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Research Article

A New Approach for Predicting Antioxidant Property of Herbal Extracts

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ABSTRACT
This study was conducted in order to determine if the herbal extract having antioxidant property. The major princible is to find out if there is any relationship between electrical current conductivity and antioxidant property of the ten Malaysian medicinal plants. The objective of this study achieved by measuring the antioxidant property of the herbal extracts, as well as to measure the ability of the herbal extracts to conduct an electrical current. Ten herbs were selected randomly, *Strobilaunthus crispa, Pereskia sacharosa, Ehretia laevis, Plectranthus ambinicus, Orthosiphon staminous, Androghophis panculata, Persicaria odorata, Clinacantus nutans, Curry leaves* and *Frankincense.* They were identified and prepared for extraction. The methanol extracts were obtained by ultrasonification extraction method. The antioxidant property was carried out using *1, 1-diphenyl-2-picrylhydrazy* (DPPH) reagent. The *pH* was measured using a *pH* meter and the phytochemical elements were also tested. Finally the electrical current conductivity was measured using a basic electrical circuit connection and the calculations of each herbal extract were obtained. The results show that all the extracts have significant amount of electrical conductivity ranging from (0.06 – 0.12 Ω·cm). All the extracts consist of high antioxidant property and that these extracts contain abundant amount of elements. This study found that there is a relationship between the electrical current conductivity and the antioxidant property. This introduces a possible new tool, which is electrical current conductivity as a predictor for the antioxidant property.

Key words: *Strobilaunthus crispa, Pereskia sacharosa, Ehretia laevis, Plectranthus ambinicus, Orthosiphon staminous, Androghophis panculata, Persicaria odorata, Clinacantus nutans, Curry leaves* and *Frankincense,* electrical current conductivity, antioxidant property, DPPH, methanol extracts, ultrasonification extraction.

INTRODUCTION

Many people today are using herbal medicine as complementary and alternative medicine along with conventional medicine. The World Health Organization (WHO) has acknowledged the increasing awareness of herbal medicines. (Mohd Yahya et al., 2008). Malaysian herbs have become one of the prime interest in most universities and research institutes in Malaysia, due to the vast areas of rainforests and tropical weather. Malaysia is also known to be the home of about 12% of all the plant species on earth, it is estimated that 15,000 known plant species, in which 3,700 are known to be useful, 2,000 species with medicinal value and the balance remains not fully exploited and cultivated. (www.news.kl.utm.my). To acknowledge this The Malaysian Government has been supporting and funding researches since 1985. There are many natural substances of nature in these herbs such as in the form of juice, gum, essential oils, fatty oils (Jamila, 2006). These herbs also have the potency to contain vital properties such as antioxidants, antibacterial agent, antifungal and even anti-cancer. However the ability of these medicinal herbs to conduct electricity is not that commonly understood and you will ask yourself why do we need to know if they have this property, and why is it beneficial to heath?

In this research the amount of electrical conductivity of the selected herbal extracts will be measured along with the antioxidant activity. The determination of the electrical conductivity could serve as an indicator for the antioxidant property of the herbal extracts. Antioxidants are molecules or nutrients in our food which can prevent or slow the oxidative damage to our body. Basically inhibit the oxidation of other molecules, meaning that they transfer electrons or hydrogen from a substance to an oxidizing agent. Because of these oxidation reactions free radicals are produced, these radicals can start chain reactions in our body cells which can cause damage or death to the cell (Arash et al., 2012). Antioxidants may also enhance immune defence and therefore lower the risk of cancer and infection (www.buzzhealth.co.uk).

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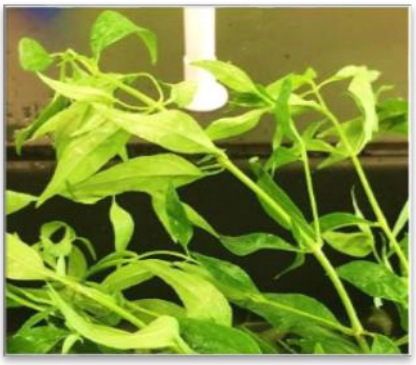
a. Strobilaunthus crispa

b. Pereskia sacharosa

c. Ehretia laevis

d. Plectranthus ambinicus,

e. Orthosiphon staminou



f. Androghophis panculata

g. Persicaria odorata,

h. Clinacantus nutans

i. Curry leaves,

j. Frankincense,

Figure 1: Plants used

Herbal extracts have the potency to act as antioxidant property and hence scavenge the free radicals (Rasha et al., 2014). Electrical conductivity (EC) is a measure of how easily an electrical current can pass through water or any solution. A conductivity probe and meter can be used to measure the accuracy in an electrical field, and with a data logger electrical conductivity can be recorded electronically. There are factors which influences the

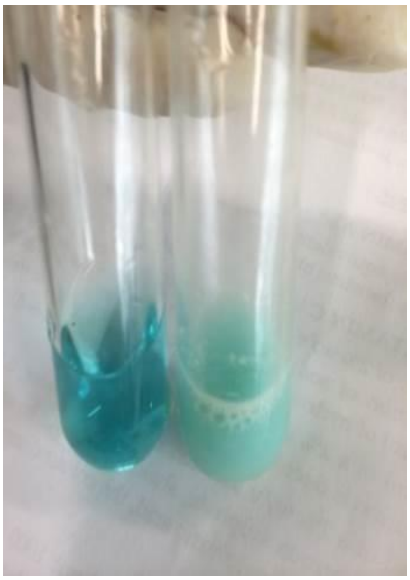
a. Test for Magnesium

b. Test for Sulphate

c. Test for Sulphate

d.Test for Nitrate

e Test for Tannins & Phenols



f Test for Gum

Figure 3 : phytochemical element tests results

electrical conductivity, such as weak electrolyte, *pH*, and temperature. Thus the presence of charged ions will determine the EC of aqueous solutions. Therefore electrical conductivity increases with the number of ions in a solution (RDD Moore et al., 2008). A selection of medicinal herbal extracts was selected randomly namely:

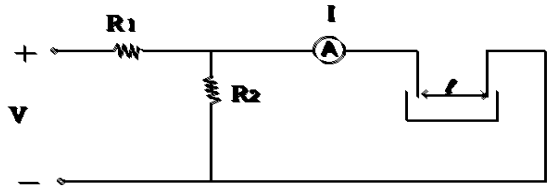


Figure 2: diagram of the electrical circuit

METHODS

In this research the herbs were selected randomly (See plate 1) and each herb was extracted and tested for its antioxidant properties, pH, and phytochemical elements. Then the herbal extractions were further tested to measure the electrical current conductivity by applying the slandered electrical circuit flow. The research was carried out in seven phases.

It is known that water molecules dissociate into ions a function of pH and temperature result in a very predictable conductivity, and is affected by the presence of extraneous ions, which are the chloride and sodium ions. However electrical conductivity is a useful indicator of total dissolved solids because the conduction of current in an electrolyte solution is primarily dependant on the ionic species (M Hayashi, (Hiscock et al., 1996) (2004)

Phase 1: Collection and Identification of the herbs

The herbs were collected from a plant nursery in

Table 1, calculation of resistance of the ten herbal extracts

No	Herbal extract	R=V/I	Results Ω
1	*S.crispa*	8/6.01	1.33
2	*P.sacharosa*	8/1.09	7.33
3	*E.laeves*	8/1.36	5.88
4	*P.ambinicus*	8/1.80	4.44
5	*O.staminuos*	8/2.63	3.04
6	*A.panculata*	8/2.90	2.75
7	*P.odorata*	8/7.9	1.01
8	*C.nutans*	8/3.40	2.35
9	*Curry leaves*	8/5.41	1.47
10	*Frankincense*	8/1.66	4.81

Table 2: calculation of the total resistant in the circuit of the ten herbal extracts

No	Herbal extract	Total resistance in the circuit $R_T=R_a+R$	Results Ω
1	*S.crispa*	6.9 + 1.33	8.23
2	*P.sacharosa*	6.9 + 7.33	14.23
3	*E.laeves*	6.9 + 5.88	12.78
4	*P.ambinicus*	6.9 + 4.44	11.34
5	*O.staminuos*	6.9 + 3.04	9.94
6	*A.panculata*	6.9 + 2.75	9.65
7	*P.odorata*	6.9 + 1.01	7.91
8	*C.nutans*	6.9 + 2.35	9.25
9	*Curry leaves*	6.9 + 1.47	8.37
10	*Frankincense*	6.9 + 4.81	11.71

Table 3: calculation of resistivity of the ten herbal extracts

No	Herbal extract	P=R/0.870	Results Ω/cm
1	*S.crispa*	8.23/0.870	9.45
2	*P.sacharosa*	14.23/0.870	16.35
3	*E.laeves*	12.78/0.870	14.68
4	*P.ambinicus*	11.34/0.870	13.03
5	*O.staminuos*	9.94/0.870	11.42
6	*A.panculata*	9.65/0.870	11.09
7	*P.odorata*	7.91/0.870	9.09
8	*C.nutans*	9.25/0.870	10.63
9	*Curry leaves*	8.37/0.870	9.62
10	*Frankincense*	11.71/0.870	13.45

Malaysia mainly in Shah Alam and Subang area. Then each sample was placed in a separate plastic bag labelled and sent for identification to The Institute of bioscience, University Putra Malaysia.

Phase 2: Preparation of the herbs for extraction

The herbs were washed under running tap water followed by distilled water and dried using oven at 40°C till they were crispy. Then the dried herbs were grinded using a grinding blender, as described in (Huda et al., 2007)

Phase 3: Extraction of the herbs

The dried grinded herbs were first weighed and then put in a beaker containing 1 part of dried leafs with 3 parts of methanol. The soaked leafs were placed in the ultrasonic water bath and left for 24 hours (Huanerg X et al., 2007) After efficient time was achieved the leaves were filtered using whatsman filter paper in a funnel the soaked leaves

Table 4: Calculation of the conductivity of the ten herbal extracts

No	Herbal extract	$\sigma=\frac{1}{\rho}$	Results Ω·cm
1	*S.crispa*	1/9.45	0.105
2	*P.sacharosa*	1/16.35	0.061
3	*E.laeves*	1/14.68	0.068
4	*P.ambinicus*	1/13.03	0.076
5	*O.staminuos*	1/11.42	0.087
6	*A.panculata*	1/11.09	0.090
7	*P.odorata*	1/9.09	0.110
8	*C.nutans*	1/10.63	0.094
9	*Curry leaves*	1/9.62	0.103
10	*Frankincense*	1/13.45	0.074

Table 5: the identification of the ten herbs

Number	Scientific Name	Local Name	Family Name
1.	*Strobilanthes crispa*	*Pecah kaca*	Acanthaceae
2.	*Pereskia sacharosa*	*Jarum tujuh bilah*	Cactaceae
3.	*Ehratia laevis*	*Tebengau*	Boraginaceae
4.	*Plectranthus amboinicus*	*Bangun-bangun*	Lamiaceae
5.	*Orthosiphon aristatus*	*Misai Kuching*	Lamiaceae
6.	*Andrographis paniculata*	*Hempedu bumi*	Acanthaceae
7.	*Persicaria odorata*	*Kesum*	Polyonaceae
8.	*Clinacanthus nutans*	*Belalai gajah*	Acanthaceae
9.	*Murraya koenigii*	*kari*	Rutaceae

were poured. Then the pure extract was taken and put for evaporation in the rotary evaporator.

Phase 4: Measurement of pH level

The *p*H was measured using a pH meter by placing the probe directly into the extracts and the results were recorded from the meter as described in Basic pH measurements.

Phase 5: Antioxidant activity of the extracted herbs

All the extractions were dissolved to make up a stock solution of 1g of extract to 10ml of methanol. Then they were diluted in serial dilutions of three, (1mg/ml, 0.1mg/ml, 0.75mg/ml, and 0.50mg/ml). The DPPH solution was also prepared by dissolving 6mg of DPPH in 100ml of methanol. Then 1ml of the extract from each dilution was added into the test tubes containing 2ml of the freshly prepared DPPH solution. The control was also prepared by adding 1ml of methanol to 2ml of DPPH solution. The mixtures were shaken vigorously and were left to stand in the dark for 30 minutes. The absorbance of the resulting solution was measured by using a spectrophotometer at absorbance of 517nm. The

Table 6, Percentage of extraction yield of the ten herbs

S. No.	Sample	Amount of Dried leaves (g)	Amount of extract yield (g)	Percentage of yield (%)
1.	*Strobilaunthus crispa*	102.82	6.95	6.75
2.	*Pereskia sacharosa*	19.71	0.75	3.8
3.	*Ehretia laevis*	50.65	5.16	10.1
4.	*Plectranthus ambinicus*	22.11	1.88	8.5
5.	*Orthosiphon staminous*	10.22	0.74	7.24
6.	*Androghophis panculata*	23.41	2.75	11.74
7.	*Persicaria odorata*	66.74	8.65	12.96
8.	*Clinacantus nutans*	45.60	0.46	1.0
9.	*Curry leaves*	29.16	3.17	10.87
10.	*Frankincense*	7.85	7.08	90.19

scavenging activity of each extract on DPPH radical was calculated using the follow equation: (Azlim A. et al., 2010) & (Molyneux, P. et al., 2004).

Scavenging activity (%) = $\left(\frac{1-absorbance\,sample}{absorbance\,control}\right) \times 100$

Phase 6: Electrical activity of the extracts

A basic electric circuit was designed to measure the current and resistance of the herbal extracts by applying a power supply of 8 volts and using 2 known resistivity connected in parallel, an ammeter to measure the current in ohms, a cathode and an anode that was inserted in the extraction bath of known length and area. After recording the reading of the current from the ammeter three formulas were applied in order to get the conductivity and there were Resistivity, Resistance and conductivity.

$R = V/I$ Equation (1)

$R = \rho \frac{\ell}{A}$ Equation (2)

$\sigma = \frac{1}{\rho}$ Equation (3)

Whereas: R= Resistance ρ = Resistivity ℓ = Length

A= Area σ = Conductivity

Observations

Area of container: 3.1cm^2

Length between two electrodes (ℓ) = 2.7cm

The total resistivity = 6.9 Ω

Phase 7: Preliminary phytochemical screening of the extracts

This is to detect some of the inorganic constituents of each extract. The methods were carried out using standard procedures described in K. R. Khandelwal, *practical phamacognosy. Techniques and Experiments* (E.R. 2010).

Table 7, MIC_{50} of the antioxidant property of the ten herbal extracts

S.No.	Herbs	MIC_{50} (mg/mL)
1	*Strobilanthes crispa*	0.03 ± 0.01
2	*Pereskia sacharosa*	0.05 ± 0.01
3	*Ehretia laevis*	0.02 ± 0.01
4	*Plectranthus amboinicus*	0.06 ± 0.01
5	*Orthosiphon staminous*	0.02 ± 0.01
6	*Andrographis paniculata*	0.07 ± 0.01
7	*Persicaria odorata*	0.07 ± 0.01
8	*Clinacantus nutans*	0.04 ± 0.01
9	*Murraya koenigii*	0.03 ± 0.01
10	*Frankincense*	0.08 ± 0.01

The tests for Magnesium, Chloride, Sulphate, Nitrate, Tannins &Phenols, Gum are

Test for Magnesium

Few drops of ammonium carbonate solution were added to 3-4ml of each extracts, which gives a white precipitate in the presence of Magnesium. See figure 9 (a).

Test for Chloride

2-3ml of lead acetate solution was added to 5ml of the diluted extracts the solubility of white precipitate in hot water indicates the presence of chloride. See figure 9 (b).

Test for Sulphate

Few drops of 5% $BaCl_2$ solution was added to 3ml of each extract white crystalline was observed in the presence of sulphate. See figure 9 (c).

Test for Nitrates

2ml of ferrous sulphate was added to 3ml of each extract and there was no brown colour observed but when adding sulphuric acid slowly from the sides of the test tube, a brown colour ring is produced at the junction of two liquids. See figure 9 (d).

Test for Tannins & Phenols

To the diluted extractions a few drops of lead acetate solution were added, white precipitation was observed. See figure 9 (e).

Test for gum

1ml of extract added into HCL and then mixed with 1ml of Benedict's solution. The mixture was then heated in a water bath for 5min. A light blue colour was developed. See figure 9 (f).

RESULTS & DISCUSSION

Herb identification

The identification of the ten herbal extracts were all sent to Institute Biosains, University Putra Malaysia, and the samples were all confirmed and identified as seen in the Appendix 1 for the certificate of confirmation of plant species. The identification of the ten herbs is summarised table 1, which shows the scientific name as well as the local name and the family name. As for *Frankincense*, it is a gum from the *Boswana* tree found in Southern Region of Oman and not found here in Malaysia.

Extraction percentage yield of the ten herbs

The ten herbs are all extracted with 99.9% of Methanol by ultrasonification and later evaporated by rotary evaporator. The calculation of the extraction yield is the weight percentage of the crude extract to the raw

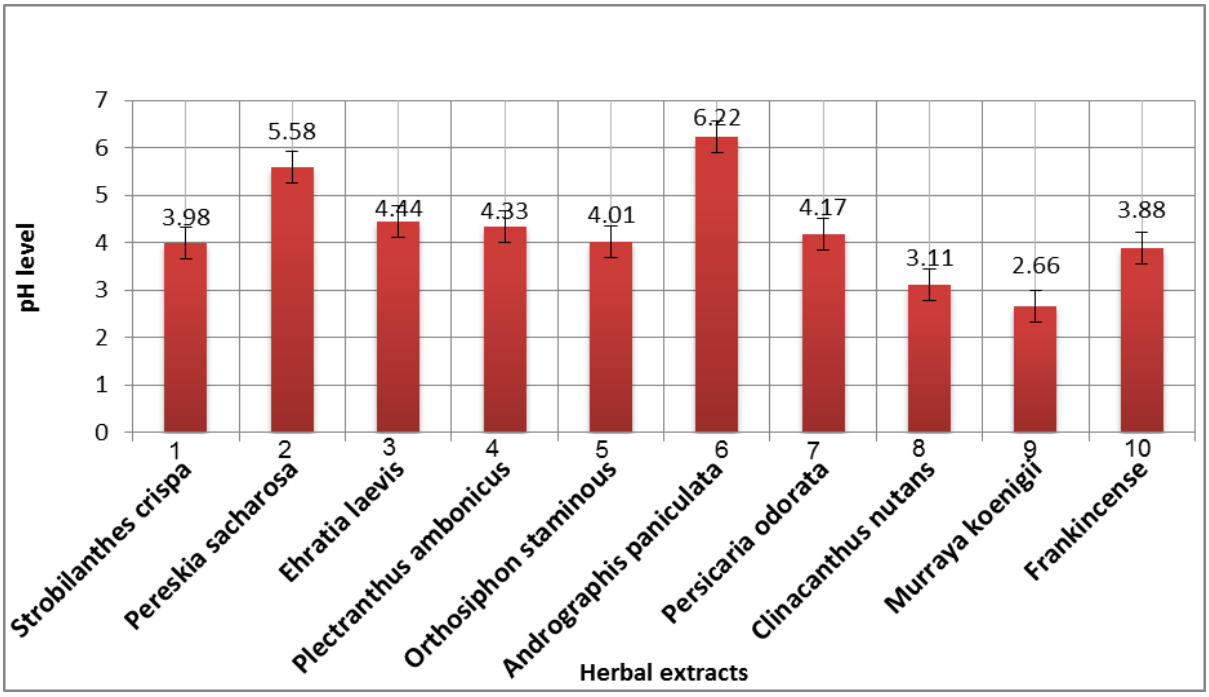


Figure 4: *pH* graph of the ten herbal extracts.

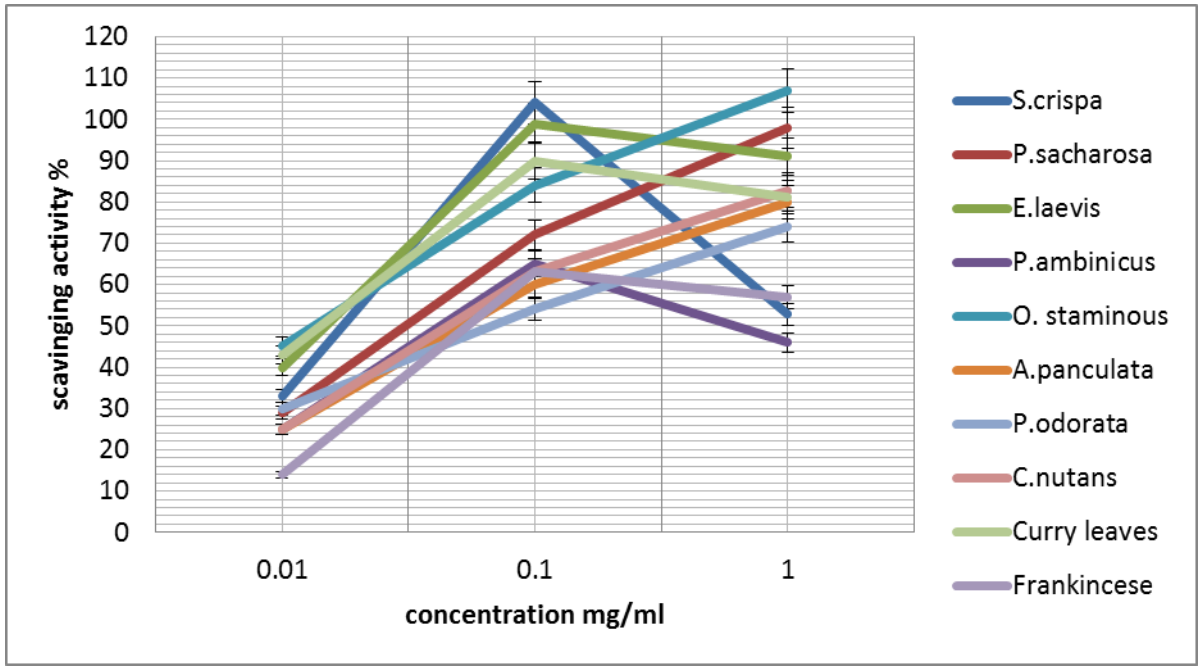


Figure 5: MIC_{50} antioxidant property graph of the ten herbal extracts

material. The percentage extraction yield was calculated as follows:

The percentage yield is calculated using the following formula :(Sasongko et al., 2001)

Percentage of extraction yield % = $\frac{Amount\ of\ extract\ yield\ (g)}{Amount\ of\ dried\ plants\ used\ (g)}$ X 100

The results were summarized in table 2,

Base on table 2, it is seen that the highest percentage yield is from the herb *Persicaria odorata*, with 12.96%, whereas the lowest is *Clinacantus nutans*, with only 1% extraction yield. As for *Frankincense* with the percentage of 90.19%, this is due to the property of gum and it is not considered as an herb. The differences in the amount and percentage of extraction yield can be explained due to the size of the particulars in the leaves. The leaves that have smaller particle size will yield more extracts compared to those with larger particle size, regardless of the extraction method used (Fonseca et al., 2006).

pH test result of the ten herbal extracts

pH is an indication of how many hydrogen ions forms in a certain volume of water. (Basic *pH* measurement). From figure 1, the *pH* graph shows that all the herbal extracts are below 5, which is in the acidic *pH* scale except for the herb number 6, (*A. panculata*). The lowest

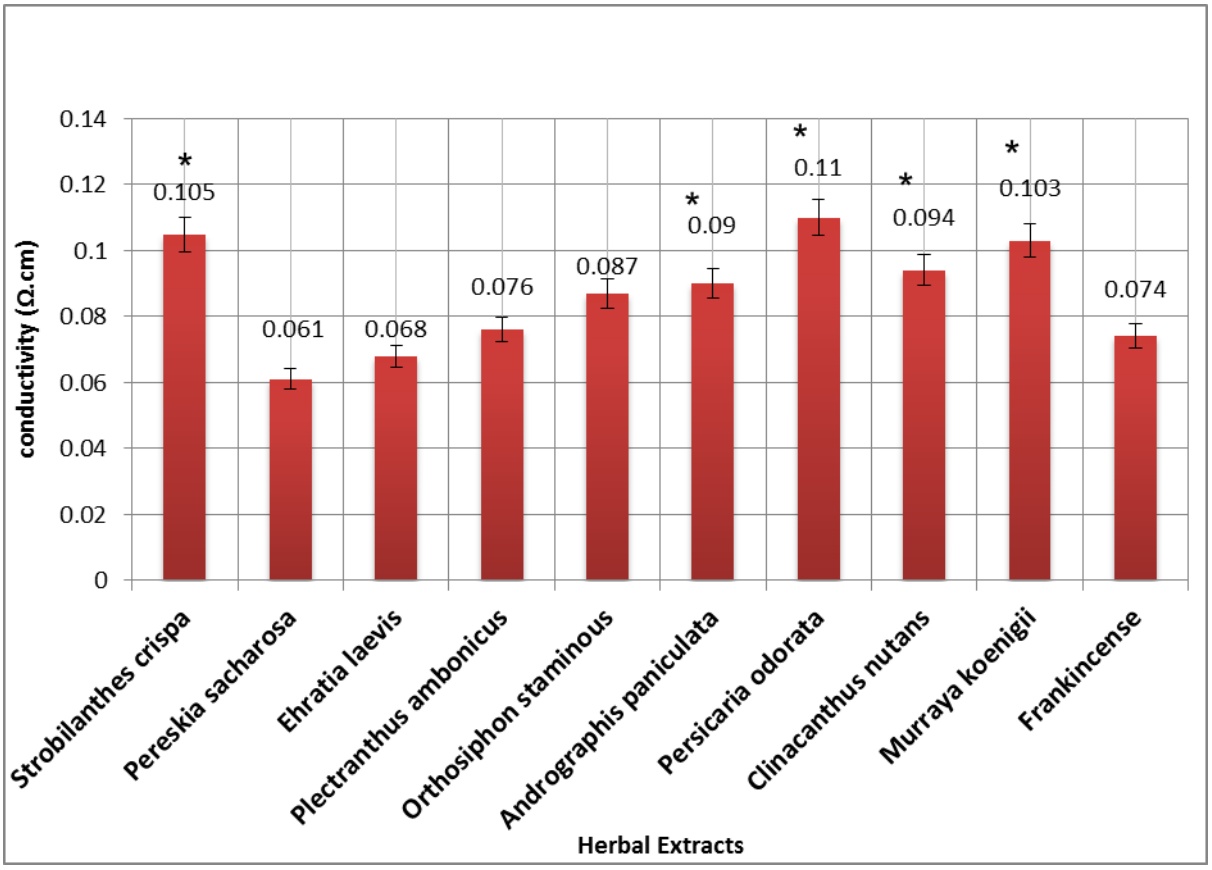


Figure 6, electrical current conductivity graph.

Table 8: results of the phytochemical element tests of the ten herbal extracts

S.No.	Herbs	Tannins and Phenols	Magnesium	Chloride	Sulphate	Nitrate
1	*Strobilanthes crispa*	+	+	+	-	+
2	*Pereskia sacharosa*	+	+	+	-	-
3	*Ehretia laevis*	+	+	+	+	+
4	*Plectranthus amboinicus*	+	-	+	-	-
5	*Orthosiphon staminous*	+	+	+	-	-
6	*Andrographis paniculata*	-	+	-	+	-
7	*Persicaria odorata*	+	-	-	+	+
8	*Clinacantus nutans*	-	-	+	+	+
9	*Murraya koenigii*	+	-	+	+	+
10	*Frankincense*	+	-	+	-	-

is *Curry leaves* with *pH* of 2.66 and the highest is *A.panculata* with *pH* of 6.22. This indicates that they contain hydrogen ions which are the characteristic of a good electrical current conductivity.

Antioxidant property using DPPH

The percentage of antioxidant property of the herbal extracts in three different concentrations is obtained, which from that the Minimal Inhibitory Concentration of herbal extracts by half amount (MIC_{50}) was achieved using a graph, (see fig2).The lowest minimal inhibitory concentration of the herbal extracts by 50% is considered to have the most antioxidant property. (Moleyneux, P. 2004). According to Moleyneux, P. 2004, from table 3, and in figure 2, showed that the MIC_{50} of the ten herbal extracts were all between the range 0.01- 0.08mg/ml. Overall all the herbal extracts show to have a good antioxidant property.

Electrical current conductivity of the ten herbal extracts

The electrical current conductivity was measured using the basic circuit with known voltage and current measured from the multimeter. Figure 3, shows the conductivity graph of the amount of electrical current conductivity of the ten herbal extracts. From the graph the herbal extract of *P. odorata* had the highest conductivity

amount 0.110Ω·cm compared to other ten herbal extracts. Whereas the lowest conductance was seen in the herbal extract of *P.sacharosa* 0.061Ω·cm. However all the extracts showed to have approximately electrical current conductivity ranging from 0.06 – 0.12Ω·cm.

Phytochemical element test of the ten herbal extracts

Phytochemicals are defined as bioactive non-nutrient plant compounds in plant foods that have been linked to reducing the risk of major chronic diseases (Doughar et al., 2009). Any herb consists of many phytochemical elements. These elements vary among each herb and have different roles and mechanism of action.

The types of phytochemical elements used are the inorganic elements which consist of mainly minerals. The tests are: Magnesium, Chloride, Sulphate, Nitrate, Tanins & Phenols. Phytochemical element tests were carried to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and coloration reaction to identify the major natural chemical groups.(Arora,2013) From table 4, it showed that the herbal extract of *E.laeves* contains all the five phytochemical elements that were tested. Whereas the herbal extract of *A.panculata* contains the least which is only Magnesium. However all the ten herbal extracts contained phytochemical elements.

CONCLUSION

As antioxidants play an important role in oxidizing the oxidative stress in our body cells caused by free radicals. It is appropriate to understand that electrical conductivity and if it could be used as a predictor for antioxidant property in the herbal extracts. Based on the results obtained, all the ten herbal extracts show to have good antioxidant property. The lowest MIC_{50} was seen in the herbal extract *E.laeves* and *O.staminous.* The *pH* of the herbal extracts was all in the acidic *pH* scale, which indicates the presence of hydrogen ions. As for the amount of electrical current conductivity in the herbal extracts, based on the results it shows that the herbal extracts contain electrical current conductivity ranging from 0.061 – 0.110Ω·cm. This is seen that the lowest electrical current conductivity from the herbal extract of *P.sacharosa* and the highest electrical current conductivity of about 0.116Ω·cm seen in the herbal extract of *P.odorata*. This indicates that a good antioxidant property contains a good amount of electrical current conductivity.

All the herbal extracts also show to have abundant amount of phytochemical elements which consists of Tannins & Phenols, Magnesium, Chloride, Sulphate, and Nitrate. Based on the results the herbal extract *E.laeves* contains all the phytochemical elements that were tested and the herbal extract *A.panculata* contains only one of the phytochemical elements.

As a conclusion it is found that there is a relationship between the antioxidant property and the electrical current conductivity of the herbal extracts.

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