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PHENOLIC CONTENT OF SOME SELECTED LAMIACEOUS EGYPTIAN MEDICINAL PLANTS: ANTIOXIDANT POTENTIAL AND ECOLOGICAL FRIEND MOSQUITO-LARVICIDAL

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Abstract

Phenol compounds are naturally occurring biologically active compounds existing in all plants had received major medical concern so development in research focused on their extraction, identification and quantification have occurred over the last 25 years; they constitute an important source of antioxidants and were used to help human body to reduce oxidative damage. Mosquitos-borne diseases constitute one of the major health problems worldwide. Control strategies involving pinpointing natural ecological friend, cheap and safe mosquitocides, mainly larvicides to stop their life cycle.

Quantitative estimation of total phenol, flavonoids, phenylethanoid and iridoid contents of sixteen selected Lamiaceous Egyptian plants for screening of their antioxidant and mosquito larvicidal effects was carried out. The results showed that the most suitable medicinal plants used as antioxidants were *Lavendula dentata* L., *Thymus capitatus* L. *and Thymus bovei* Benth., which contain adequate mixture of total phenol, flavonoid and phenylethanoid contents, with distinct larvicidal effect in a descending order was *T. capitatus* L.*, T. bovei* Benth. and *L. dentata* L. by their adequate mixture of total phenol, flavonoid, iridoid and phenylethanoid glycoside content.

Key words: Lamiaceae, Polyphenols; Flavonoid; Phenylethanoid; Iridoid; Antioxidant; *Anopheles*, Larvicides

Introduction

Labiatae family (Lamiaceae) is one of the largest most distinctive families of flowering plants, with about 220 genera and almost 4000 species worldwide, it has an almost cosmopolitan distribution (Naghibi *et al,* 2005); Lamiaceous plants were reported to contain unique phenol-rich fractions which may provide potential sources of natural antioxidants to be utilized in food and beverage products for inhibition of lipid oxidation (Abou Elfotoh *et al,* 2013; Proestos *et al,* 2013). Reactive oxygen species (ROS) such as •O2 (superoxide anion), H_2O_2 (hydrogen peroxide), and •OH (hydroxyl radical) are closely involved in various human diseases such as Alzheimer's disease, aging, cancer, inflammation, rheumatoid arthritis and atherosclerosis (Moein *et al,* 2008, El-Hela *et al,* 2013). Synthetic antioxidants as butyleted hydroxyanisole and butylated hydroxytoluene may be liver damaging and carcinogenic (Karsheva *et al,* 2013) so it is preferred to be replaced by natural ones.

On the other hand, mosquitoes top all arthropod-borne diseases (WHO 1996). The use of synthetic organic larvicides resulted in damage to the environment, pest resurgence and toxic effects on non-target organisms (El-Bahnasawy *et al,* 2013b), plant derived insecticides encompasses an array of chemical compounds thus, the chance of insects developing resistance to such insecticides are less and ideal safe ecological friend insect controllers (Ghosh *et al,* 2012).

The study aimed at the quantitative estimation of polyphenol compounds of sixteen selected Egyptian Lamiaceous plants and

screening for their antioxidant potential and methanol extracts were tested for Anopheline mosquito larvicidal efficacy.

Materials, Equipment and Methods

Leaves of *Ocimum basilicum* and *O. sanctum* were collected from the research Garden, Al-Azhar Faculty of Agriculture, *Lavendula dentata* L. and *Salvia officinalis* L. were purchased from an ornamental plant station, Kerdasa, Giza, *Lavendula angustifolia* Mill. and *Thymus vulgare* L. were purchased from the Medicinal Plants Station, Cairo Faculty of Pharmacy, *Mentha microcorphylla* were collected form Gharbia Governorate, while others were collected from Saint Catherine, South Sinai, at the flowering time and kindly identified by Dr. Moneer Abdel-Ghany Prof. of Plant Taxonomy, Cairo Faculty of Science.

All samples were air-dried, powdered and kept in clean tightly closed amber colored glass containers in a dark place at low temperature. Voucher specimens were kept in the Herbarium Museum, Department of Pharmacognosy, Al-Azhar Faculty of Pharmacy.

For total phenol content: Folin-Ciocalteu reagent (Sigma Chemical Co., St. LouisMO, USA) and Gallic acid (E. Merck, Darmstadt, Germany). For total flavonoid content: Quercetin and Aluminium chloride (Merck Co. Darmstadt, Germany. For total phenylethanoid glycosides: Arnow reagent (Dhar and Rosazza, 2000) and Verbascoside (Sigma-Aldrich Quimica South Madrid Spain). For total iridoids: Trim and Hill reagent (Trim and Hill 1952) and Herbagoside (Sigma-Aldrich Quimica South Madrid, Spain). For antioxidant effect: DPPH, Butylated hydroxyl toluene (BHT) (Sigma-Aldrich Quimica, South Madrid, Spain), Silica gel 60 F254 (Merck, Darmstadt, Germany) and mobile phase [butanol: acetic acid: water (40: 10: 50)]. For mosquito larvicidal effect eggs of *Anopheles* species were collected Al-Fayium Governorate water bodies, laboratory reared and tested as given by El-Hela *et al*. (2013).

Apparatus: Soxhlet, Chromatographic glass jars, Spectrophotometer (Perkin-Elmer Lambada 3) for quantitative determination of antioxidant effect, Rotatory evaporator (BUCHI Rotavapor® R-210/R-215, Germany), 96 Micro-well™ Plates, Conical Wells, Thermo Fisher Scientific USA, Genesys Spectrophotometer (Milton Roy, INC., Rochester, NY) for quantitative estimation of total phenolics, flavonoids, phenylethanoids and iridoids, Centrifuge.

Preparation of extracts: 50g of each dried powdered plant under investigation was extracted separately by soxhlet for 24hr with methanol. After filtration, extracts were concentrated under vacuum then washed within hexane until the chlorophyll was completely removed; the washed methanol extracts were filtered and used for study.

Determination of total phenolic compounds; Spectrophotometric assay using the Folin-Ciocalteu′s reagent which is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic compounds and polyphenol antioxidants (Abdel-Hady *et al,* 2011). Determination of total flavonoids: Colourimetric assay using aluminum chloride solution according to (Gorogawa *et al,* 2002, Abdel-Hady *et al,* 2011). Determination of total phenylethanoid glycosides: Colourimetric assay by using Arnow's reagent (1937), Abdel-Hady *et al.* (2011). Determination of the total iridoids; Colourimetrically assay using Trim and Hill reagent after Trim and Hill (1952) and Abdel-Hady *et al,* 2011.

Determination of antioxidant effect; Qualitatively and quantitatively using stable DPPH radical assay (Cavin *et al,* 1998; Gialvez *et al,* 2003; Abdel-Hady *et al,* 2011)

Results

The results are shown in tables 1 & 2.

Table; 1: Total phenol, flavonoid, phenylethanoid glycosides and iridoid contents of methanol extracts of selected Lamiaceous Egyptian medicinal plants.

No.	Medicinal Plants	Total Phenol Content (µg%)	Flavonoid Content (µg%)	Iridoid Content (µg%)	Phenylethanoid Content (µg%)
1	*Ballota undulata* (Fresen.) Benth.	86.29±2.15	86.18±1.50	----	218.30±2.66
2	*Lavendula angustifolia* Mill.	152.50±2.21	104.95±1.87	----	212.76±3.26
3	*Lavendula dentata* L.	167.10±2.30	116.15±1.91	----	178.45±2.89
4	*Mentha microcorphylla* Koch.	47.80±1.19	28.09±1.04	----	152.61±2.73
5	*Ocimum basilicum* L.	56.28±1.53	26.37±1.08	----	218.72±2.96
6	*Ocimum gratissimum* L.	54.96±2.83	27.95±1.17	----	212.80±3.02
7	*Ocimum sanctum* L.	42.56±1.91	22.45±1.18	----	216.50±2.99
8	*Origanum syriacum* L.	91.45±3.06	45.71±1.23	----	136.35±2.48
9	*Salvia deserti Decne.*	136.55±2.90	35.26±1.39	22.61±1.15	122.60±1.75
10	*Salvia langiera Poir.*	147.85±2.93	37.60±1.59	19.25±1.27	120.45±2.39
11	*Salvia officinalis* L.	156.72 ±3.05	35.01±1.22	24.30±1.25	136.48±2.41
12	*Teucrium decaisnei* C. Presl	76.35±2.39	85.70±1.80	10.46±0.72	184.37±1.92
13	*Teucrium leucocladum* Boiss.	74.24±2.30	88.45±1.75	13.36±0.87	167.20±1.85
14	*Thymus bovei Benth.*	105.20±2.06	122.90±2.16	95.14±2.70	150.50±1.92
15	*Thymus capitatus* L.	112.09±1.99	127.47±2.05	100.05±2.81	155.28±1.86
16	*Thymus vulgare* L.	121.35±2.18	115.11±2.27	127.55±2.54	167.33±1.95

Table, 2: Antioxidant potential and larvicidal activity of methanol extracts of Egyptian Lamiaceous plants:

No	Medicinal Plants	Antioxidant effect		Larvicidal effect
		EC_{50}(µg ml^{-1})	Scavenging%	LC_{50} (mg %)
1	*Ballota undulata* (Fresen.) Benth.	0.085±0.003	88.35±2.76	17.90±1.05
2	*Lavendula angustifolia* Mill.	0.094±0.001	89.15±2.78	17.25±1.27
3	*Lavendula dentate* L.	0.047±0.001	97.18±2.75	12.35±1.03
4	*Mentha microcorphylla* Koch.	0.092±0.09	82.57±2.20	23.60± 1.14
5	*Ocimum basilicum* L.	2.53±1.10	63.29±2.09	65.25±1.99
6	*Ocimum gratissimum* L.	2.84±0.85	62.35±1.86	69.20±2.30
7	*Ocimum sanctum* L.	2.18±0.07	62.10±2.01	64.20±1.50
8	*Origanum syriacum* L.	2.09±0.06	58.20±1.85	60.38±1.35
9	*Salvia deserti Decne.*	1.90±0.05	81.19±2.73	23.95±1.20
10	*Salvia langiera Poir.*	1.75±0.05	85.25±2.05	22.15±1.15
11	*Salvia officinalis* L.	1.64±0.06	86.25±2.13	20.02±1.06
12	*Teucrium decaisnei* C. Presl	2.02±0.05	57.40±1.79	60.25±1.33
13	*Teucrium leucocladum* Boiss.	1.92±0.04	84.50±2.18	21.30±1.09
14	*Thymus bovei Benth.*	0.058±0.001	92.48±2.90	10.60±1.10
15	*Thymus capitatus* L.	0.050±0.001	95.36±2.60	9.70±1.50
16	*Thymus vulgare* L.	1.65±0.05	87.40±2.15	18.29±1.20

M±SD of triplicate experiments, EC50 for Butyl hydroxyl toluene; antioxidant standard=0.054µgml-1

Discussion

Quantitative estimation of total phenol, flavonoid, iridoid and phenylethanoid content of selected Egyptian Lamiaceous plants proved that *L. dentata* L., *T. capitatus* L .and *T. bovei* Benth. exhibited the highest phenol and flavonoid contents with adequate phenylehenoid contents; adequate iridoid content existed in *T. capitatus and T. bovei* while *L. dentata* L., was proved to have no iridoid content, the greatest percent scavenging activity was recorded for the previous three plants respectively suggesting that the antioxidant effect is directly related to total phenol, flavonoid and phenylehenoid contents (Abdel-Hady *et al*, 2011, El-Hela *et al,* 2013).

The screening of larvicidal activity highlighted *T. capitatus, T. bovei* and *L. dentata* L. respectively where iridoid containing

plants *T. capitatus, T. bovei* exhibited greater potency suggesting that iridoid combination with other existing phenol compounds potentiate their larvicidal effect (El-Hela *et al,* 2013).

In this study, the anopheline larvae were selected due to the presence of a malaria focus in Al-Fayium (El-Bahnasawy *et al,* 2013a) and the reported of three *Anopheles* species in Toshka District bordering Sudan where the chloroquine-resistant malignant malaria is still a health problem there (El-Bahnasawy *et al,* 2011).

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