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Effect of different solvents on phytochemical and antimicrobial potential of licorice extract

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ABSTRACT

Effect of the solvents ethanol, acetone, and distilled water at several concentrations on the antimicrobial potential, antioxidant activity, total flavonoid and phenolic content, and biochemical screening was studied for licorice extract. All extract at 100% solvent concentration showed the presence of phenols, carbohydrates, alkaloids, steroids, coumarins, tannins, saponins, and flavonoids followed by 75% and 50% concentrations except fatty acids, proteins, and quinones. The highest total phenolic content (114.17 mg/GAE) was observed in 100% ethanol extract and total flavonoid content (56 mg/QE) in 100% distilled water extract. The ethanol 100% extract had greatest antioxidant activity (62.3%) at 400µg/mL concentration. Antimicrobial effect was recorded higher against *Streptococcus pyogenes* (21mm) and *Staphylococcus aureus* (20mm) in 100% ethanol extract. Gram-negative bacteria showed resistance against all licorice extract. Due to various bioactive compounds in licorice extract it would be a natural antioxidant and antimicrobial agent in the pharmaceutical and food industry with multiple health benefits.

Keywords: Antioxidant; extraction; flavonoid; medicinal; phenolic, preservation.

INTRODUCTION: The extraction and application of natural compounds have been the main field of study in the pharmaceutical and food industry (Sohail *et al.,* 2017). Plants contain several active compounds that act as secondary metabolites includes saponins, flavonoids, alkaloids, tannins, phenolic, and many others that play a vital role for the plant itself, leads to treatment of various disorders in humans, and exhibit great potential in the food industry due to strong antioxidant and antimicrobial actions (Iqbal *et al.,* 2017). Medicinal plants contain a diverse variety of phytochemicals that have been adapted to perform different biological functions. Natural resources are much considering for the treatment of several ailments successively (Lim and Mohamed, 2016).

Licorice is a root of the medicinal plant *Glycyrrhiza glabra* L. belongs to the family *Fabaceae.* It is also called "Sweetwood" due to its sweet taste, soft and fibrous structure, and yellow color inside (Sohail *et al.,* 2017). It is cultivated in many parts of the world including the Mediterranean basin of Africa, USA, Europe, India, Pakistan, and several other regions (Badr et al., 2013). Extract from roots of this plant has been widely used for the cure of several ailments due to strong ethnopharmaceutical characteristics. One of the two most important features that relate with licorice in concern through its therapeutic actions is the capability to transmit antioxidant and antimicrobial activity. Licorice has many phytochemicals includes alkaloids, flavonoids, anthocyanin's, tannins, quinones, phenolics, terpenoids, saponins, carbohydrates, proteins, amino acids, fatty acids, sterols, coumarins, chalcones, and others in regards to its healing properties (Chandra and Gunasekaran, 2017) including antioxidant, antimicrobial, anti-inflammatory, anti-tumor, anti-allergic, gastroprotective activity, anti-malarial, anti-hyperglycemic, anti-fungal, enzyme inhibitory, skin lightening activity, antiviral and various others (Zadeh *et al.,* 2013).

Antimicrobial characteristic is another key action that is exhibited by the licorice. It is critically assumed that licorice has several biological functions against varied kinds of disease affecting organisms including *Bacillus subtilis*, *Escherichia coli*, *Klebsiella aerogenes*, *Staphylococcus aureus*, *S. pneumonia and P. aeruginosa* (Alwan *et al.,* 2015). This is the reason that licorice is considered as medicinal agent for gastrointestinal complications like cough, bronchitis, arthritis, and in folk medicines. It is still used for peptic ulcers, respiratory infections, and gastritis, traditionally (Batiha *et al.,* 2020). Most importantly due to flavonoids and phenolic components licorice has strong antioxidant and antimicrobial properties (Iqbal *et al.,* 2017).

The best common technique is the extraction that separates the material from the plant using solvent by an appropriate method. The collection of extraction solvent depends on storage conditions, the chemical composition of raw materials, method and parameter of extraction, size and contamination degree of a sample, and presence of interfering factors (Azwanida, 2015). A polar solvent like ethanol, acetone, ethyl acetate, methanol, and others are commonly used. It is moreover recommended that the water and other organic solvents can extract equally high and low polarity constituents (Iqbal *et al.,* 2017).

OBJECTIVES: Presently no available material is presented associating with improved use of solvents for separating and investigating the possible bioactive components of licorice. The purpose of this study was to increase the awareness of licorice to observe the influence of different solvents on antimicrobial

potential and phytochemical profile including antioxidant activity, total flavonoid and phenolic content, and biochemical screening of licorice extract.

MATERIALS AND METHODS: Selection and preparation of raw material: Sample (licorice) was collected from the National Agriculture Research Centre (NARC), Islamabad, Pakistan. Authentication was carried out by the Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi. Roots (500g) were washed, dried, and then ground into powder form, packed in the air-tight polythene zip bag, and stored at room temperature.

Chemicals and bacterial strains: All chemicals were lab-grade provided by the Institute of Food and Nutritional Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, and bacterial strains were provided by Benazir Bhutto Hospital, Rawalpindi, Pakistan.

Preparation of extract: Seven different extracts were prepared by dissolving 20g powder with 100 mL of 100%, 75%, 50% (w/v) ethanol, acetone, and distilled water, each separately in Erlenmeyer flasks, cover with aluminum foils, and placed in an orbital shaker at 150rpm speed for 48 hr at room temperature (NarasingaRao and Kaladhar, 2014). Whatmann filter paper No. 1 was used for filtration of extract and solvent evaporated by rotary evaporator to reduce its volume (¼ of original) and kept in airtight bottles in the refrigerator at 4°C.

Phytochemical analysis: Biochemical screening: Different biochemical tests of each extract was performed to check out the presence and absence of various phytochemicals like alkaloids, flavonoids, saponins, steroids, tannins carbohydrates, proteins, amino acids, fatty acids, coumarins, phenols, and quinones as described previously (Dhawan and Gupta, 2016). These tests have given the qualitative analysis of the compounds.

Alkaloids: To check the presence of alkaloids, 2mL of each extract was dissolved in the 2mL of Wagner's reagent in a test tube. The endpoint was the formation of red-brown precipitates that specify the presence of alkaloids.

Flavonoids: For flavonoids, in tests tubes, each extract of 1 mL was added with the addition of few drops of NaOH. After adding few drops of H_2SO_4, a yellow colored appeared before immediately disappeared that showed the occurrence of flavonoids.

Saponins: In the test tubes, each extract of 0.5 mL was dissolved in 1mL of distilled water. After shaking the test tubes for about 10 min. foam formation appeared on the top that showed the occurrence of saponins.

Steroids: To check the presence of steroids, 1mL of each extract was taken in the sideways of glass tubes with the addition of 1mL of concentrated H_2SO_4. The endpoint was the dark reddish green color that indicated steroids.

Tannins: 0.1mL of each extract was dissolve with 2mL (45% ethanol) for tannins detection. Then the addition of 1mL of ferric chloride solution takes place after boiling for 5 minutes. The endpoint is greenish to black color that indicated tannins.

Coumarins: For coumarins 2mL of each extract was taken and then 3mL of 10% sodium hydroxide NaOH was added to it. The endpoint was the yellow color that confirms coumarins.

Phenols: For phenols 1mL of each extract was taken and then 2mL of ethanol was added to it. After that, a pinch of $FeCl_3$ was added and the formation of greenish-yellow color was the endpoint that indicated phenol's presence.

Carbohydrates: For carbohydrates presence 2mL of each extract was taken with the addition of 2 drops of molisch's reagent. Test tubes were shaken well. Then conc. H_2SO_4 2mL was added to the extract by the sides of test tubes. The formation of the reddish-violet-colored ring, in the end, showed carbohydrates occurrence.

Proteins: About 2mL of biuret reagent was added to each extract for protein detection. The formation of a violet color ring is the endpoint that confirmed the occurrence of peptide linkage.

Fatty Acids: The ether 5mL was mixed with 0.5mL of each extract for fatty acids indication. Then drops of extract and ether solution were put on the filter paper and allowed for evaporation. The endpoint was the transparence of the extract on the filter paper which indicated fatty acids present.

Amino acids: To indicate amino acids, in the test tubes each extract 2mL was taken with the addition of 2mL ninhydrin reagent. Then boiling of test tubes were takes place in a hot water bath for about 20 min. The endpoint is a purple color that showed amino acids present.

Quinones: To indicate quinones, 2mL of each extract was taken in the test tubes with the addition of 3mL conc. H_2SO_4. The endpoint is a green color that indicates the presence of quinones.

Total phenolic and flavonoid content: Determination of total phenolic content of licorice extract was carried out by procedure described previously (Mohammed, 2014). Firstly, standards of the Gallic acid were made and used for the calibration curve. Each extract of 0.5 g was dissolved in 5mL ethanol to make a solution. After that 2.5mL of Folin-Ciocalteau's reagent (10%) was added in 0.5 mL of each extract and then for neutralizing 2.5mL Na_2CO_3 (7.5%) was added in it and place in an incubator for 30 min. The first blank was run i.e. ethanol and then sample in a spectrophotometer at 765nm wavelength. The absorbance was calculated as mg/Gallic acid equivalents/g of extract. Estimation of total flavonoid content was done according to the method described earlier (Mohammed, 2014). Firstly, standards of Quercetin were made and used for the calibration curve. 1mL of each extract was dissolved in 4mL of distilled water in a 10mL round bottom flask and 0.3mL $NaNO_3$ (5%) was added to it. After 5 min, 0.3mL of $AlCl_3$ (10%) was mixed in it. After 6 min 1M NaOH (2mL) was added to it. Then volume was raised to 10 mL in the flask. Then mixing was done carefully, and absorbance was calculated at 415nm wavelength. Total flavonoid content was calculated as mg/Quercetin equivalents/g of extract.

Antioxidant activity: DPPH (2-2 diphenyl 1- picrylhydrazyl) radical scavenging assay was followed to check the antioxidant activity of licorice extract (Cakmak *et al.,* 2016). For stock solution 24mg of DPPH was added in methanol and volume was made up to 100mL. Different concentrations of each extract were made (400µg/mL to 50 µg/mL) by using 0.02g from each extract. After that DPPH solution absorbance was measured by spectrophotometer. Then 3mL of DPPH solution was added to each extract. The reaction mixture was mixed thoroughly and incubated in a dark place for 30 min. Antioxidant activity was checked through a spectrophotometer at the wavelength of 517 nm. Percentage of antioxidant activity was calculated as:

$$\text{Antioxidant activity}(\%) = \left[\frac{A - B}{A}\right] \times 100$$

Here, A = absorbance of DPPH solution, B = absorbance of the sample

Antimicrobial activity: Estimation of antimicrobial activity of licorice extract was done by Disc Diffusion method (Varsha *et al.,* 2013) and *S. aureus*, *S. pyogenes, Escherichia coli*, and *Pseudomonas aeruginosa* were used to study antimicrobial activity. Firstly, Muller-Hinton media plates were prepared for the growth of bacteria's and then the test organism was spread on it separately. Then wells were prepared on the media plates with the help of an aluminum bore (6 mm diameter). After that 500µL of each extract (diluted) was taken in the wells and the solvents (Ethanol, acetone, and distilled water) were used as negative controls. At the concentration of 30µg antibiotics (Moxifloxacin, Amikacin, and Augmentin) were used as a positive control. Then all the plates were kept in an incubator at 37° C temperature for 48 h. After the completed incubation period antimicrobial activity was recorded as zone of inhibition in (mm) around the wells.

Statistical analysis: The attained results data were analyzed by analysis of variance (ANOVA) at a 5% level of significance ($\rho < 0.05$) followed by the Tukey test through statistical software "Statistix 8.0".

RESULTS: Phytochemical analysis: Biochemical screening: To check the absence and presence of different phytochemicals in each licorice extract qualitative biochemical tests were performed. All extract prepared from 100% solvent concentration showed the presence of steroids, phenols, saponins, tannins, coumarins, flavonoids, carbohydrates, and alkaloids followed by 75% and 50% solvent concentrations separately, but fatty acids, protein, and quinones were not detected by these extract (table 1).

Total phenolic and flavonoid content: Results of total phenolic and flavonoid content of each licorice extract are shown in (table 2). For both TPC and TFC statistical results were found to be significantly different in each extract. The TPC contents ranged from 114.17 to 34.57 as mg/GA equivalent/g of extract and TFC contents 56.00 to 31.68 as mg/Quercetin equivalents/g of extract. Total phenolic content was observed maximum in ethanol 100% extract (114.17mg/GAE) and minimum in ethanol 50% extract (34.57mg/GAE) while total flavonoid content reported maximum in distilled water 100% extract (56 mg/QE) and minimum in ethanol 50% extract (31.68 mg/QE) with significant ($p < 0.05$).

Antioxidant activity: The result of the antioxidant activity of each licorice extract is shown in (table 3). The statistical result was found to be significantly different in each extract. Antioxidant activity of licorice extract ranged from 62.30% to 13.33% followed by each extract. Highest antioxidant activity was reported in 100% ethanol (62.30%), acetone (60.33%), and distilled water (58.40%) extract at 400 µg/mL concentration and it decreases gradually at 50µg/mL concentration with significant ($p < 0.05$) difference.

Antimicrobial activity: The result of the antimicrobial activity of each licorice extract is shown in (table 4). Maximum zone of inhibition was measured in 100% ethanolic extract (20mm) against *Staphylococcus aureus* and (21mm) against *Streptococcus pyogenes*, followed by acetone 100% and distilled water 100% extracts and the minimum was observed in 75% and 50% extract concentrations. No zone of inhibition was recorded against gram-negative bacteria. Negative controls (solvents) were found to give no inhibition effect against all bacteria. Antibiotics (positive control) gives a zone of inhibition against all bacteria except Augmentin which was resistant to *Escherichia coli* (table 5).

DISCUSSION: Licorice is the root of the well-known therapeutic plant *Glycyrrhiza glabra* L. Roots of this plant have been especially used for the cure of many ailments in the pharmaceutical industry. Due to its strong phytochemistry, it has various health benefits. Results of biochemical screening highlighted those solvents (ethanol, acetone, distilled water) and their concentrations (100%, 75%, 50%) have not effect on detection of phytochemicals of extract. All phytochemicals were detected by licorice extracts except fatty acids, protein, and quinones, this may be due to the poor solubility of these components in ethanol, acetone, and distilled water (table 1). This observation validated the previous study that reported the presence of phytochemicals like flavonoids alkaloids, tannins, and others in the aqueous and alcoholic extract of licorice (Siddiqui *et al.,* 2015). However, that previous research also showed that the solvent and its concentration did not affect the indication of these chemical constituents. Hence, all these phytochemicals revealed in licorice extract have great therapeutical properties. Previous chemical studies also reported that licorice had phytochemicals includes chalcones, phenols, sterols, saponins, and many others that had strong antioxidant, antibacterial activity, and various others (Alam *et al.,* 2017). Another earlier study showed the presence of phytochemicals steroids, alkaloids, tannins, terpenoids, flavonoids, and others in chloroform extract of *Glycyrrhiza glabra* (Chandra and Gunasekaran, 2017). Similarly, the presence of steroids, saponins, fats, flavonoids, alkaloids, triterpenoids, and glycosides were reported in ethanolic, toluene, tetrahydrofuran, hexane and, ethyl acetate extract of *Glycyrrhiza glabra* (Iqbal *et al.,* 2017). Hence, from the current and previous studies, it has cleared that all polar and non-polar solvents with different concentrations can indicate the existence of phytochemicals in licorice extract.

Additionally, all these phytochemicals have therapeutic applications for several diseases and many problems like healing effects, astringent properties, anti-inflammation, and many more. Alkaloids are natural compounds having nitrogen in them and could reduce stress and depression. They have also analgesic, antibacterial and antispasmodic actions. Tannins are phenolic constituents that occur organically in nature, can bind proteins, and shows anti-inflammatory and healing effects (Das *et al.,* 2020). Saponins are active compounds that occur sweet taste and provides medicinal effects on the physiology of humans but their large intake causes gastroenteritis problems (Chandra and Gunasekaran, 2017).

Flavonoids are the most important active compounds that have multiple actions including antioxidant, antiviral, anti-inflammatory, and various others (Sharifi-Rad *et al.,* 2021). Therefore, licorice extract contains various phytochemicals reported in this study would be helpful for treatment of several ailments and provide beneficial effects on human health. Total phenolic and flavonoid content were increased or decrease with the highest and lowest concentration and polarity of the solvent

Phytochemicals	Test/Reagent used	E 100%	E 75%	E 50%	A 100%	A 75%	A 50%	D.W 100%
Alkaloids	Wagner reagent	+	+	+	+	+	+	+
Flavonoids	NaOH test	+	+	+	+	+	+	+
Saponins	Foam test	+	+	+	+	+	+	+
Steroids	H_2SO_4 test	+	+	+	+	+	+	+
Tannins	Ferric chloride test	+	+	+	+	+	+	+
Carbohydrates	Molisch's reagent	+	+	+	+	+	+	+
Protein	Biuret reagent	-	-	-	-	-	-	-
Amino acids	Ninhydrin reagent	+	+	+	+	+	+	+
Fatty acids	Ether	-	-	-	-	-	-	-
Coumarins	NaOH test	+	+	+	-	+	+	+
Phenols	Ferric chloride test	+	+	+	+	+	+	+
Quinones	HCL test	-	-	-	-	-	-	-

Table 1: Biochemical screening of licorice extract.
Indication: (+) = Presence, (-) = Absence, E = ethanol, A = acetone, and D.W = distilled water licorice extracts

Licorice extracts	TPC (mg/GAE/g of extract)	TFC (mg/QE/g of extract)
E 100%	114.17 ± 0.1[a]	50.69 ± 0.4[b]
E 75%	75.32 ± 0.2[c]	47.92 ± 0.6[d]
E 50%	34.57 ± 0.2[g]	31.68 ± 0.5[g]
A 100%	88.17 ± 0.1[b]	48.58 ± 1.1[c]
A75%	68.40 ± 0.2[d]	43.50 ± 0.2[e]
A 50%	40.18 ± 0.6[f]	37.24 ± 1.4[f]
D.W 100%	64.84 ± 0.7[e]	56.00 ± 1.5[a]

Table: 2 Total phenolic and flavonoid content of licorice extract
Note: All values are means of three replications with ± SD, values with different letters significantly different at $p < 0.05$, Indication: E = ethanol, A = acetone, D.W = distilled water

Conc.µg/mL	Licorice extract (Antioxidant activity %)						
	E 100%	E 75%	E 50%	A 100%	A 75%	A 50%	D.W100%
400	62.3±0.2[a]	45.5±0.1[f]	48.5±0.03[d]	60.3±0.2[b]	33.15±0.5[g]	46.7±0.2[e]	58.4±0.3[c]
200	46.4±1.0[b]	38.4±3.0[c]	37.9±0.3[d]	50.3±0.7[a]	26.4±0.6[g]	27.6±0.2[f]	37.1±0.01[e]
100	36.2±0.1[a]	26.4±0.1[c]	23.7±0.03[e]	35.6±0.1[b]	18.8±0.01[f]	17.4±0.05[g]	26.2±0.02[d]
50	20.2±0.1[a]	17.4±0.3[d]	14.7±0.01[f]	19.6±0.2[b]	15.5±0.5[e]	13.4±0.04[g]	18.3±0.1[c]

Table: 3 Antioxidant activity of licorice extract
Note: Conc. = Concentration, E = ethanol, A = acetone, and D.W = distilled water licorice extract, all these values are means of three replications with ± Standard deviation, values with different letters significantly different at $p < 0.05$

Bacteria used	E 100%	E 75%	E 50%	A 100%	A 75%	A 50%	D.W100%
Staphylococcus aureus (+)	20±1.2[a]	18±0.9[c]	14±1.7[g]	16±2.3[b]	13±1.4[d]	11±2.0[f]	19±2.1[e]
Streptococcus pyogenes(+)	21±2.7[b]	18±0.6[c]	17±1.6[a]	19±1.4[g]	15±2.3[f]	10±1.7[e]	16±1.5[c]
Escherichia coli(-)	0±0.0	0±0.0	0 ±0.0	0 ±0.0	0±0.0	0±0.0	0±0.0
Pseudomonas aeruginosa(-)	0±0.0	0±0.0	0±0.0	0 ±0.0	0±0.0	0±0.0	0±0.0

Table: 4 Anti-microbial activity of licorice extract
Note: (0) = No zone of inhibition (No effect), E = ethanol, A = acetone, and D.W = distilled water licorice extracts, all these values are means of three replications with ± Standard deviation, values with different letters significantly different at $p < 0.05$

Bacteria used	Antibiotics (Zone of inhibition in mm)					
	Augmentin	Moxifloxacin	Amikacin	Ethanol	Acetone	Distilled water
S. aureus(+)	>17	16	>17	-	-	-
S. pyogenes(+)	>17	>21	>21	-	-	-
E. Coli(-)	Resistant	>16	>17	-	-	-
P. aeruginosa(-)	>16	>17	>17	-	-	-

Table: 5 Effect of positive and negative control against bacteria

Results indicated that solvents (ethanol, acetone, distilled water) and their concentrations (100%, 75%, 50%) influenced significantly ($p \leq 0.05$) on TPC and TFC of extract. Hence, the Tukey post hoc test showed the difference between TPC and TFC that varied with the trend 100% > 75% > 50% of solvent and its concentration (table 2). This is due to the polarity of

extracting solvent and the solubility of chemical constituents in the solvent, impact of the dielectric constant, structure of organic solvent, and biochemical nature of phytoconstituents (Iqbal *et al.,* 2017). Hence, 100% ethanol, acetone, and distilled water extracts were better than 75 % and 50% that gives higher TPC and TFC in licorice extract. Similarly, ethanol, acetone, and distilled water were better solvents for detections of polyphenols from plant sources reported in various studies (Mokrani and Madani, 2016). TPC was reported earlier higher in ethanolic extract 185.7 µg/GAE and lower in aqueous extract 75.7 µg/GAE, respectively (Tohma and Gulçin, 2010) was much correlate with TPC observed in this study. Similarly, the TFC of this study was also in line with the previous study that reported higher total flavonoid contents 67 as µg of standard equivalent/mg in aqueous extract of licorice (Velvizhi and Annapurani, 2018). Moreover, licorice flavonoids and phenolics are the most effective natural antioxidant components that showed antioxidants act through a different mechanism including the donation of hydrogen, scavenging free radicals, chelation of metals, and also due to polyphenolics, licorice act as a critical medicinal agent and exhibited several therapeutic functions (Ciganović *et al.,* 2019).

Antioxidants are the active compounds that scavenge the free radicles in the human body and provide protection from various diseases. Our results indicated that solvents (ethanol, acetone, distilled water) and their concentrations (100%, 75%, 50%) influenced significantly ($p \leq 0.05$) on antioxidant activity of the extract. Hence, the Tukey post hoc test showed the difference between antioxidant activity that varied with the trend 100% > 75% > 50% of extract solvent and concentration (table 3). This might be due to the solvent polarity and its increasing or decreasing concentration. Hence, 100% ethanol, acetone, and distilled water extracts were better than 75 % and 50% that gives the higher antioxidant activity of licorice extract. Besides it was confirmed that ethanol, acetone, and distilled water were better solvents to indicate antioxidant activity from plant sources (Mokrani and Madani, 2016). The present study also confirms higher phenolic and flavonoid contents due to which antioxidant activity also depends. This study showed compatibility with previous research that reported the highest antioxidant activity 92% in ethanolic extract of licorice followed by other solvents (Iqbal *et al.,* 2017). Similarly, our results were also compatible with another study that showed the highest antioxidant activity 88.7% in methanolic extract of licorice at 800 µg/mL concentration and showed that activity increases or decreases with low and high concentration and solvent type (Ercisli *et al.,* 2008). Previously, antioxidant activity was observed higher 80% in the methanolic extract at the concentration of 100 µg/mL concentration (Velvizhi and Annapurani, 2018) was much correlated with the antioxidant activity of the current study. Our observation also validated the previous study that reported antioxidant activity of licorice in aqueous 52.2% and ethanolic extract 54.2%, separately (Tohma and Gulçin, 2010). Moreover, free radicals contain singlet oxygen and hydrogen peroxide that cause several mutations in the human body leads to multiple serious ailments. In the present study, licorice extracts showed the highest antioxidant activity leads to protection from free radicals and prevention from many critical diseases (Sharifi-Rad *et al.,* 2021).

Antimicrobial activity of licorice extract against different pathogenic bacteria was observed in the present study. Our results highlighted those solvents (ethanol, acetone, distilled water) and their concentrations (100%, 75%, 50%) influenced significantly ($p \leq 0.05$) on antimicrobial activity of licorice extract. Therefore, the Tukey post hoc test showed the difference between antimicrobial activity that varied with the trend 100% > 75% > 50% of extract solvent and concentration (table 4). Hence, 100% ethanol, acetone, and distilled water extracts were better than 75 % and 50% that gives a higher zone of inhibition of licorice extract against gram-positive bacteria only. This result of antimicrobial activity correlated with previous findings that showed antimicrobial activity of licorice aqueous, acetone, and ethanolic extract against different microbes including *E. coli, S. pyogenes*, *P. aeruginosa, S. aureus*, and others (Alwan *et al.,* 2015). These researchers also showed that antimicrobial activity was higher or lower depending upon the solvent polarity and its concentration. Similarly, a previous study reported zone of inhibition had greatly affected by increases or decreases in the concentration of solvent and its polarity. They also reported the highest zone of inhibition of licorice methanolic extract in 100% concentration against gram-positive bacteria (Varsha *et al.,* 2013). Gram-negative bacteria showed resistance against licorice extract in this current study, this might be due to the difference in the cell wall in these bacteria. The cell wall of both gram-negative and positive bacteria is made up of peptidoglycan but there is an additional layer composed of murine proteins and membrane proteins in gram-negative bacteria, attached to the outer layer to the cell wall that provides more resistance to this bacterium (Bailey, 2018). Another study also reported no effect of licorice extract against gram-negative bacteria due to their different growth pattern (Awamie and Rees, 2016). Also, maybe licorice extract is less effective to gram -ive bacteria and more beneficial against gram +ive bacteria. A previous study also revealed that licorice ethanolic extract has more inhibitory action against gram positive bacteria than negative bacteria (Sedighinia *et al.,* 2012), that correlate with our findings. Moreover, bioactive compounds like glabridin, glycyrrhizic acid, and many other compounds in licorice extract exhibit antimicrobial effects (Alwan *et al.,* 2015). So, licorice extract showed higher phenolic and flavonoid content in this study owing to the antimicrobial activity leads to the cure of many infections in humans against different pathogenic bacteria.

In the current study, solvents were used as negative control and antibiotics as a positive control. Antibiotics give a zone of inhibition against all bacteria except Augmentin which was resistant to *E. coli* and no zone of inhibition was recorded from negative controls. The use of Augmentin was first reported in this current study. Results from this study showed that the antibacterial activity of licorice all extracts was significantly greater than the negative control. These results also indicated that the antibacterial activity of Augmentin, Moxifloxacin, and Amikacin, a well-known antibacterial agent was not significantly greater than licorice extracts (table 5). All these antibiotics are normally used for the cure of infection caused by these bacteria. Similarly, antibiotics penicillin G and Amoxicillin were used to check the antibacterial activity against different microbes, and they also showed no significant difference between the zone of inhibition of licorice extract and antibiotics

(Geetha and Roy, 2012). Further, the antibacterial effect of licorice and leaves extract against various microbes were reported previously that also showed zone of inhibition of standard antibiotics Gentamycin and Keflex and revealed that antibiotics and licorice extract had no higher difference to control the activity of microbes (Alwan *et al.,* 2015). Hence, extract of licorice had abundant antimicrobial activity against gram +ive and -ive bacteria so it could be used as a natural therapy for controlling the infections against these pathogenic bacteria without harmful effects.

CONCLUSION: Extraction of solvent and its concentration significantly affected on total phenolic and flavonoid content, antioxidant, and antimicrobial activity of extract except screening of biochemical compounds. The occurrence of bioactive compounds critically depends upon solvent polarity and concentration. It is recommended that organic solvents with higher polarity would be a better solvent for the preparation of extract with no harmful effects. Due to multiple phytochemicals present in licorice extract, it would be a natural antioxidant and antimicrobial agent in medicines for reduction of oxidative stress, protection from several pathogenic bacteria's and numerous other ailments, and in the food industry for preservation purpose.

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