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PHYTOCHEMICAL ANALYSIS, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY DETERMINATION OF *MOMORDICA CHARANTIA*

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INTRODUCTION

Momordica charantia is commonly known as bitter melon or bitter gourd, with Ayurveda name: karela.[1] It is found in the tropical and subtropical regions of the world, distributed in Asian countries and widely cultivated as a vegetable crop. All parts of the plant including the fruit, taste is bitter.[2] However, it is a common food and remedy as folk medicine to treat different disease, used in tropical region such as China, India, Africa and Southern East Asia.[1-3] *Momordica charantia* is cultivated up to an altitude of 1500m during warm in April to July by using 2-3 seeds in a pit. Only one plant is retained and seedlings are watered once or twice a week. Plants begin to flower 30-35 days after sowing and the fruits are ready for harvesting 15-20 days after flowering.[4] *Momordica charantia* is commonly known as bitter melon or bitter gourd, with Ayurveda name: karela.[1,7] It is found in the tropical and subtropical regions of the world, distributed in Asian countries and widely cultivated as a vegetable crop.[2,7] All parts of the plant including the fruit taste bitter.[2] It is a flowering vine in the family Cucurbitaceae.[2]

The fruit can be in ovoid, ellipsoid, or spindle shaped, dehiscent irregularly as a three valved fleshy capsule or indehiscent.[4] The fruit has a distinct warty looking exterior and an oblong shape.[4] The fruit appears as hanging down egg-shaped cylindrical shape and covered with longitudinal ridges and warts, showing green or white color.[8] It may turn orange in color when they are ripened.[8]

It can be in different shapes and sizes depending on different species. Some bear miniature fruit for example *charantia C.B. Clarke* (karala) has only 6–10 cm in length.[2] The bitter melon more typical of India such as *muricata (*Willd*), Hybrid green* has a narrower shape with pointed ends, and a surface covered with jagged, triangular "teeth" and ridges.[2,4] The typical Chinese phenotype is 20–30 cm long, oblong with bluntly tapering ends and pale green in color, with a gently undulating, warty surface.[4] The fruit is hollow in cross-section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large flat seeds and pith.[4] Seeds and pith appear white in unripe fruits, ripening to red. The flesh is crunchy and watery in texture and the skin is tender and edible.[4] Its leaves can be grown up to 5 cm long and usually 5-7 lobed.[8] It has spiral tendrils unbranched or two branched and are carried singly along the stem.[8] The tendril bearing vine can grow up to 5 meter and can be either slightly hairy or hairless.[4,8]

It is a common remedy used in tropical region such as China, India, Africa and Southern East Asia and widely used in folk medicine to treat different disease.[2] It also acts as a source of carbohydrate, proteins, mineral, vitamins and other nutrients for human to maintain health.[2] Many health benefits and medicinal properties can be shown by this plants for example it reduces blood sugar, kills bacteria, balance hormones, reduce inflammation, kill viruses, fights free radicals and so forth.[7, 9,10]

METHODOLOGY

Collection and preparation of plant material

After collection of fruit from the plant, material was washed with clean distilled water thoroughly and the fruits were cut longitudinally into half by using a knife. The contents inside the fruit including the seeds and the fibrous material were removed and discarded. The fruits were further cut into small pieces. This was to increase the surface area of fruits to expose extracting solvents. Then the small cut pieces were kept in a tightly closed plastic bag and placed in the refrigerator to preserve the freshness as long as possible.

Soxhlet extraction with absolute alcohol

Soxhlet is one of the extraction methods, which is known as hot continuous extraction. The small cut pieces of raw material were placed in a thimble made of strong filter paper that can be placed in the chamber of Soxhlet apparatus.[23] Approximately 700 g of small cut pieces of raw materials was weighed and dried under shade for five days, crushed in the form of powder and placed into the thimble of Soxhlet apparatus. As an extracting solvent, 350 ml of

absolute ethanol was dropped. The extraction process was started and the temperature maintained at 70°C. This process was carried out for about 8 hours. After the complete extraction, the extract was collected and evaporated by using Rotary evaporator at a temperature of 70°C at 100 rotations per minute (rpm) to 100 ml. The concentrated extract was kept in a beaker, closed with aluminum foil and properly labeled. The extract was further dried by using water bath until there was a formation of semi-solid state.

Maceration extraction with absolute alcohol

Maceration is one of the extraction methods that soak raw materials in the extracting solvent. The mixture is allowed to stand at room temperature with frequent agitation. After a period of time, the active constituents will be extracted out from raw materials into the extract solvent.[11]

Approximately 700 g of small cut pieces of raw materials, previously dried under shade were weighed six days. A 5000 ml of round bottom flask was prepared and cleaned with distilled water and ethanol. All the dried raw material was added into the flask and followed by 700 ml of absolute ethanol until all the raw material was fully soaked and dipped in with ethanol. A small sheet of poked aluminum foil was covered the mouth of round bottom flask. The round bottom flask was shaken gently 3 times a day with duration of 1 to 2 minutes for seven days. Then extract was filtered from the raw material by using two clean muslin cloths and was collected in 800 ml of flask. The extract was then evaporated by using rotary evaporator at a temperature of 70 °C at 100 rotations per minute (rpm) to 100 ml. The concentrated extract was kept in the flask closed with aluminum foil and properly labeled. The extract was further dried by using water bath until there was a formation of semi-solid state.

Phytochemical Screening

Both the Soxhlet and maceration extracts were subjected to qualitative phytochemical screening to determine the presence of alkaloids, reducing sugars, saponins, terpenoids, steroids, anthraquinones, glycosides, tannins, flavonoids, carbohydrate and phenols. The results of the tests were observed and recorded accordingly as shown in Table 1.

Anti-microbial activity screening

Anti-microbial activity of *Momordica charantia* extract was carried out against four different strains (*Bacillus subtilis, Staphylococcus pyogenes, Pseudomonas aeruginosa* and *Escherichia coli)*. Mueller-Hinton agar was used as medium to screen anti-bacterial activity.

In this case, well diffusion technique was used for screening of antibacterial activity. Inoculum of different strains (*Bacillus subtilis, Staphylococcus pyogenes, Pseudomonas aeruginosa* and *Escherichia coli)* were prepared by transferring 1 colony of each test organism to 5 ml of Mueller-Hinton broth in sterilized individual universal bottles. Mueller-Hinton broth was used to prepare inoculum, which is also known as bacterial suspension. The concentration of inoculum was standardized by comparing with McFarland standards. 0.5 McFarland standards was prepared by mixing 9.95 ml 1% sulphuric acid in Mueller-Hinton broth and 0.05 ml 1% barium chloride in distilled water, in order to estimate bacterial density which is 1.50 x 10^8 CFU/ml. The broth was then incubated at 37 °C for 24 hours in incubator shaker. The turbidity of the bacterial suspension was standardized by comparing to 0.5 McFarland standards.[17] The results of antimicrobial assay are shown in Table 2.

24 petri dishes were prepared and filled with molten Mueller-Hinton agar and kept in cold room for one night. The petri dishes were then taken out and labelling was done by using marker pen in laminar airflow cabinet. With the Bunsen burner on, one strain of the prepared inoculum was spread evenly over plate with sterile loop. A standard cork borer of 6 mm diameter was used to cut 5 uniform wells on the surface of the agar. 40 µl of different concentration of Soxhlet extract (1, 5 and 10 mg/ml), negative control (sterile distilled water) and positive control (Ciprofloxacin, 5µg) were introduced in the different well. All the steps were triplicate for each strain and repeated for maceration extract. The 24 inoculated plates were incubated at 37 °C for 24 hours. Zone of inhibition was measured to the nearest millimeter (mm) and mean was calculated.[17]

ANTIOXIDANT ACTIVITY SCREENING

DPPH scavenging activity

The free radical scavenging capacity of ethanolic extract was assayed by using 1, 1-Diphenyl-2-picrilhydrazyl (DPPH) method. The degree of DPPH purple decolorization to DPPH yellow indicated the scavenging efficiency of the extract. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.[13]

Total Phenolic Content

Total phenolic content was determined according to the Folin-Ciocalteu method, using Gallic acid as standards and determined by using the Folin-Ciocalteu reagent.[14] The phenolic compounds are oxidized to phenolates by the reagent at alkaline pH in a saturated solution of sodium carbonate resulting in a blue molybdenum-tungstate complex.[14]

Different concentrations of Soxhlet extract (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) were prepared. 0.5 ml of different concentrations of extract solution and also control (methanol, without extract) were added into individual centrifuge tubes. They were individually mixed with 2.5 ml of 0.75% w/v Sodium bicarbonate solution and 2.5 ml of 1% v/v Folin-Ciocalteau reagent. The mixtures were vortexed for few seconds and left to stand in the dark at room temperature for 15 minutes. The absorbance was measured at 765 nm against a blank by using UV-visible spectrophotometer.[14,15] The total phenolic content was expressed as milligrams of Gallic acid equivalents per grams (mg GAE/g).

RESULTS

Table 1: Results of phytochemicals present in ethanolic extracts of maceration and Soxhlet processes.

No.	Analysis Tests	Ethanolic maceration		Ethanolic Soxhlet Sample
		Filtrate	Residue	
1.	Alkaloid Test	+	+	+
2.	Test for Reducing Sugar	-	-	-
3.	Test for Saponins	-	-	-
4.	Test for Terpenoids	+	+	+
5.	Test for Antraquinones	-	-	-
6.	Test for Glycosides	-	-	-
7.	Test for Tannins	-	-	-
8.	Test for Flavonoids	-	-	-
9.	Test for Carbohydrate	+	+	+

+ = Present - = Absent

Table 2: Results for anti-microbial activity of ethanolic Soxhelt and maceration extractions.

Micro-organisms	Extraction process	Conc. Of Ethanolic Extracts (mg/ml)	Zone of inhibition on Mueller Hinton Agar, mm in diameter	Ciprofloxacin (Positive Control), mm in diameter	Sterile Distilled Water (Negative Control), mm in diameter
Bacillus subtilis	Soxhlet	1.0	-	24 ± 0.2	-
		5.0	-	24 ± 0.2	-
		10.0	-	24 ± 0.2	-
	Maceration	1.0	-	24 ± 0.2	-
		5.0	-	24 ± 0.2	-
		10.0	-	24 ± 0.2	-
Staphylo-coccus pyogenes	Soxhlet	1.0	-	24 ± 0.2	-
		5.0	-	24 ± 0.2	-
		10.0	-	24 ± 0.2	-
	Maceration	1.0	-	24 ± 0.2	-
		5.0	-	24 ± 0.2	-

		10.0	-	24 ± 0.2	-
Escherichia coli	Soxhlet	1.0	-	24 ± 0.2	-
		5.0	-	24 ± 0.2	-
		10.0	-	24 ± 0.2	-
	Maceration	1.0	-	24 ± 0.2	-
		5.0	-	24 ± 0.2	-
		10.0	-	24 ± 0.2	-
Pseudo-monas aeruginosa	Soxhlet	1.0	-	24 ± 0.2	-
		5.0	-	24 ± 0.2	-
		10.0	-	24 ± 0.2	-
	Maceration	1.0	-	24 ± 0.2	-
		5.0	-	24 ± 0.2	-
		10.0	-	24 ± 0.2	-

In present antibacterial study, two different extracts with three different concentrations were investigated to detect the zone of inhibition against *Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Escherichia coli*. Ciprofloxacin was used as a positive control and sterilized distilled water was used as a negative control in this study.

Anti-oxidant activity

DPPH radical-scavenging activity

The following results showed the absorbance and percentage scavenging value of both extractsand BHT along with the graphs with IC 50 calculated. Each Soxhelt ethanolic extract concentrations with its absorbance value is shown in Table 3 while each macerated ethanolic extract concentrations with its absorbance value is tabulated in Table 4. Each Soxhlet ethanolic extract concentrations with its percentage scavenging is shown in Table 5 with that of each macerated ethanolic extract concentrations with its percentage scavenging is shown in Table 6.

A. Soxhlet ethanolic extract

Table 3: Each Soxhlet ethanolic extract concentrations with its absorbance value.

Sample conc. (mg/ml)	Absorbance (WL517nm)
Blank	0.627
0.03125	0.616
0.0625	0.605
0.125	0.603
0.25	0.586
0.5	0.579
1	0.56

Table 4: Each macerated ethanolic extract concentrations with its absorbance value.

Sample conc. (mg/ml)	Absorbance (WL517nm)
Blank	0.59
0.03125	0.575
0.0625	0.572
0.125	0.565
0.25	0.55
0.5	0.535
1	0.526

Table 5: Each Soxhlet ethanolic extract concentrations with its percentage scavenging.

Sample conc. (mg/ml)	Percentage Scavenging (%)
0.03125	1.75
0.0625	3.51
0.125	3.83
0.25	6.54
0.5	7.66
1	10.69

B. Macerated ethanolic extract

Table 6: Each macerated ethanolic extract concentrations with its percentage scavenging.

Sample conc. (mg/ml)	Percentage Scavenging (%)
0.03125	2.54
0.0625	3.05
0.125	4.24
0.25	6.78
0.5	9.32
1	10.85

C. Butyl Hydroxyl Toluene (BHT) (Control)

Table 7 indicates each BHT concentrations with its absorbance value while Table 8 shows each BHT concentrations with its percentage scavenging.

Table 7: Each BHT concentrations with its absorbance value.

Sample conc. (mg/ml)	Absorbance (WL517nm)
Blank	0.556
0.03125	0.461
0.0625	0.332
0.125	0.245
0.25	0.106
0.5	0.03
1	0.024

Table 8: Each BHT concentrations with its percentage scavenging.

Sample conc. (mg/ml)	Percentage Scavenging (%)
0.03125	17.09
0.0625	40.29
0.125	55.94
0.25	80.94
0.5	94.6
1	95.68

Figures 1, 2, 3 and 4 are showing the standard graph of BHT, percentage scavenging (%) against sample concentration of Soxhelt ethanolic extract, percentage scavenging (%) against sample concentration of macerated ethanolic extract and percentage scavenging (%) of two ethanolic extracts and BHT, respectively.

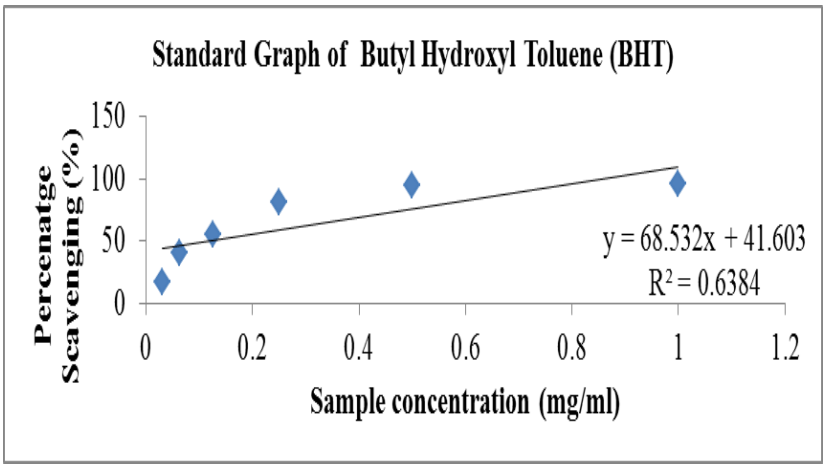

Figure 1: Standard graph of BHT.

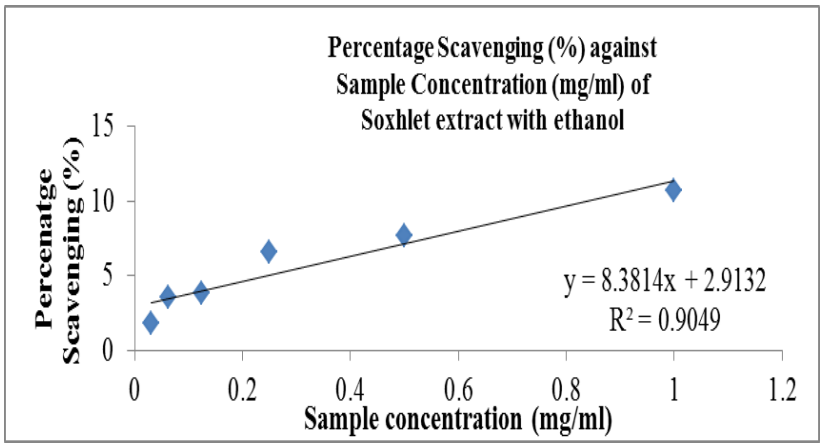

Figure 2: Percentage Scavenging (%) against sample concentration of Soxhlet ethanolic extract.

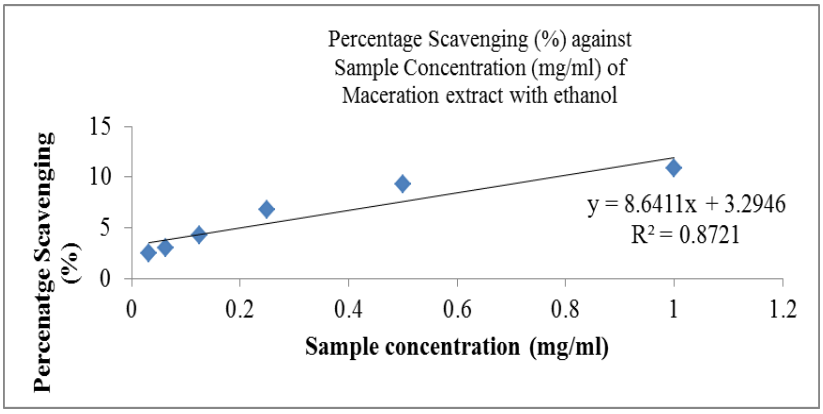

Figure 3: Percentage Scavenging (%) against sample concentration of macerated ethanolic extract.

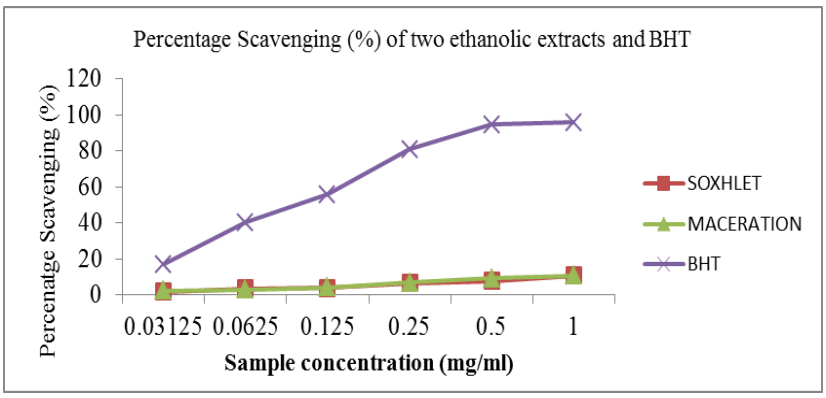

Figure 4: Percentage Scavenging (%) of two ethanolic extracts and BHT.

Comparison of percentage scavenging (%) of two ethanolic extracts and BHT is shown in Table 9 and comparison of IC 50 of two ethanolic extracts and BHT is tabulated in Table 10.

Table 9: Comparison of Percentage Scavenging (%) of two ethanolic extracts and BHT.

Sample conc. (mg/ml)	Percentage Scavenging (%)		
	SOXHLET	MACERATION	BHT
0.03125	1.75	2.54	17.09
0.0625	3.51	3.05	40.29
0.125	3.83	4.24	55.94
0.25	6.54	6.78	80.94
0.5	7.66	9.32	94.60
1.0	10.69	10.85	95.68

Table 10: Comparison of IC 50 of two ethanolic extracts and BHT

Sample type	IC 50 (mg/ml)
Soxhlet	5.6180
Maceration	5.4050
BHT	0.1225

Total Phenol Content

A. Soxhlet ethanolic extract

In the determination of total phenolic extract, Figure 5 shows the results obtained.

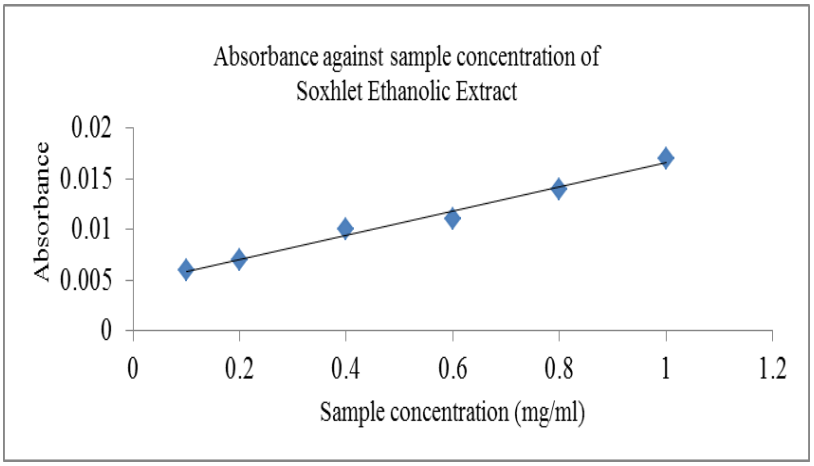

Figure 5: Absorbance against sample concentration of Soxhlet ethanolic extract.

Each Soxhelt and macerated ethanolic concentrations with absorbance value is shown in Table 11 and 12, respectively while each Gallic acid concentrations with absorbance value is indicated in Table 13. The calibration curve of the Gallic acid is shown in Figure 6.

Table 11: Each Soxhlet ethanolic extract concentrations with its absorbance value

Sample conc. (mg/ml)	Absorbance (WL765nm)
0.1	0.006
0.2	0.007
0.4	0.010
0.6	0.011
0.8	0.014
1.0	0.017

B. Macerated ethanolic extract

Table 12: Each macerated ethanolic extract concentrations with its absorbance value

Sample conc. (mg/ml)	Absorbance (WL765nm)
0.1	0.010
0.2	0.017
0.4	0.022
0.6	0.028
0.8	0.031
1.0	0.036

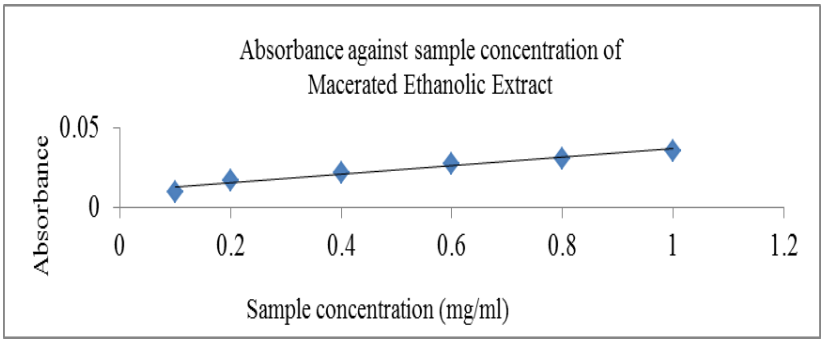

Figure 7: Absorbance against sample concentration of macerated ethanolic extract.

B. Gallic Acid:

Table 13: Each Gallic Acid concentrations with its absorbance value.

Sample conc. (mg/ml)	Absorbance (WL765nm)
0.1	0.387
0.2	0.405
0.4	0.452
0.6	0.462
0.8	0.472
1.0	0.479

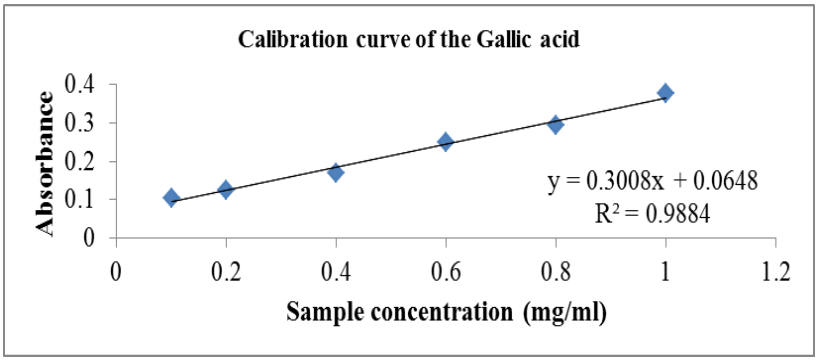

Figure 8: Calibration curve of the Gallic acid.

The total phenolic content results of Soxhelt ethanolic extract, macerated ethanolic extract and of Gallic acid are tabulated in Table 14 a, b and c, respectively.

Table 14 (a): Total Phenolic Content results of Soxhlet ethanolic extract.

Sample type	Sample conc. (mg/ml)	Absorbance	Total Phenolic Content (mg GAE/g)
Soxhlet Ethanolic Extract	0.1	0.006	-0.195
	0.2	0.007	-0.192
	0.4	0.010	-0.182
	0.6	0.011	-0.179
	0.8	0.014	-0.169
	1.0	0.017	-0.159

Table 14(b): Total Phenolic Content results of Macerated ethanolic extract.

Sample type	Sample conc. (mg/ml)	Absorbance	Total Phenolic Content (mg GAE/g)
Macerated Ethanolic Extract	0.1	0.010	-0.182
	0.2	0.017	-0.159
	0.4	0.022	-0.142
	0.6	0.028	-0.122
	0.8	0.031	-0.112
	1.0	0.036	-0.096

Table 14 (c): Total Phenolic Content results of Gallic Acid.

Sample type	Sample conc. (mg/ml)	Absorbance	Total Phenolic Content (mg GAE/g)
Gallic Acid	0.1	0.387	1.071
	0.2	0.405	1.131
	0.4	0.452	1.287
	0.6	0.462	1.320
	0.8	0.472	1.354
	1.0	0.479	1.377

DISCUSSION

The objective of this research was to carry out preliminary and extended investigation on *Momordica charantia* (bitter gourd). Two extraction methods were carried out on the fruit of *Momordica charantia*, which are Soxhlet method and maceration method by using absolute ethanol as solvent.

Before the extraction method carried out, the fruits were washed and air-drying for few days. This was to ensure the removal of excess water and unnecessary materials in the plant so that it would give lesser error compared to fresh one. Drying also inhibit enzyme action or biochemical reaction present in the cell that may cause any further degradation. The raw material was cut into smaller pieces to increase the surface area in order to increase contact with extracting solvent for both extraction methods.

The Soxhlet ethanolic extract showed the presence of alkaloids, triterpenoids and carbohydrate. Macerated ethanolic extract was divided into 2 portions, filtrate and residue. The filtrate showed same active compounds with Soxhlet ethanolic extract whereas the residue showed presence of alkaloids, saponins, tri-terpenoids and phenol. From that, the fruit may contain several important major compounds such as Vicine which was glycol alkaloid and charantin which was triterpenoids which have medicinal benefit.[13] There were actually

many phytochemicals present in this *Momordica charantia* reported by many studies.[2,10, 13,15, 10,17]

Anti-microbial activity was carried out against different bacteria which include *Bacillus subtilis* and *Staphylococcus pyogenes* as Gram-positive bacteria and *Pseudomonas aeruginosa* and *Escherichia coli* as Gram-negative bacteria. Well diffusion method had been used to screen for anti-microbial activity. All the extracts showed no zone of inhibition on Mueller-Hinton agar against the tested bacteria and hence this study reported that *Momordica charantia* fruit did not show anti-microbial activity as shown in Table 2. It was stated that the presence of phenolic compounds in raw material may have anti-microbial activity, so in this case, there was absence of phenol found in both extractions that showed in Table 1 and hence this was why there is no anti-microbial activity shown.[2]

Anti-oxidant activity was tested on *Momordica charantia* and two types of tests were carried out, namely DPPH radical-scavenging activity and Total Phenolic Content. For DPPH radical-scavenging activity, the percentage scavenging of each concentration of extracts were calculated by using equation of Radical scavenging (%) = $[(A_0 - A_1 / A_0) \times 100]$ as well as IC 50 of different extracts were determined. Percentage scavenging of macerated ethanolic extract was slightly higher than Soxhlet ethanolic extract. The higher the percentage scavenging, the higher the hydroxyl radical scavenging activity, in which indicate that macerated ethanolic extract showed higher hydroxyl radical scavenging activity was compared with Soxhlet ethanolic extract, macerated ethanolic extract was having 5.4050 mg/ml of IC 50 which was lower than Soxhlet ethanolic extract of having 5.6180 mg/ml. IC 50 was indicated as the effective concentration of the sample that was required to scavenge 50% of the DPPH free radicals.(32) Therefore, the lower the concentration needed to scavenge 50% of the DPPH free radicals, the better the anti-oxidant activity. All the extracts were increased hydroxyl radical scavenging activity in an increase dose manner. However, the results of all the extracts were significantly lower than the positive control Butyl Hydroxyl Toluene (BHT).

For Total Phenolic Assay, the amount of Total Phenolic Content of both Soxhlet and macerated ethanolic extracts were determined in the unit of mg GAE/g, by using two equation of y = 0.1011x + 0.3906 and $C = \frac{A}{B} \times \text{Dilution factor}$. By comparison, total phenolic content of macerated ethanolic extract showed higher value which indicated better

anti-oxidant activity. All the extracts were increased anti-oxidant activity in an increase dose manner. However, the results of all the extracts were lower than the positive control Gallic acid.

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