 40 ^{0}C by a rotary evaporator. The fractions obtained were kept in the freezer until further analysis.[12]

Total phenol content (TPC)

The determination of antioxidant activity through TPC was carried out according to the method of.[13] About 100 µL leaf and stem extracts was added with 0.4 mL distilled water and 0.5 mL diluted Folin-Ciocalteu reagent. The samples prickly pear pulps with Folin-Ciocalteu reagent were left for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbance was taken at 765 nm wavelength with spectrophotometer after 2 hours. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of dry sample (mg GA/100 g of DW).

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Ferric reducing antioxidant power (FRAP)

The determination of antioxidant activity through FRAP was carried out according to the method of.[13] FRAP reagent was prepared fresh as using 300 mM acetate buffer, pH3 .6 (3.1 g sodium acetate trihydrate, plus 16 mL acetic acid made up to 1:1 with distilled water); 10 mM TPTZ (2,4,6-tris (2-pyridyl)-*s*-triazine), in 40 mMHCl and 20 mM $FeCl3•_6H_2O$ in the ratio of 10:1:1 to give the working reagent. About 1 ml FRAP reagent was added to 100 µL prickly pear pulp and the absorbance was taken at 595 nm wavelength with spectrophotometer after 30 minutes. Calibration curve of Trolox was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of dry sample (mg TE/100 g of DW).

DPPH radical scavenging activity

The determination of antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system was carried out according to the method of.[13] Stock solution was prepared by dissolving 40 mg DPPH in 100 ml methanol and kept at -20°C until used. About 350 mL stock solution was mixed with 350 ml methanol to obtain the absorbance of 0.70±0.01 unit at 516 nm wavelength by using spectrophotometer (Epoch, Biotek, USA). About 100 µL prickly pear pulp with 1 ml methanolic DPPH solution prepared were kept overnight for scavenging reaction in the dark. Percentage of DPPH scavenging activity was determined as follow: DPPH scavenging activity (%) = [(A blank –A sample) / A blank] × 100. Where A is the absorbance.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS was carried out according to the method of.[14] GC-MS analyses of 80% methanolic extract of prickly pear pulps was performed using an Agilent 7890A GC system coupled with an Agilent 5975 C mass selective detector, equipped with a HP-5MS GC column (5% phenyl methyl siloxane, Agilent 19091S 433, 30 m × 250 µm internal diameter, 0.25 µm film thickness). Helium was used as a carrier gas at flow rate of 1.21 mL/min. The instrument was operated in the electron impact (EI) mode at 70 eV and ion source temperature 230°C in the scan range of 50-500 m/z. The initial column temperature was set at 40°C held for 2 min, ramped at a rate of 4°C/ min to 270°C and held for 5.5 min (total run time 65 min). Dilute sample solutions of the extracts were prepared in HPLC grade methanol, and a volume of 5µL was injected. The constituents were identified by comparing the mass spectra available in a MS database of National Institute Standard and Technology (NIST 08).

Antibacterial assay

Staphylococcus aureus and *Escherichia coli* were used in experiment. Mueller Hinton agar was used in antibacterial assay. Plant extracts were dissolved in methanolic and acetone to obtain a concentration of 300 mg/mL. Antibacterial assays were conducted using the disc diffusion method as previously described by.[15] Negative controls were prepared using the same solvent employed to dissolve the plant extract. Zones of inhibition around the discs were measured in mm. The experiment was repeated in triplicate and the mean of diameter of the inhibition zones was calculated.

Statistical analysis

Data were expressed as the means of three independent experiments. Statistical comparisons of the results were performed by one-way ANOVA using SPSS ver.19. Significant differences ($P<0.05$) among the fractions were analyzed by Duncan 'triplicates range test.

RESULTS AND DISCUSSIONS

Phytochemicals identified by GC-MS of prickly pear pulp extract

Gas Chromatography-Mass analysis of prickly pear pulp extract revealed the presence of various groups of bioactive compounds. The bioactive compounds with their retention time (RT), molecular formula, area, molecular weight and biological activity are exhibited in Table 1.

Aluminum, triethyl-, 2,4-Difluorobenzene, 1-benzyloxy-, 2-Azido-2,4,4,6,6-pentamethylheptane, Dodecane, Aziridine, 2-methyl-2-(2,2,4,4-tetramethylpentyl, (R)-lavandulyl acetate, D-Limonene, Decanoic acid, 3-hydroxy-, methyl ester, Phenylethyl Alcohol, Acetic acid, phenylmethyl ester, 1,5,5-Trimethyl-6-methylene-cyclohexene, Cyclohexanone, 5-methyl-2-(1-methylethylidene)-, 1-(2-Vinylphenyl) ethanone, Caryophyllene, Phenol, 3,5-bis(1,1-dimethylethyl)-, Isoamyl salicylate, Diethyl Phthalate, Cinnamaldehyde, .alpha.-pentyl-, Disulfide, di-tert-dodecyl, tert-Hexadecanethiol, Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-, n-Hexadecanoic acid, 1-Heptatriacotanol, Phytol, Retinoic acid, methyl ester, Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-, Tetrapentacontane, 1,54-dibromo-, Nonacosane, Dotriacontane, Hentriacontane, Ethyl iso-allocholate, Octadecane, 3-ethyl-5-(2-ethylbutyl)-, gamma.-Sitosterol, Cholestan-3-one, and cyclic 1,2-ethanediyl aetal, (5.beta.). In the present study, some of the identified compounds has been reported to have several biological activities. The identified compounds from *prickly pear*-pulp extracts have been described to have therapeutics properties by some researchers. For example,D-Limonene, Caryophyllene, Phenol, 3,5-bis(1,1-dimethylethyl)-, Isoamyl salicylate -, n-Hexadecanoic acid, 1-Heptatriacotanol, Oxiraneundecanoic acid, Phytol, Retinoic acid, methyl ester, Phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-methyl-, Tetrapentacontane, 1,54-dibromo-, Nonacosane, Dotriacontane, Hentriacontane, antimicrobial, anticancer, antioxidants, anti-diabetic, anit-inflammatory.[16-19]

Total phenol content and antioxidants activity

The content of phenolic compounds and antioxidants activity in different fraction extracts (acetone, ethanol and methanol) of prickly pear pulp extract shown in Table 2. Methanol extract gave the highest phenolic content (228.50 ± 3.67 mg/GAE/100 g DW) when compared with acetone and ethanol 194.67 ± 1.53 and 176.53 ± 2.67 mg/GAE/100 g DW respectively. After methanol, acetone fraction had high content of phenolic compounds in prickly pear pulps extract. Thus, the polarity of solvents has an indirect function in the extraction process, because it can raise the solubility of antioxidant compounds.[20] It was impossible to improve a standard solvent that was suitable for the all types of antioxidants extraction of medicinal plants. It was also known the phenolic content was affected by the type of the plant or materials, the

Figure 1: Prickly pear fruits.

Table 1: Phytochemicals identified by GC-MS of prickly pear pulp extract.

No.	Compound name	Formula	RT	Area %	M.w	Biological Activity
1	Aluminum, triethyl-	$C_6H_{15}Al$	4.812	1.54	114.16	
2	2,4-Difluorobenzene, 1-benzyloxy-	$C_{13}H_{10}F_{2O}$	4.145	2.30	220.21	Antifungal
3	2-Azido-2,4,4,6,6-pentamethylheptane	$C_{12}H_{25}N_3$	6.79	1.48	211.35	Antitumor, Anti-inflammatory
4	Dodecane	$C_{12}H_{25}F$	7.54	2.40	170.33	Antioxidants
5	Aziridine, 2-methyl-2-(2,2,4,4-tetramethylpentyl)-	$C_{12}H_{25}N$	8.77	1.60	183.33	Antioxidant
6	(R)-lavandulyl acetate	$C_{12}H_{20}O_2$	9.69	2.71	196.29	Ant parasitic, Antimicrobial
7	D-Limonene	$C_{10}H_{16}$	10.45	2.38	136.23	Antimicrobial
8	Decanoic acid, 3-hydroxy-, methyl ester	$C_{11}H_{22}O_3$	11.39	2.14	202.29	Antibacterial
9	Phenylethyl Alcohol	$C_8H_{10}O$	12.08	0.25	122.16	Antibacterial
10	Acetic acid, phenylmethyl ester	$C_9H_{10}O_2$	12.91	4.29	150.17	Antimicrobial
11	1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$	13.37	1.90	136.23	Antimicrobial
12	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	$C_{10}H_{16}O$	14.16	0.65	152.23	Antimicrobial
13	1-(2-Vinylphenyl)ethanone	$C_{10}H_{10}O$	15.83	0.97	146.19	
14	Caryophyllene	$C_{15}H_{24}$	16.74	5.87	204.35	Anticancer, Antimicrobial Antioxidant
15	Phenol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	17.79	11.15	452.6	Antioxidants, Antimicrobial
16	Isoamyl salicylate	$C_{12}H_{16}O_3$	18.16	3.75	208.25	Antimicrobial
17	Diethyl Phthalate	$C_{12}H_{14}O_4$	18.90	5.13	222.24	Antimicrobial
18	Cinnamaldehyde, .alpha.-pentyl-	$C_{14}H_{18}O$	19.49	1.16	202.29	Antimicrobial
19	Disulfide, di-tert-dodecyl	$C_{24}H_{50}S^2$	20.05	3.78	402.8	Antimicrobial
20	tert-Hexadecanethiol	$C_{16}H_{34}S$	21.04	4.13	258.51	Antibacterial
21	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	$C_{19}H_{36}O_3$	22.30	2.65	312	Antioxidants, Antimicrobial
22	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	22.71	4.24	256.42	Antioxidant
23	1-Heptatriacotanol	$C_{37}H_{76}O$	23.36	2.90		Antimicrobial
24	Phytol	$C_{20}H_{40}O$	24.13	1.48	296.5	Antimicrobial
25	Retinoic acid, methyl ester	$C_{21}H_{30}O_2$	26.18	1.08	314.5	Antioxidant
26	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	$C_{23}H_{32}O_2$	26.86	4.49	340.49	Antioxidant
27	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	29.40	1.77	917.2	Antioxidants
28	Nonacosane	$C_{29}H_{60}$	30.29	8.58	408.8	Antimicrobial, Antioxidant
29	Dotriacontane	$C_{32}H_{66}$	31.54	4.23	450.9	Antioxidant, Antibacterial
30	Hentriacontane	$C_{31}H_{64}$	31.92	2.91	436.8	Anti-inflammatory, Antimicrobial
31	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	33.58	4.99	436.6	Antimicrobial
32	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	34.04	1.08	366.7	Antioxidant, Antibacterial
33	Gamma-sitosterol	$C_{29}H_{52}O_2$	34.83	1.54	414.7	Antidiabetic
34	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5.beta.)-	$C_{29}H_{50}O_2$	36.39	2.30	430.7	Antimicrobial

Table 2: Antioxidant activity of prickly pearpulp estimated by TPC, FRAP and DPPH.

Fraction	TPC (mg GAE/g)	FRAP(mg TE/g)	DPPH %
Ethanol	176.53 ± 2.67^c	92.79 ± 2.54^c	79.67 ± 1.49^c
Acetone	194.67 ± 1.53^b	102.04 ± 2.91^b	87.65 ± 1.08^b
Methanol	228.50 ± 3.67^a	118.63 ± 3.86^a	92.81 ± 2.16^a

$^{a-c}$ Different letters within each column indicate significant difference ($p<0.05$).

Table 3: Antibacterial activity of prickly pear pulp extract.

Fraction	Minimum inhibitory concentration (mg/ml)	
	Staph. aureus	E.coli
Ethanol	6.00 ± 0.00^c	5.00 ± 0.00^c
Acetone	9.21 ± 1.53^b	7.51 ± 0.28^b
Methanol	14.45 ± 0.67^a	11.86 ± 0.50^a

$^{a-c}$ Different letters within each column indicate significant difference ($p<0.05$).

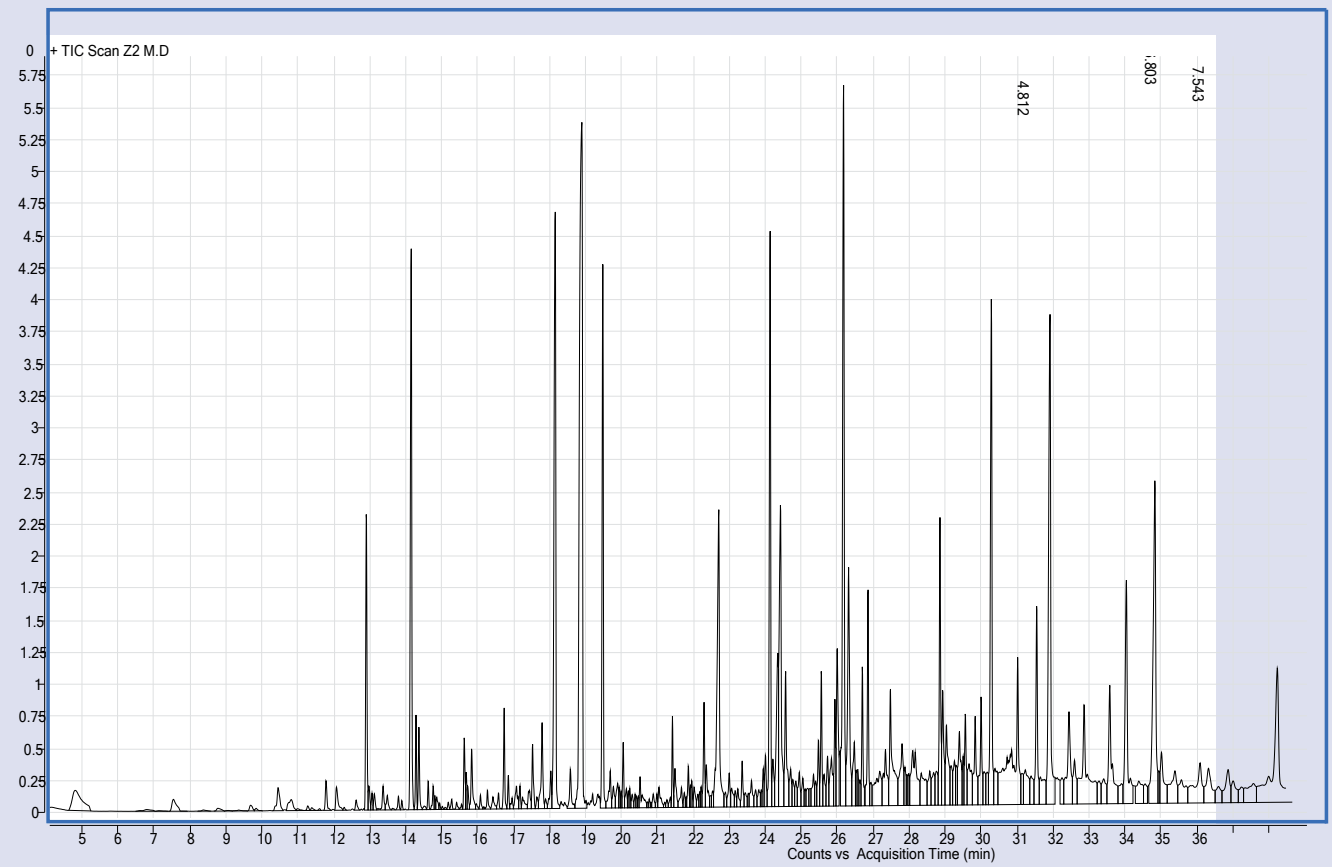

Figure 2: Gas chromatogram of methanolic *prickly pear* pulp extract.

usage of solvent polarity.[21-22] For amount of the reductive ability, the Fe_3^+- Fe_2^+ transformations in the presence of prickly pear pulp extract sample was examined. Table 2 shows FRAP values for three different solvents. The result ranged from to 92.79 ± 2.54 118.63 ±3.86 to mg TE/100 g DW. Significant differences (P<0.05) in FRAP values were found between the different solvent. Methanol was the best solvent for finding extracts with higher antioxidant activity. The FRAP value obtained by acetone was higher significantly (P<0.05) than the extract obtained by ethanol. The results showed that methanol extract of prickly pear pulp is having significantly (P<0.05) higher scavenging activity compared to acetone and ethanol. The results in Table 2 showed that antioxidant activity were sensitive to extraction solvents; generally, methanol presented the highest extraction recovery. DPPH values of prickly pear pulp extract in all organic solvent extracted increase with increase in the polarity. Methanol was the best solvent for obtaining extracts with high antioxidant activates in prickly pear pulp followed significantly (p>0.05) with acetone (92.81 and 87.65 %) respectively. Correlation has been observed between radical scavenging power of plant extract with total phenolics content.[23] There are several studies that reported a significant correlation between phenolic compounds and antioxidant activity by DPPH method.[24-25] In current study, strong antioxidant activity of prickly pear pulp extract perhaps due to the phenolic contents.[26] The different results obtained from the previous studies may be attributed to different cultivars, growing conditions, ripening stage at harvest, or the storage conditions and time elapsed before the fruits were analysed.

Antibacterial activity

Natural products may be a mainly rich source of anti-infective agents. The antibacterial activity on pathogenic strains of *Staphylococcus aureus* and *E. coli* bacteria of prickly pear pulp extracts was assessed in the current study (Table 3). The antibacterial activity of the prickly pear pulp extracts varied depending on the bacterial species used. The most sensitive organism was *Staphylococcus aureus* being the most resistant. The diameter of the inhibition zone varied ranging from (14.45 mm) to (11.86 mm) for methanolic extract as compared to (6.00mm) to (5.00 mm) for ethanol extract (Table 3). The antimicrobial activity of the prickly pear pulp extract was found highest against *Staphylococcus aureus* while lowest activity was found against *E. coli.* Furthermore, the antibacterial activity of the leaf and stem extracts could also be associated with their higher total phenolic contents (Table 2). This result agrees with several other studies that have shown that the inhibitory effect of phenolic content from natural products extracts are more strong to Gram-positive bacteria than Gram-negative. Generally, phenolic compounds (phenolic acids, flavonoids and anthocyanin) potentially disturb the function of bacterial cell membranes which causes delay of growth and multiplication of bacteria. Further phenolic compound involved in protein and cell wall binding, enzyme inactivation and intercalation into the cell wall and/or DNA during inactivation of pathogens.[27] The extraction yield was affected by the extraction method, polarity, solubility, concentration, pH, temperature and extraction solvents.[28] Other studies reported the occurrence of phenolics, alkaloids and tannins in plant extracts and they are related with antimicrobial activity.[29]

CONCLUSION

The GC-MS profiling of prickly pear pulp extract revealed the presences of numerous phytochemicals that have been known to possess therapeutics properties. The study recommends that total phenol content played essential role in antibacterial activity and antioxidants

(FRAP and DPPH). It was revealed that methanol fraction from prickly pear pulp extract possessed the strongest antibacterial and antioxidant activity because of the highest total phenol content. Further, the acetone fraction of prickly pear pulp also showed the most important phenolic compounds. From these results, it could be concluded that prickly pear pulp extract shave various bioactive compounds, total phenol content, strong antioxidant and antibacterial activity.

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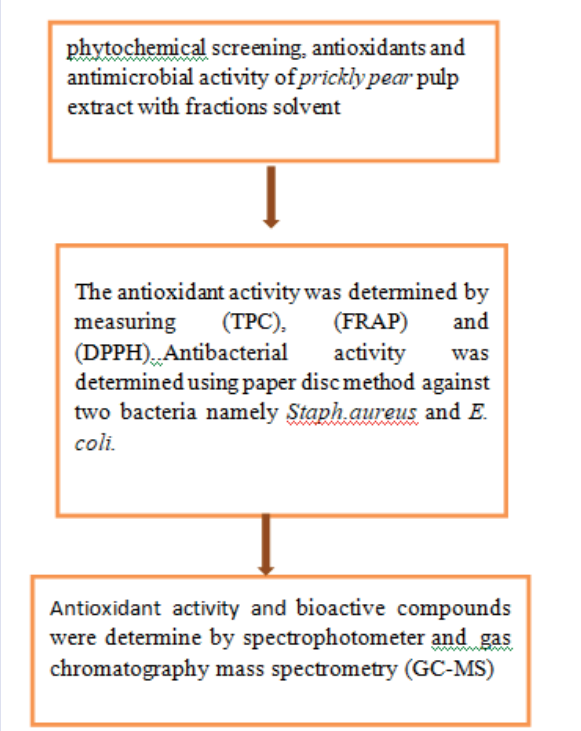
GRAPHICAL ABSTRACT



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