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ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS AND THE BIOLOGICAL EFFECTS OF SELECTED PLANTS ON STAPHYLOCOCCUS AUREUS

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ABSTRACT: *Several natural products especially secondary metabolites have formed the basis of medicines, although it's been often difficult to justify the presence of these compounds in the biochemistry of plant; this as posited by Cragg et al., in their presentation on Ethnobotany and the Search for New Drugs, Ciba Foundation Symposium draws a challenge to scientists. It has been suggested that these compounds may have been synthesized by the plants as part of the defense system of the plant; a good example is that plants are known to produce phytoalexins as a response to attack by bacteria and fungi. Cragg and Newmann in Biodiversity: A continuing source or novel drug leads, support this though stating that the compound produced provides an invaluable resource that has been used to find new drug molecules This paper is set to identify the antimicrobial activity of these medicinal plants and their biological effects. The sampled out plant extracts include Thymus vulgaris, Berberis vulgaris and Calluna vulgaris with which inhibition or activation by different chemical catalysts is performed to establish their effects in the tested natural extracts. Experimental design is used where the reagents are determined and chemical reactions performed in the procedures as outlined in the methodology section. The results of the microbial activity based on the level of concentration is then recorded for every crude extracts and the effects of each with different antibiotics against Staphylococcus aureus. This study reflects that berberine showed excellent synergistic activity against Staphylococcus aureus at relatively low concentration and thus can be used against staphylococcus infections.*

KEYWORDS: Antimicrobial, Thymus Vulgaris, Berberis Vulgaris, Calluna Vulgaris, Metabolites, Staphylococcus Aureus

INTRODUCTION

Medicinal Plants Antimicrobial Activity, Antidiabetic, Anticholinergic and Antioxidants Effects

Natural compounds may be feasible alternatives for the treatment of diabetes or reinforcements to currently used treatments. They may as well reduce the risk of the disease. Large amounts can be consumed in every day diet, which is a positive aspect. There are a large number of plants and natural biomolecules that have been discussed in various literatures for their anti-diabetic effects. Recently, more research is being focused on elucidating the action of plants and their active constituents with diabetes (Soumyanath, 2006). Plants contain a wide variety of free radicals scavenging molecules including phenols, flavonoids, vitamins and terpenoids that are rich in antioxidant activity (Madssen & Bertelsen, 1995). Many plants are the source of flavonoids, phenolics which posses ability to scavenge the free radicals in human body. Significant antioxidant properties have been recorded in phytochemicals that are necessary for the reductions in the occurrence of many diseases (Hertog & Feskens, 1993).

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There are a few synthetic medicines, e.g. tacrine, donepezil, and the natural product-base drivastigmine for treatment of cognitive dysfunction and memory loss associated with AD (Oh et al, 2004). These compounds have been reported to have their adverse effects including gastrointestinal disturbances and problems associated with bioavailability (Schulz, 2003) which necessitates the interest in fi2004) better AChE inhibitors from natural resources. Medicinal plants are potential antimicrobial crude drugs and are a source for natural compounds that are new anti-infection agents. The antimicrobial activity of plant extracts are found in essential oils, isolated compounds such as alkaloids, flavonoids, sesquiterpene lactones, diterpenes and triterpenes (Recio et al.,1989a). Review on the most relevant articles showed that phenolics are the predominant active chemicals in certain species of plants, with gram positive bacteria being the most sensible germs. Ngwendson et al. (2003) focused on determining the antimicrobial activity of plant extract found in folk medicine, examples are EO (Alma et al., 2003), alkaloids (Klausmeyer et al., 2004), flavonoids (Sohn et al., 2004), sesquiterpene (Lin et al., 2003), diterpenes (El-seedi et al., 2002) and triterpene (Katerere et al., 2003).To understand the activity of plants used in folk medicine in different parts of the world articles on microbial activity and medicinal plants have been compiled by Pubmed. Examples of such articles that have been published recently include study of medicinal plants from Brazil (Duarte et al., 2005), Thailand (Wannissorn et al., 2005**)** Ghana (Konning et al., 2004), India (Jeevan Ram et al., 2004), Uganda (Olila et al., 2001) and Lebanon (Barbour *et al.*, 2004). Specific studies of the activity of plant against concrete pathological microorganisms have been conducted. These studies focuses on activity against *Candida albicans* (Duaute et al., 2005), *Helicobacterpylori* (O'Gara et al., 2000), enterohaemorrhagic *Escherichia coli* (Voravuthikunchai et al., 2004), sexually transmitted diseases including *Naiserria gonorrhea* (Shokeen et al., 2005) and bacteria resistant to known antibiotic such as *Staphylococcus aureus* which is resistant to methicilin (Machado et al., 2003).

Mechanisms of Action of Bioactive Compounds

Two principle mechanisms of action have been proposed for natural antioxidants (Ingold, 1968). The first is a chain-breaking mechanism, by which the primary antioxidant donates an electron to the free radical present in the system (e.g. lipid radical). The second mechanism involves removal of ROS initiators (secondary antioxidants) by quenching chain-initiating catalysis. Alpha-glucosidase inhibitors act by delaying the liberation of D-glucose of oligosaccharides and disaccharides from dietary complex carbohydrates and retard glucose assimilation, reducing postprandial plasma glucose levels (Matsuura et al., 2004). The bioactive compounds may inhibit microorganisms, interfere with some metabolic processes or may modulate gene expression and signal transduction pathways (Kris-Etherton et al., 2002; Manson 2003). Bioactive compounds and essential oils may exhibit different modes of action against bacterial strains, such as interference with the phospholipids bilayer of the cell membrane which has as a consequence permeability. Increase and loss of cellular constituents, damage of the enzymes involved in the production of cellular energy and synthesis of structural components, and destruction or inactivation of genetic material. Generally mechanism of action against bacteria is considered to be the disturbance of the cytoplasm membrane, disrupting the proton motive force, electron flow, active transport and coagulation of cell contents (Kotzekidou et al., 2008).

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METHODOLOGY

Biochemical Assay

Determination of inhibitory/stimulatory effect of tested extracts on liver thioberbutaric acid reactive species (TBARS) induction

Principal

Malondialdehyde (MDA), the byproduct of L.P.O forms adduct with TBA. On boiling produces pink colored complex.

Reagents

- Trichloroacetic acid (TCA), 15%
- Thiobarbituric acid (TBA), 0.7%
- Ferrous sulphat heptahydrated, 0.5mM
- Butylated Hydroxy Toluene (BHT), 0.5%

Procedure

2 mL of each extract (test), the organic solvent (control) or distilled water (ddH_2O) (blank) were incubated with equal volume of liver homogenate for about 45 min at 37°C. *In vitro* tissue lipid peroxidation was induced by adding H_2O_2 and ferrous sulphate ($FeSO4{\cdot}7H_2O$) at a final concentration of 1 and 0.5 mM, respectively, in both test and control reaction mixtures. After an incubation period of about 30 min at 37°C, BHT at a final concentration of 0.02% was added and mixed carefully to stop the peroxidation reaction. The mixtures were centrifuged at 3000 rpm for 15 min, and then 1 mL of the resultant supernatant was mixed with 1 mL of TCA (15%) followed by centrifugation at 3000 rpm for 10 min.

TBARS was determined in liver, according to the method described by (Tappel & Zalkin, 1959). 1mL of supernatant was mixed with 500 µL TBA (0.7%), heated in boiling water bath for 45 min, cooled and the color in the supernatant diminished at 532 nm. The TBARS level was calculated against control without the extract according to the following equation: TBARS level (nmol/ml) = At / 0.156.

Determination of inhibitory/stimulatory effect of tested extracts on beta glucosidase activity

Principal

þ-nitro phenyl- $^{\beta}$- ^{D}-glucosidase (PNPG) in the presence of beta glucosidase enzyme yields ^{P}-nitro phenol (PNP) and D-glucose.

Reagents

- þ-nitro phenyl- $^{\beta}$- ^{D}-glucopyranoside (PNPG)

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- 0.1M Phosphate buffer pH 7.4
- Sodium carbonate

Procedure

Method mentioned by (Han & Srinivasan, 1969) was carried out .100µl of the extract (test), organic solvent (control) or distill water (blank) were diluted with 2.5mL 0.1M phosphate buffer pH 7.4 ,then 100µl of liver homogenate were added, mixed well and incubated in water bath with the reaction mixture at 30C° for 5 min. Then 500µl of the substrate (PNPG), 5mM, were added and the reaction was allowed to proceed for 15 min. The reaction was stopped by addition of 2mL of 1M sodium carbonate. The produced color adduct was spectrophotometrically detected at 400 nm.

Determination of inhibitory/stimulatory effect of tested extracts on acetylcholine esterase (AChE) activity

Principal

Acetyl cholinesterase (AChE) catalyzes the hydrolysis of acetylthiocholine (ATCh) upon hydrolysis, this substrate analog produces into acetate and thiocholine. Thiocholine, in the presence of the highly reactive dithiobisnitro-benzoate (DTNB) ion generates a yellow color, which is visible and can be quantitatively monitored by spectrophotometric absorption at 405 nm.

Reagents

- Dithiobis 2-nitrobenzoic acid (DTNB)
- Phosphate buffer (0.1M pH 7.4)
- Acetylthiocholin iodide (ACTI)-substrate

Procedure

AChE activity was measured according to the method of Ell man *et al*, (1961), 150 µl of phosphate buffer (0.1M,pH 8) were added to a mixture of 60µl of liver homogenate and 20µl of the extracts (test), or organic solvent (control) then incubated for 45 min at 37°C.Five micro liter of substrate ACTI(75mM) were added, mixed well and incubated for 15min at 37°C, 60µl of DTNB(0.32mM) were added and left for 5 min to stop the reaction. The absorbance was measured against the blank at 405 nm and specific AChE activity was calculated as follows:

The values obtained were analyzed; blank reading was subtracted from sample readings

Specific enzyme activity = [A] x [Total volume in cuvette in µL] / [Molar extinction coefficient of DTNB] x [Volume of liver homogenate in µL] x [Protein concentration in mg/ mL]

Where, specific activity = moles of substrate hydrolyzed / min / mg of proteinA = absorbance Molar extinction of DTNB = 1.36 x 104.

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RESULTS

The antioxidants or pro-oxidants effects of tested natural fractions (TBARS % inhibition or activation)

The antioxidant potential of the three medicinal fractions were evaluated through the TBARS assay based on formation of MDA, a sub product of lipid peroxidation. Table 1 clearly shows that all the fractions significantly decreased the TBARS formation at $p<0.05$. *Berberis vulgaris* extracts showed the highest inhibitory activity. The PEG fraction of *Berberis vulgaris* had the highest antioxidant by 87.6% while the 20 % ethanol fraction of *Thymus vulgaris* had the lowest antioxidant effect by 2.5%.

Table 1. The percentage inhibition or activation on TBARS level in the presence of tested natural fractions

Crude Extract	Solvent	% of inhibition/ activation± SD
Thymus vulgaris	water	-37.78±11.15
	PEG	-31.66±07.87
	20% ethanol	-2.55±00.88
	95% ethanol	-16.37±02.45
Berberis vulgaris	water	-64.89±12.99
	PEG	-87.62±05.65
	20% ethanol	-64.28±08.03
	95% ethanol	-63.82±11.03
Calluna vulgaris	water	-22.70±12.54
	PEG	-27.87±15.87
	20% ethanol	-42.68±13.41
	95% ethanol	-30.15±10.34

Values represent the mean of three replicates ± standard deviation

Negative sign (-) indicates inhibition

The cholinergic/ anti-cholinergic effect of tested natural extracts

Most of the tested fractions extracts showed potent inhibitory effect toward AChE except PEG and 95% ethanol fractions of *Thymus vulgaris* that showed stimulatory effect by 19% and 11.5% respectively at $p<0.05$. The highest inhibitory effect was shown in PEG fraction of *Berberis vulgaris* by 32% whereas water fraction of *Calluna vulgaris* showed the lowest effect by 2% at $p<0.05$.

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Table 2. The percentage inhibition or activation on acetylcholine esterase (AChE) in the presence of tested natural fractions

Extract	Solvent	% activation / inhibition ±SD
Thymus vulgaris	water	-13.20±4.79
	PEG	+19.00±3.90
	20% ethanol	-12.84±5.00
	95% ethanol	+11.50±4.80
Berberis vulgaris	water	-16.60±1.80
	PEG	-31.83±4.20
	20% ethanol	-30.35±1.80
	95% ethanol	-4.25±1.24
Calluna vulgaris	water	-2.02±3.41
	PEG	-20.22±8.34
	20% ethanol	-15.87±4.83
	95% ethanol	-13.93±4.70

Values represent the mean of three replicates ± standard deviation

Negative sign (-) indicates inhibition, Positive sign (+) indicate activation

The diabetic/ anti-diabetic effect of tested natural extracts

All the fractions showed significant enzyme inhibitory activity against α-glucosidase enzyme at $p<0.05$. *Berberis vulgaris* had the highest inhibitory activity in water fraction by 95.8% whereas 95% ethanol fraction of *Calluna vulgaris* had the lowest inhibitory effect by 6.9%.

Table 3. The percentage inhibition or activation on diabetic/anti-diabetic effect of tested natural extracts

Extract	Solvent	% Concentration±SD
Thymus vulgaris	water	-26.36±7.02
	PEG	-16.59±6.01
	20% ethanol	-36.01±14.1
	95% ethanol	-34.71±15.9
Berberis vulgaris	water	-95.83±10.47
	PEG	-12.15±0.42
	20% ethanol	-29.50±7.05
	95% ethanol	-81.71±39.10
Calluna vulgaris	water	-38.19±13.52
	PEG	-20.08±25.39
	20% ethanol	-73.96±7.46
	95% ethanol	-6.91±4.3

Values represent the mean of three replicates ± standard deviation

Negative sign (-) indicates inhibition

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Single and combined activity of alkaloid fraction from *Thymus vulgaris* and *Berberis vulgaris* with different antibiotics against *Staphylococcus aureus*

At a higher concentration tested 5µg alkaloid, fraction of *Berberis* alone and in combination with Amp and Km showed maximum zones of inhibition compared to T*hymus* alkaloid fraction in respective order: Single; *Berberis* (15.3±1.25mm); *Thymus (*13.7±1.25mm) Combined : *Berberis* (Amp+Alk 31.0±0.82mm) ; km+Alk (34.0±0.8mm). *Thymus* (Km+Alk 32.3±1.25mm; Amp+alk (28.3±3.40mm).

Table 4. Effect of alkaloid fraction and antimicrobial drugs against *Staphylococcus aureus*

Agent tested	Concentration of Alkaloid			
	Thymus vulgaris		*Berberis vulgaris*	
	2 µg	5 µg	2 µg	5 µg
Alkaloid fraction	12.7±1.25	13.7±1.25	9.0±0.82	15.3±1.25
Amp.	18.7±1.25	36.7±1.25	16.0±0.82	38.0±0.82
Km	25.0±0.82	34.0±2.16	20.0±0.82	37.7±1.70
Amp. +Alk.	13.0±0.82	28.3±3.40	13.7±2.05	31.0±0.82
Km+Alk.	14.7±1.25	32.3±1.25	11.7±1.70	34.0±0.82

Km=Kanamycin; (Amp) =ampicillin; (Alk) =Alkaloid

Single and combined activity of flavonoid fraction from Thymus vulgaris and Berberis vulgaris with different antibiotics against Staphylococcus aureus

At a higher concentration tested 5 µg flavonoid, fraction of both *Thymus and Berberis* alone and in combination with Amp and Km exhibited weak antimicrobial activity. Inhibition zones were as follows: Single: *Thymus* (12.7±1.25mm), *Berberis* (11.3±1.70mm).Combined; *Thymus* (Amp+Fla(13.7±1.70mm);
Km+Flav(14.0±0.82mm);*Berberis*(Amp+flav15.0±0.82mm;Km+Flav14. 7±1.70mm).

Table 5. Effect of flavonoid fraction and antimicrobial drugs against *Staphylococcus aureus*

Agent tested	Concentration of Flavonoid			
	Thymus vulgaris		*Berberis vulgaris*	
	2 µg	5 µg	2 µg	5 µg
Flavonoid fraction	11.0±0.82	12.7±1.25	9.7±1.25	11.3±1.70
Amp.	15.0±0.82	23.3±1.70	16.7±1.70	25.0±3.27
Km	17.7±1.25	32.0±0.82	14.0±0.82	23.3±2.87
Amp. +Flav.	10.3±1.70	13.7±1.70	11.7±1.70	15.0±0.82
Km. +Flav.	9.0±0.82	14.0±0.82	12.7±1.70	14. 7±1.70

Km=Kanamycin; (Amp) =ampicillin ;(Flav) =flavonoid

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Single and combined activity of carbohydrate fraction from Thymus vulgaris and Berberis vulgaris with different antibiotics against Staphylococcus aureus

At a higher concentration tested 5 µg carbohydrates, fraction of *Berberis* and *Thymus* alone and in combination with Amp and Km showed almost the same zones ofinhibition. Single: *Thymus (*10.0±0.82mm), *Berberis (*13.7±0.47mm).Combined: Thymus (Amp+Carboh.) 13.7±2.05mm Km+Carboh.14.0±0.82mm); *Berberis* (Amp+car13.7±0.47mm; Km + Carboh.15.0±0.82mm).

Table 6. Effect of carbohydrate fraction and antimicrobial drugs against *Staphylococcus aureus*

Agent tested	Concentration of Carbohydrate			
	Thymus vulgaris		*Berberis vulgaris*	
	2 µg	**5 µg**	**2 µg**	**5 µg**
Carbohydrate fraction	8.0±0.82	10.0±0.82	10.3±1.25	13.7±0.47
Amp.	15.0±0.82	17.0±0.82	19.3±1.70	23.7±1.70
Km.	15.3±1.25	19.7±0.94	23.0±0.82	24.0±0.82
Amp. +Carboh.	8.0±0.82	13.7±2.05	12.3±1.25	13.7±0.47
Km+Carboh.	7.7±0.47	14.0±0.82	14.7±0.47	15.0±0.82

Km=Kanamycin; (Amp) = ampicillin ;(Carboh.) = carbohydrates

Single and combined activity of berberine with different antibiotics against Staphylococcus aureus

Berberine fraction showed highly potent zones of inhibition both singly and in combination of antibiotics at the highest concentration tested 5µg compared to the control single: **(**18.7±1.25mm); combination: (Amp+Berberin (34.7±1.25); Km+Berberin (36.3±1.25mm).

Table 7. Effect of berberine and antimicrobial drugs against *Staphylococcus aureus*

Agent tested	Berberine	
	2 µg	**5 µg/disc**
Berberin fraction	15.0±0.82	18.7±1.25
Amp.	29.3±3.30	40.0±0.82
Km	29.0±0.82	40.0±0.82
Amp. +Berberin	17.7±1.70	34.7±1.25
Km+Berberin	21.3±2.05	36.3±1.25

Km=Kanamycin; (Amp) =ampicillin

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DISCUSSION

Cholinergic and Anti-Cholinergic Effect

The fractions of *Thymus vulgaris,calluna vulgaris and Berberis vulgaris* were evaluated for their effect on two important enzymes AChE (E.C.3.1.1.7) and α- glucosidase (E.C.3.2.1.20). The first enzyme is important for breaking acetylcholine (see Table 2) which is a substrate of this enzyme that acts on a neurotransmitter in both the peripheral nervous system and central nervous system (Perry et al., 2002). Since AD, one of the most common cause of death worldwide, has become a threat to public health, new treatment strategies based on medicinal plants have been focused (Howes et al., 2003; Orhan et al., 2004). In abnormal activation of AChE, acetylcholine will degrade rapidly, especially in the brain and this is associated with Alzheimer's disease (AD). In this study, it was found that most of the extracts fraction significantly inhibited the activity of AChE enzyme especially *Berberis vulgaris* which showed the highest inhibitory effect. These data is in agreement with that of Ghareeb et al., (2010). It has been suggested that, the main functional components found in *Berberis* extract are alkaloids such as berberine which is the main active ingredient of *Berberis vulgaris* (Gorval & Grishkovets, 1999), therefore the compound might have a therapeutic potential for treatment of Alzheimer's disease. Studies concerning the AChE inhibitory activity and chemical composition of commercial essential oils of *Thymus vulgaris* performed by Dohi et al. (2009) demonstrated for the first time that eugenol was a potent AChE inhibitor.

Recently, a study concerning the AChE inhibitory of *Thymus vulgaris e*ssential oil was reported. Such results proved the diversity of results depending on the chemical composition (Ruberto & Baratta, 2002). Contrary to this, some fractions of *Thymus vulgaris* showed stimulatory effect of the enzyme, this effect is in agreement with other researchers who have reported its use in memory improvement that is treatment of hyperactive disorder, therefore the compound might have a therapeutic potential for treatment and other diseases involving AChE hyperactivity, where it was stated (Asai et al., 2007).

AChE inhibitors are general chemical classes, such as rivastigmine, tacrine and donepezil (Aricept®), and have been tested for symptomatic treatment of AD (Becker et al., 1998). However, the non-selectivity of those drugs, and their limited efficacy due to their short half lives, poor bioavailability, nausea, vomiting, diarrhea and hepatotoxicity are some of the severe limitations to their therapeutic success (Bores et al., 1996).

There have been a number of reports on the designing and development of synthetic AChE inhibitors (Catto et al., 2006), which were necessary for other studies on AChE inhibitors derived from medicinal plants (Markmee et al., 2006). 'From our results and pervious group published data we recommended that *Berberis vulgaris* extract being one of the best AD treatment medicinal plant which could be used for treatment purposes'.

Diabetic and Anti-Diabetic Effect

Diabetes management can be done by using different strategies. Proper management requires control in preprandial and postprandial hyperglycemia. α- glucosidase enzymes (EC 3.2.1.20), located in the brush-border surface membrane of intestinal cells, catalyze then hydrolysis of the α-glycosidic bond of oligosaccharides to liberate the monosaccharide units from dietary sources (Gao et al., 2008). Hence, α-glucosidase inhibitors can delay the liberation of D-glucoseof oligosaccharides and disaccharides from dietary complex carbohydrates and retard

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glucose assimilation, reducing postprandialplasma glucose levels (Matsuura et al., 2004). The inhibitors delay, but do not prevent, the absorption of ingested carbohydrates,reducing the postprandial glucose and insulin peaks (Andrade-Cetto et al., 2008). Therefore, it is a method of choice to control elevated glucose level in blood to treat diabetes and prevent other cardiovascular complications (Matsuura et al., 2004). Its inhibitors are used as drugs for treatment of diabetis mellitus type 2 because they will decrease glucose reflux into the blood.

In this paper, all the fractions of *Thymus vulgaris,Calluna vulgaris* and *Berberis vulgaris* showed significant enzyme inhibitory activity against α- glucosidase enzyme (see Table 3). The results also showed that the *Berberis vulgaris* fraction possesess significantly higher inhibitory activity and was most potent among the the other fractions simply because it is known to posess diverse pharmacological properties. This results are in accordance with Yanxia et al. (1995) who researched on use of *Berberis vulgaris* for diabetes and he found out that berberine had the therapeutic effect in hyperglycemia condition. Rao and Muralikrishna, (2000) and Friedmann (1977) showed that phenolic compounds of *Calluna vulgaris* have antiglycemic properties, this results are in agreement with the same findings of the previous study which showed inhibitory effect of α- glucosidase. Morimitsu **(**1995) showed that methanoic extract of *Thymus vulgaris* that contained high flavanoid content inhibited the non enzymatic glycation of bovin serum albumin,thus levels of advanced glycation end products were surpressed,hence showing its potency in treatment of diabetis. The results are in accordance with previous reported works that polyphenolic compounds, especially flavonoids, are among the classes of compounds that have received the most attention (Soumyanath, 2006) with regard to their antidiabetic properties.

Antioxidant and Pro-oxidant Effect

Antioxidants protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage (Mattson & Cheng, 2006), diabetic pregnancy, rheumatic disorder, DNA damage and ageing . Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischema, carcinogenesis, neurodegenerative disorders **(**Jayasri et al., 2009). Antioxidants exert their activity by scavenging the 'free-oxygen radicals' thereby giving rise to a fairly 'stable radical'. Observation of earlier studies by Chen et al. (2006) and Uddin et al. (2008) highlighted that free radicals are involved in a number of diseases including: tumour inflammation, hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases (e.g. parkinsonism, Alzheimer's diseases), AIDS and even early senescence.

In this study therefore, the antioxidant potential of the three plant fractions were evaluated through the TBARS assay based on the formation of MDA, a sub-product of lipid peroxidation. The lipid peroxidation was stimulated with FeSO4 addition to liver homogenate, and the extracts were able to significantly decrease the MDA formation at all concentrations tested as can be observed in (see Table 1). Similarly, all the fractions decreased the TBARS level, but the decrease was significant in case of *Berberis* extract where it exhibited the highest concentration. In this respect, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Kahkonen et al., 1999). All these phytochemical constituents would act in synergy in order to increase *Berberis* bioactivity such as antioxidant activity. In

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vitro and in vivo studies demonstrated by (Dussossoy et al., 2011) indicates that most compounds identified in *Calluna vulgaris* extract, had important antioxidant activity due to high amount of phenolic compounds which is in accordance with the results of the present study which showed heather as an effective antioxidant due to presence of phenolic compounds too. Reports by other authors indicate that heather contains phytochemicals such as caratenoids and flavanoids which are beneficial for protecting the skin from free radicles. On the other hand *Thymus vulgaris* showed moderate capacity for scavenging radicals, the effect is in agreement with the researcher Ruberto and Baratta, (2002) who found out that antioxidant activity of *Thymus vulgaris* may be explained by the presence of phenolic compounds (thymol and carvocrol) with known antioxidant activity.

Significant antioxidant properties have been recorded in phytochemicals that are necessary for the reduction in the occurrence of many diseases (Hertog & Feskens, 1993; Anderson & Teuber, 2001).

CONCLUSION

All the three medicinal plants fractions tested revealed the presence of phytochemical components, alkaloids, flavonoids, proteins, amino acids and total phenols. According to this study, biochemical effect results showed that, all the fractions of *Thymus vulgaris, Berberis vulgaris* and *Calluna vulgaris* showed significant inhibitory effect activity against α-glucosidase enzyme.*Berberis vulgaris* had the highest inhibitory effect while *Calluna vulgaris* showed a minimal effect. Most of the tested fractions showed inhibitory effect toward AChE except some fractions of *Thymus vulgaris* that had stimulatory effect. The highest inhibitory effect was shown in *Berberis vulgaris* fraction whereas *Calluna vulgaris* effect was minimal. All the fractions of *Thymus vulgaris, Calluna vulgaris* and *Berberis vulgaris* significantly decreased TBARS formation.*Berberis vulgaris* fraction exhibiting the highest inhibitory activity while *Thymus vulgaris* inhibited moderate activity.

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