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### RESEARCH ARTICLE

#### ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *MENTHA ARVENSIS* EXTRACT SDEPENDING ON ETHANOL CONCENTRATION.

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#### Abstract

This study was performed to investigate antioxidant and antimicrobial activities of *Mentha arvensis* ethanol extracts, which were extracted depending on ethanol concentrations. Yield of total extract showed the highest level with 17.3% in the 30% ethanol extract. Total polyphenolic contents in the 50% and 70% ethanol extracts were detected by relatively more amounts when compared with those of the other extracts, whereas total flavonoid in 100% ethanol extract was showed by the most content. As a result consistent with the total polyphenol and flavonoid contents, the highest activities from DPPH, ABTS and FRAP assays were detected in the 70%, 100%, and 50% ethanol extracts, respectively. Antimicrobial activities against *Clostridium perfringens* and *Escherichia coli* were strongly detected from the 70% and 100% ethanol extracts. Therefore, we suggest that total polyphenol and flavonoid contents from the *M. arvensis* ethanol extracts are directly associated with antioxidant and antibacterial activities.

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#### Introduction:-

Recently, consumers have increased interest in the nutritional balance and safety for food, but not the quantity of food, with the improvement of educational and economic levels (Plastow *et al.* 2014; Sharma *et al.* 2015). Furthermore, average life expectancy has been extended by development of medical standards, and a growing interest in well-being has been increased the efforts to discover the functional food. However, since the discovery of functional materials are necessary to precede the development of functional food, many studies for functional materials has been mainly performed from natural products to be identified for safety (Mussatto 2014; Webb *et al.* 2004). Plant maintains a large number of variously useful components including secondary metabolites such as polyphenol and flavonoid compounds. However, since many parts have not yet been scientifically investigated to date, various natural resources in plant have been tried for search of the functional materials to have physiological activity (Lao *et al.* 2014; Lim *et al.* 2014; Qin *et al.* 2014; Wu *et al.* 2014). Recently, material research from natural products has been discovered antioxidants as a part of the various functional food, which the antioxidants play important roles for maintenance of self-defense mechanism and for protection of human body from active oxygen (Chen *et al.* 2010; Chaudhari *et al.* 2014).

Mint, *Mentha arvensis*, is a perennial plant belonging to family Lamiaceae. *M. arvensis* propagates via the long roots in underground as a perennial plant of 20~60 cm in height, and the leaves maintain many of parenchyma cells to

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carry a lot of essential oils. The various species of genus *Mentha* grow as wild plants in many places including Europe, Asia and North America. *M. arvensis* is a major-cultivated species is indigenous in Asia, but *M. piperita* is native in Europe region. The main components of peppermint have been well-known to menthol, menthone, 1,8-cineole, isomenthone, methyl acetate, neomenthol and limonene (Windholz 1983).

Peppermint has been often used as edible, medicinal and perfume main ingredients from ancient Egypt, and in a variety of symptoms such as indigestion, nausea, laryngitis, diarrhea, headache and abdominal pain in the East and West (Yoon *et al.* 2005). Peppermint maintains anti-inflammatory and anti-allergic activities and inhibitory effect of biofilm formation (Shin and Kim 1998; Sandasi *et al.* 2011; Lim *et al.* 2012). In addition, peppermint has been variously used for cookies, gum, cosmetics and toothpaste owing to specific refreshing feeling (Shin and Park 1994). The essential oil of peppermint is various efficacies such as analgesic, antiseptic, anti-excitement, removal of intestinal gas, strengthening of concentration, respiratory disease, mild anesthetic effect, normalization of ovarian function and dermatitis (Micklefield *et al.* 2003; Kim 2006). The improvement of symptoms through inhalation of peppermint essential oil has been reported from anorexia and vomiting of cancer patients (Jung and Lee 2004; Woo 2010). On the other hand, the mint's essential oil is the inhibitory activities against *Escheria coli*, *Helicobacter pylori*, *Salmonella typhimurium* and *Staphylococcus aureus* (Lee *et al.* 2002; Woo *et al.* 2013).

In this study, as a preceding study for the application in the meat products to induce an improved functionality, we prepared *M. arvensis* extracts depending on ethanol concentrations and examined antioxidant and antibacterial activities for each extract.

## Materials and Methods:-

### Preparation and yield of *M. arvensis* ethanol extract:-

*M. arvensis* was purchased from traditional market in South Korea. Fifty grams of aerial part were extracted by immersion for 1 week at room temperature with 0%, 30%, 50%, 70% and 100% ethanol concentrations. The extracts were filtered by Watman No.1 filterpaper, concentrated under vacuum in a rotary evaporator (RW-0252G 4000/G1, Heidolph, Germany), and then made into powder by lyophilization (PVTFD 10R, IISinBioBase, Korea). Each extract after lyophilization was applied by dilution into 0~1,000 µg/mL for assay. Yields of the extracts depending on ethanol concentrations were presented by percent of lyophilized power/dried material (w/w) weight ratio.

### Determination of total polyphenol content:-

Total polyphenol content was measured according to method of Peschelet *et al.* (2006). An aliquot of the extract (0.1 mL of 100 µg/mL) was mixed with 7.9 mL of distilled water and 0.5 mL of Folin-Ciocalteu's phenol. After reaction for 2 min, 1.5 mL of 20% sodium carbonate solution was added, and then reacted for 2 h. Total polyphenol content was measured by a microplate reader (Multiscan GO, Thermo Scientific co. Ltd., USA) at 765 nm in wavelength. Total polyphenol content was presented as an mg gallic acid/g equivalent.

### Determination of total flavonoid content:-

Total flavonoid content was measured according to method of Chang *et al.* (2002). An aliquot of the extract (0.5 mL of 100 µg/mL) was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water, and then reacted for 30 min at room temperature. Total flavonoid content was measured by a microplate reader (Multiscan GO, Thermo Scientific co. Ltd., USA) at 415 nm in wavelength. Flavonoid content was presented as an mg catechine/g equivalent.

### DPPH radical scavenging activity:-

DPPH radical scavenging activity was determined by reducing power of sample to a free radical scavenging for 2,2-Diphenyl-1-picrylhydrazyl by a few modified method of Brand-Williams *et al.* (1995). The modified method was briefly described as follows. An aliquot of the extract (500 µg/mL) was well-mixed for 10 sec with the equivalent amount of DPPH (D9132, SIGMA, USA) solution, and after reaction for 20 min, the treated solution was measured by a microplate reader (Multiscan GO, Thermo Scientific co. Ltd., USA) at 517 nm in wavelength. Ascorbic acid and butylated hydroxyanisole (BHA) were employed as positive controls. Free radical scavenging activity was presented by absorbance ratio between sample and control, and calculated as follows:

$$\text{DPPH radical scavenging capacity (\%)} = (1 - (AS - AS_0)/A_0) \times 100\%$$

Where A<sub>0</sub> is the absorbance of the negative control group without sample, AS<sub>0</sub> is the absorbance of the sample solution and AS is the absorbance of the treatment group with sample.

**ABTS<sup>+</sup> radical scavenging activity:-**

ABTS<sup>+</sup> radical cation discoloration assay was examined for the radical scavenging activity of the mint extract according to method of Re *et al.* (1999). The ABTS<sup>+</sup> stock solution (A9941-100TAB, SIGMA, USA) was diluted by 50% ethanol solution to prepare an ABTS<sup>+</sup> working solution. The ABTS<sup>+</sup> working solution was adjusted to 0.700±0.05 at 732 nm in wavelength at room temperature. An aliquot of the extract (2 µL of 500 µg/mL) was mixed with 50 µL of ABTS<sup>+</sup> working solution, reacted for 10 min, and then measured by a microplate reader (Multiscan GO, Thermo Scientific co. ltd., USA) at 734 nm in wavelength. Ascorbic acid and butylated hydroxyanisole (BHA) were employed as controls. Cation radical scavenging was presented by absorbance ratio between sample and control, and calculated as follows:

$$\text{ABTS}^+\text{ radical scavenging capacity (\%)} = (1 - (\text{AS} - \text{AS}_0)/\text{A}_0) \times 100\%$$

Where A<sub>0</sub> is the absorbance of the negative control group without sample, AS<sub>0</sub> is the absorbance of the sample solution and AS is the absorbance of the treatment group with sample.

**FRAP assay:-**

FRAP (ferric-reducing antioxidant power) assay was determined depending on method of Benzie *et al.* (2006). The extract (500 µg/mL) was reacted with FRAP solution [0.3 M sodium acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O by ratio of 10:1:1], and then measured by a microplate reader (Multiscan GO, Thermo Scientific co. ltd., USA) at 595 nm in wavelength. FRAP activity was determined as an µM FeSO<sub>4</sub>·7H<sub>2</sub>O/µg equivalent.

**Determination of antibacterial activity:-**

Antibacterial activity was examined by 8 mm disc dispersion method (Barry 1976). The ethanol extracts were dissolved by 20 mg/mL concentrations. The applied bacteria including *Staphylococcus aureus* (ATCC 112692), *Clostridium perfringens* (ATCC 13124), *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 11775), *Listeria monocytogenes* (ATCC 19114), *Bacillus cereus* (ATCC 1178), *Vibrio parahaemolyticus* (ATCC 17802D-5), and *Candida albicans* (ATCC 1023) were allocated from KCTC (Korean Collection for Type Cultures). The allocated bacteria were subcultured in Nutrient broth (Difco, USA). The subcultured bacteria were smeared on Nutrient agar, and positioned by 8 mm disc accumulated with 0.4, 1, 2, and 4 mg of each extract, and then incubated for 24 h at 37°C. After incubation, antibacterial activity was calculated by size of clear zone around the disc.

**Statistics analysis:-**

Individual comparisons among least squares means (LSM) for significant differences were made according to the multiple range test of Duncan. All analyses were performed within the SAS statistical software package (version 9.1, SAS Inst., Inc., USA), and differences were considered significant at  $P < 0.05$ .

**Results and Discussion:-****Total polyphenol and flavonoid contents:-**

In order to examine total extract yield depending on ethanol concentration, yields from *M. arvensis* were presented by weight ratio of dry extract/raw material. The yields extracted from 0%, 30%, 50%, 70% and 100% ethanol concentrations were detected by 15.80, 17.28, 17.18, 14.94 and 7.38% (w/w), respectively (Fig. 1). As a result, the extract yield was the highest content at 30% ethanol extract. Especially, it is assumed that the extremely low extraction yield in 100% ethanol is totally reduced owing to the low extraction yield of hydrophilic substances.

It has been well-known that polyphenolic compounds and flavonoids are closely associated with antioxidant activity (Macheix *et al.* 1990; Lee *et al.* 2002; Chung 1999; Fidrianny *et al.* 2013). Therefore, to analyze antioxidant activity in this study, we examined total polyphenol and flavonoid contents from the mint extracts (Table 1). Total polyphenols from the 50% and 70% ethanol extracts were detected by relatively high contents, 74.97 and 77.86 mg gallic acid/g, respectively, whereas total flavonoid in 100% ethanol was extracted to the most content, 62.51 mg catechin/g.

Taken together, it is estimated that the 50, 70 and 100% ethanol extracts to maintain high polyphenol and flavonoid contents are related with high antioxidant activities.

**DPPH radical scavenging activity:-**

DPPH activity was examined for analysis of free radical scavenging ability from the mint extracts. DPPH activity depending on ethanol concentration was showed at Fig. 2. DPPH activities of the extracts exhibited totally lower values than those of ascorbic acid and BHA, but the 70% ethanol extract to maintain the most total phenolic content exhibited the strongest DPPH radical scavenging activity among the extracts. This result is consistent with a report that the activity increases proportional to the polyphenol content from the correlation between antioxidant content and free radical scavenging activity (Chung 1999).

Furthermore, in this study, the 30% and 50% ethanol extracts to maintain comparative high polyphenol content were detected by comparative high DPPH radical scavenging activities among the extracts. However, although total polyphenol content in the 0% (cold-water) ethanol extract was detected by a value higher than that of the 100% ethanol extract, the 0% ethanol extract showed the lowest value of DPPH activity. It is assumed that the extracted materials in 0% ethanol concentration have differential chemical properties to exhibit differential DPPH activity with the other ethanol extracts. Therefore, we suggest that polyphenolic compounds in the mint extract is directly associated with DPPH radical scavenging activity.

**ABTS<sup>+</sup> radical scavenging activity:-**

We examined ABTS<sup>+</sup> activity to analyze anionic free radical scavenging activity. ABTS<sup>+</sup> radical scavenging activities of the mint extracts depending on ethanol concentration were shown in Fig. 2. The highest activity was detected by the 100% ethanol extract with 9.65%, and the 70% ethanol extract was observed by relatively high value.

Flavonoids have antioxidant abilities by various patterns according to extraction solvent or extract materials, and in particular the materials extracted from *n*-hexane are associated with high ABTS<sup>+</sup> radical scavenging activity (Fidrianny *et al.* 2013). In addition, the prenylated flavonoid reacts strongly with ABTS<sup>+</sup> radical, but does not react with DPPH radical (Lee *et al.* 2006). Since anionic radical scavenging ability in this study was detected by relatively high values from the 70% and 100% extracts among the *M. arvensis* ethanol extracts, and it is predicted which the 70% and 100% ethanol extracts contain lower polarities than those of the other extracts in this study, we suggest that materials to have relatively low polarity in the extracted fractions maintain ABTS<sup>+</sup> radical scavenging activity. When compared with ascorbic acid, very low activities were totally observed in the extracts, but the 70% and 100% extracts showed activities higher than that of BHA.

From these results, we suggest that ABTS<sup>+</sup> activity is associated with flavonoid content because of maintaining relatively high flavonoid content in the 70% and 100% ethanol extracts when compared with the other extracts. However, since the 0% ethanol extract showed the lowest value of ABTS<sup>+</sup> activity, in the same way as in the DPPH activity, it is assumed that the extracted materials in the 0% ethanol have differential chemical properties to show differential ABTS<sup>+</sup> activity with the other ethanol extracts.

**FRAP activity:-**

We examined to analyze Ferric-reducing antioxidant power (FRAP; %) from the mint extracts. FRAP activities for the mint extract depending on ethanol concentration were presented at Fig. 4. FRAP activities of the extracts were totally detected by very lower levels when compared with ascorbic acid and BHA. However, among activities of the extracts, the 50% ethanol extract was showed by relatively high value.

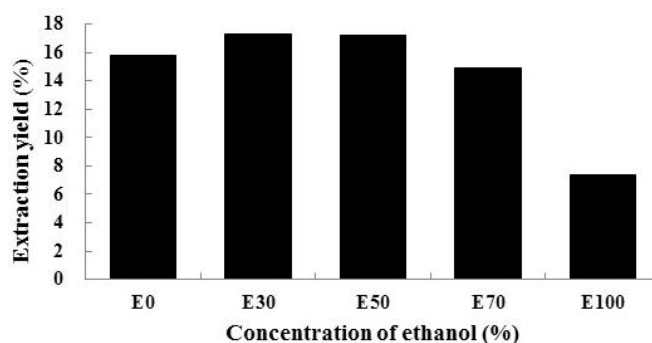
Phenolic compound is a secondary metabolite to maintain the most abundance in fruit as hydrophilic antioxidants (Macheix *et al.* 1990). Antioxidant activities measured by DPPH and FRAP assays are highly associated with total polyphenol contents in nectarines, peaches and plums (Gil *et al.* 2002). Furthermore, the correlation between total polyphenol content and antioxidant activity is determined by FRAP or electron spin resonance spectroscopy in fruit juices (Gardner *et al.* 2000). Therefore, we suggest that the higher antioxidant activities of the 0, 30, 50 and 70% ethanol extracts than that of the 100% extract are owing to higher total polyphenol content.

**Antibacterial activity:-**

In order to examine antibacterial activities from the extracts, we evaluated antibacterial activities by various bacterial strains including Gram positive, Gram negative and yeast. Antibacterial activities of the mint extracts depending on ethanol concentration was shown at Table 2. The results of clear zone assays surrounding disc appeared antibacterial activity against *C. perfringens* from all the extracts, except for the 0% extract. Especially, the

70% and 100% extracts showed strong activities against *C. perfringens*. On the other hand, the 70% and 100% extracts showed strong activities against *E. coli*. However, we didn't observe the activities against the other-applied strains.

The peppermint oil is potential antibacterial activities against pathogenic bacteria including *H. pylori* and *S. aureus* (Woo *et al.* 2013). As the peppermint contains many contents of isomenthol (26.84%) and menthol (25.48%), the oil presents excellent antibacterial activities against *E. coli* and *S. typhimurium* (Lee *et al.* 2002). Therefore, we suggest that the extracts applied for this study have differential activity with the previous study due to the differentially extracted components depending on solvent used for extraction.

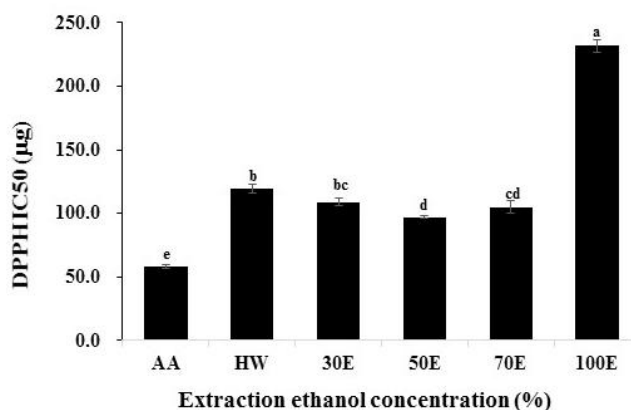


**Fig. 1:- Extraction yields of *M. arvensis* depending on ethanol concentration.** The quantified *M. arvensis* was extracted depending on each ethanol concentration. X- and Y-axes indicate ethanol concentration and extraction yield, respectively. E0; cold-water extract (0% ethanol), E30, E50, E70 and E100; 30, 50, 70 and 100% ethanol extracts, respectively.

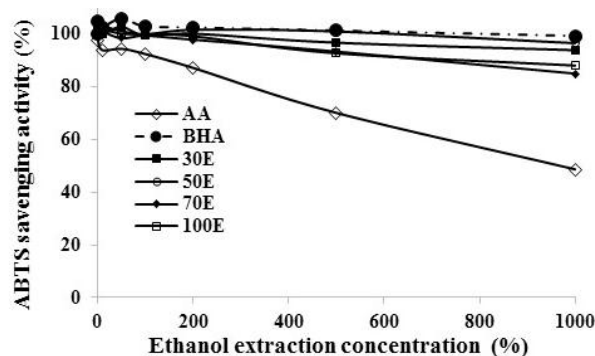
A

AA	E0	30E	50E	70E	100E
57.7±1.48	119.54±3.07	109.05±3.31	96.98±1.21	104.95±5.02	231.74±4.88

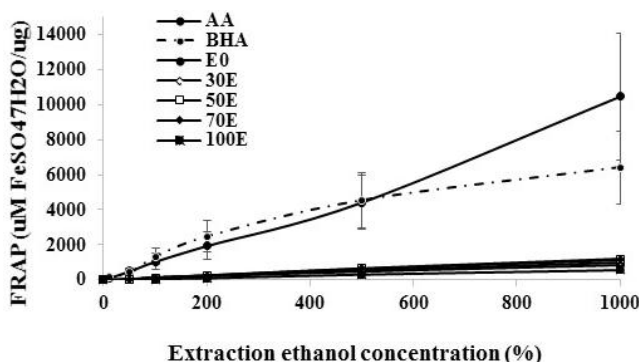
B



**Fig. 2:- DPPH radical scavenging activities of the *M. arvensis* ethanol extracts.** The extracts of 0, 1, 10, 50, 100 and 1,000 µg/mL were applied for assay of DPPH. X- and Y-axes indicate ethanol concentration and 50% inhibitory concentration (IC<sub>50</sub>) of DPPH radical scavenging activity, respectively. Ascorbic acid (AA) was applied as a positive reference for DPPH activity. E0; cold-water extract (0% ethanol), E30, E50, E70 and E100; 30, 50, 70 and 100% ethanol extracts, respectively. <sup>a-e</sup>Values indicate significant difference among the samples ( $P < 0.05$ ).



**Fig. 3:-** ABTS<sup>+</sup> radical scavenging activities of the *M. arvensis* ethanol extracts. The extracts of 0, 1, 10, 50, 100 and 1,000 µg/mL were applied for assay of ABTS<sup>+</sup>. X- and Y-axes indicate ethanol concentration and ABTS<sup>+</sup> radical scavenging activity, respectively. Ascorbic acid (AA) and BHA were applied as positive references for ABTS<sup>+</sup> activity. E0; cold-water extract (0% ethanol), E30, E50, E70 and E100; 30, 50, 70 and 100% ethanol extracts, respectively.



**Fig. 4:-** Assays for Ferric reducing ability of power from the *M. arvensis* ethanol extracts. The extracts of 0, 1, 10, 50, 100 and 1,000 µg/mL were applied for FRAP assay. X- and Y-axes indicate ethanol concentration and FRAP activity, respectively. Ascorbic acid (AA) and BHA were applied as positive references for FRAP activity. E0; cold-water extract (0% ethanol), E30, E50, E70 and E100; 30, 50, 70 and 100% ethanol extracts, respectively.

**Table 1:-** Total polyphenol and flavonoid contents from the *Mentha arvensis* ethanol extracts

Applied ethanol concentration (%)	Total polyphenol (mg GAE/g)	Total flavonoid (mg QE/g)
0	12.69±0.15 <sup>b</sup>	1.52±0.05 <sup>b</sup>
30	12.90±0.44 <sup>b</sup>	0.08±0.01 <sup>d</sup>
50	14.28±0.17 <sup>a</sup>	0.40±0.10 <sup>c</sup>
70	14.74±0.23 <sup>a</sup>	1.42±0.07 <sup>b</sup>
100	7.07±0.08 <sup>c</sup>	3.15±0.11 <sup>a</sup>

<sup>a-d</sup> Means±SD indicate significant difference within the same row.

**Table 2:-** Antimicrobial activities to the *Mentha arvensis* ethanol extracts

Extract type	E0				E30				E50				E70				E100				
	0.4	1	2	4	0.4	1	2	4	0.4	1	2	4	0.4	1	2	4	0.4	1	2	4	
Micro organisms																					
<i>E. coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	++	++	-	+	++	++
<i>S. typhimurium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>V. parahaemolyticus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>B. cereus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>C. perfringens</i>	-	-	-	-	-	-	-	++	-	-	-	++	++	++	++	++	++	++	++	++	++
<i>L. monocytogenes</i>	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+
<i>S. staphylococcus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>C. albicans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\**Escheria coli* (ATCC 11775), *Salmonella typhimurium* (ATCC 14028), *Vibrio parahaemolyticus* (ATCC 17802D-5), *Clostridium perfringens* (ATCC 13124), *Bacillus cereus* (ATCC 1178), *Listeria monocytogenes* (ATCC 19114), *Staphylococcus aureus* (ATCC 112692), and *Candida albicans* (00 432 ATCC 1023 IFO 1594). \*\* -; No antimicrobial activity, +; Very slight antimicrobial activity to clear zone of 8.1-9.0 mm diameter, ++; Moderate antimicrobial activity to clear zone of 9.1-10.9 mm; +++; Clear antimicrobial activity to clear zone of 11.0-12.9 mm; ++++; Strong antimicrobial activity to clear zone of 13.0-15.9 mm; +++++; Very strong antimicrobial activity to clear zone above 16.0 mm.

### Conclusions:-

The yield of extraction for *M. arvensis* appeared the most amount at the 30% ethanol extract. Total polyphenol content was detected by relatively high values at the 50 and 70% ethanol extracts, whereas total flavonoid content was showed by the most amount at the 100% ethanol extract. As a result consistent with these contents, DPPH, ABTS and FRAP activities were assayed by the highest values at the 70, 100 and 50% extracts, respectively. However, ABTS and FRAP activities, except for the activity of DPPH, were very low in comparison with ascorbic acid and BHA. Antibacterial activity was detected only for *E. coli* and *C. perfringens*.

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