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RESEARCH ARTICLE

Assessment of Antioxidant, Anti-Inflammatory, Anti-Cholinesterase and Cytotoxic Activities of Tulsi (*Ocimum Sanctum*) Leaves

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ABSTRACT

This study evaluated antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of extracts with different polarities (hexane, dichloromethane, ethyl acetate, ethanol and methanol) obtained from *Ocimum sanctum* leaves. Total phenolics (8.8–127.3 mg gallic acid equivalent/g dry weight), flavonoids (1.2–76.9 mg quercetin equivalent/g dry weight), tannins (63.7–260.8 mg catechin equivalent/kg dry weight) and anthocyanins (0.41–3.73 mg cyanidin-3-glucoside equivalent/g dry weight) of different extracts were evaluated. 5-lipoxygenase (5-LOX), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition activities were obtained for the ethanol extract (IC_{50} values of 6.20, 14.83 and 2.65 mg/l, respectively) and the best cytotoxic activity against MCF-7 cells was obtained for the methanol extract (IC_{50} = 31 mg/l). These important biological activities showed that *Ocimum sanctum* leaves could be a potential source of the active molecules intended for applications in pharmaceutical industry, but only after additional in vivo experiments.

KEYWORDS

Ocimum sanctum, Anti-inflammatory, Antioxidant, Cytotoxic, Cholinesterase, Extraction

INTRODUCTION

Oxidative stress is defined as the imbalance between pro-oxidants and antioxidants, in favour of the former. Numerous studies have shown that the oxidative stress contributes significantly to the development and the progression of wide variety of diseases such as diabetes reported that free radicals are likely an important factor in the pathogenesis of Alzheimer Disease (AD). The brains of AD patients present several signs of free radical attacks such lipid peroxidation, protein oxidation, damage to mitochondrial and nuclear DNA.

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Also, the crucial role of superoxide production in the pathogenesis of diabetes was pointed out[7]. New clinical and epidemiological data consider the oxidative stress as an important determinant in the development of chronic inflammation and cancer through activation of variety of transcriptional factors (Reuter et al., 2010; Toullec et al., 2010) that antioxidants play a key role in protecting against oxidative damage. Thus, antioxidants may be present in fruit and vegetable were well recommended to reduce cardiovascular and neurodegenerative risks (McCall et al., 2011). These positive health effects were attributed to a variety of compounds such as phenolics and terpenoids. In recent years, considerable attention has been directed to study these active compounds from medicinal plants for various pharmacological

purposes (Dorman et al., 2004). The different parts of Tulsi (*Ocimum sanctum*) have been known as a reservoir of bioactive compounds with potential biological activities. Tulsi, especially the leaves of Tulsi, decreased the dyslipidemia of obesity and cardiovascular risk factors (Lei et al., 2007). Anti-parasitic, anti-microbial and antioxidant activities of Tulsi leaves extracts were reported (Egharevba et al., 2010; El-Shennawy et al., 2010 and Wang et al., 2013). Several papers were reported on the ability of Tulsi leaves extracts to fight obesity (Al-Muammar and Fozia Khan, 2012), cancer and other human diseases (Lansky and Newman, 2007). Phenolics in Tulsi leaves are thought to contribute in their health benefits (Lan et al., 2009). The objectives of this study were to determine the quantification of the main classes of polyphenolic compounds of Tulsi leaves extracts prepared with different polarity solvents and to compare antioxidant, anti-cholinesterase, anti-inflammatory and cytotoxic activities of different extracts.

MATERIALS AND METHOD

Chemicals Used

All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma, Aldrich, Fluka (Saint-Quentin France).

Plant Sample

The leaves of *Ocimum sanctum*, belonging to Lamiaceae family, were collected in February 2013.

Preparation of the Extracts

Air-dried Tulsi leaves were ground to fine powder and successively extracted with solvents of increasing polarity (hexane, dichloromethane, ethyl acetate, ethanol and then methanol). Thus, 10 g of leaves powder were placed in hexane (100 ml) for 16 h under frequent agitation at ambient pressure and temperature. The mixture was filtered using Whatman Filter Paper (GF/A, 110 mm) and the experiment was repeated twice. The solvent was evaporated using a rotary evaporator under vacuum at 35 °C. Then, the firstly extracted powder was extracted again with dichloromethane under the same conditions as with hexane. The same procedure was applied for the following solvents: ethyl acetate, ethanol and methanol. Extracts were kept in amber vials and stored at 4 °C for further analysis.

Quantification of Total Phenolic Content

The total phenolics of each extract were determined by the Folin–Ciocalteu method (Georgé et al., 2005). Briefly, the diluted solution of each extract (0.5 ml) was mixed with Folin–Ciocalteu reagent (0.2 N, 2.5 ml), rested at room temperature for 5 min and then sodium carbonate solution (75 g in water, 2 ml) was added. After 1 h of incubation, the absorbance was measured at 765 nm against water blank. A standard calibration curve was plotted using gallic acid (0–300 mg/L). Results were expressed as mg of gallic acid equivalents (GAE) per g of dw.

Quantification of Total Flavonoids Content

The total flavonoids content were estimated according to the Arvouet-Grand et al. method (1994) using a microplate reader. To 96 well plates, 100 µl of each variety extract was mixed with a solution (100 µl) of aluminium trichloride ($AlCl_3$) in methanol (2%). The absorbance of the mixture was measured at 510 nm against a reagent blank of methanol (100 µl) and plant extract (100 µl) without $AlCl_3$. Different concentrations of quercetin solution were used for calibrations and results were expressed as mg of quercetin equivalents (QE) per g of dw.

Quantification of Total Condensed Tannin Content

The condensed tannin content of tulsi flower extract was determined by the vanillin method (Naczk et al., 2000) with modifications: 50 µl of extract solution was mixed with 150 µl of vanillin (1% in 7 M H_2SO_4) in an ice bath and then incubated at 25 °C. After 15 min, the absorbance of the solution was read at 500 nm. Results were expressed as mg catechin equivalents (CE) per g of dw from a calibration curve.

Quantification of Total Anthocyanins Content

Total anthocyanin contents were determined by a pH differential method (Cheng and Breen, 1991) using two buffers: hydrochloric acid–potassium chloride (pH 1.0, 0.2 M) and acetic acid-sodium acetate (pH 4.5, 1 M) using 96-well plates. 20 µl of Tulsi extract was mixed with 180 µl of corresponding buffers and the absorbance was measured at 510 and 700 nm after 15 min of incubation. Absorbance (A) was calculated using $A = [(A_{510} - A_{700})$ pH 1.0 - $(A_{510} - A_{700})$ pH 4.5] using a molar extinction coefficient of 29600. The final results were expressed as mg cyanidin-3-glucoside equivalent (C3GE) per g of dw.

Determination of DPPH Radical Scavenging Activity

Antioxidant scavenging activity was studied using 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by Blois (1958) with some modifications: 20 µl of various dilutions of the test materials (pure antioxidants or plant extracts) were mixed with 180 µl of a 0.2 mM methanolic DPPH solution. After an incubation period of 30 min at 25 °C, the absorbance at 520 nm and the wavelength of maximum absorbance of DPPH, were recorded as A (sample). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as A (blank). The free radical-scavenging activity of each solution was then calculated as percentage of inhibition according to the following equation: % inhibition ¼ 100 A (blank) – A (sample).

Extracts' antioxidant activity was expressed as IC_{50}, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. Quercetin was used as a standard. All measurements were performed in triplicate.

Determination of ABTS Radical-Scavenging Activity

The radical scavenging capacity of the samples for 2, 20-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS) radical cation was determined as described by Re et al. (1999). ABTS was generated by mixing a 7 mM stock solution of ABTS at pH 7.4 (5 mM NaH_2PO_4, 5 mM Na_2HPO_4 and 154 mM NaCl) with 2.5 mM potassium persulfate (final concentration) followed by storage in the dark at room temperature for 16 h before use. The mixture was diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm using spectrophotometer. For each sample, diluted methanol solution of the sample (20 µl) was allowed to react with fresh ABTS solution (180 µl), and then the absorbance was measured 6 min after initial mixing. Quercetin was used as a standard and the capacity of free radical scavenging was expressed by IC_{50} (mg/l) values calculated denote the concentration required to scavenge 50% of ABTS radicals. The capacity of free radical scavenging IC_{50} was determined using the same previously described equation for the DPPH method. All measurements were performed in triplicate.

Anti-Inflammatory Activity

The anti-inflammatory activity of Tulsi leaves was determined on Soybean lipoxygenase as described by Bylac and Racine (2003) with modifications. Various concentrations of 20 µl of tulsi leaf extracts was mixed individually with sodium phosphate buffer (pH 7.4) containing 5-LOX and 60 µl of linoleic acid (3.5 mM), yielding a final volume of 1 ml. However the blank does not contain the substrate, but will be added 30 µl of buffer solution. All extracts were re-suspended in the DMSO followed by dilution in the buffer so that the DMSO does not exceed 1%. The mixture was incubated at 25 °C for 10 min, and the absorbance was determined at 234 nm. The absorption change with the conversion of linoleic acid to 13-hydroperoxyoctadeca-9, 11-dienoate (characterized by the appearance of the conjugated diene at 234 nm) was flowed for 10 min at 25 °C. Nordihydroguaiaretic acid (NDGA) was used as positive control. The percentage of enzyme activity was plotted against concentration of the leaf extract. The

IC_{50} value is the concentration of the flower extract that caused 50% enzyme inhibition.

Anti-Cholinesterase Activity

Cholinesterase (ChE) inhibitory activities were measured using Ellman's Method, as previously reported (Akkol et al., 2012) with modifications. In this study, 50 µl of 0.1 M sodium phosphate buffer (pH 8.0), 25 µl of AChE (or BuChE) solution, 25 µl of leaf extract and 125 µl of DTNB were added in a 96-well microplate and incubated for 15 min at 25 °C. All extracts were re-suspended in the DMSO followed by dilution in the buffer so that the DMSO does not exceed 1%. The reaction was then initiated with the addition of 25 µl of acetylthiocholine iodide (or butyrylthiocholine chloride). The hydrolysis of acetylthiocholine iodide (or butyrylthiocholine chloride) was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm. The concentration of the extracts which caused 50% inhibition of the AChE (or BuChE) activity (IC_{50}) was calculated by nonlinear regression analysis. The percentage of inhibition was calculated from (1-S/E)-100, where E and S were the respective enzyme activities without and with the test sample, respectively. Galanthamine was used as positive control.

Cytotoxicity Evaluation

Cytotoxicity of sample was estimated on human breast cancer cells (MCF-7) as described by Natarajan et al. (2011) with modification. Cells were distributed in 96-well plates at 3104 cells/well in 100 µl, and then 100 µl of culture medium containing sample at various concentrations were added. Cell growth was estimated by The MTT (3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay. MTT is a yellow water-soluble tetrazolium salt. Metabolically active cells are able to convert the dye to water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring. All extracts were re-suspended in the DMSO followed by dilution in the buffer so that the DMSO does not exceed 1%. Doxorubicin was used as positive control.

RESULTS AND DISCUSSION

Preliminary Phytochemical Study

Extraction Yields

In this study, different solvents of increasing polarity were used: hexane, dichloromethane, ethyl acetate, ethanol and methanol (Fig. 1). The yields of polar fractions (ethanol and methanol) of *Ocimum sanctum* leaves are more important than the non-polar fractions (hexane and dichloromethane). The highest extraction yield was obtained with the methanolic extract (35.04%) of the plant material whereas the lowest was recorded with the ethyl acetate extract (0.78%). We suggested that the variation in the yields of different extracts can be attributed to the polarities of the different compounds in the Tulsi leaves. Kaneria et al. (2012) extracted successively leaves of Indian Tulsi, collected in August 2010, using different solvents. Similar to our findings, they found that the extraction yield from Tulsi leaves dependents on the solvent: petroleum ether (1%), toluene (1.5%), ethyl acetate (2%), acetone (6%) and was higher in water (16%). These values are lower than those reported in our study. Several reports indicated that the extraction yield depends on solvents, time and temperature of extraction as well as the chemical nature of the sample. Under the same time and temperature conditions, the solvent used and the chemical property of sample are the two most important factors (Baravalia et al., 2009). We tried to detect the presence of alkaloids qualitatively and all the samples do not contain this family.

Total Phenolics Content

Total phenolics content of Tulsi leaves extracts was depicted in Fig 1. and varied from 8.8 ± 0.3 to 127.3 ± 1.6 mg GAE/g dw. Ethyl acetate extract contained the high value of total phenolics (127.3 ± 1.6 mg GAE/g dw), followed by methanol (85.2 ± 2.4 mg GAE/g dw) and ethanol extracts (82.6 ± 1.5 mg GAE/g dw). The lowest values were 9.9 ± 0.6 and 8.8 ± 0.3 mg GAE/g dw in dichloromethane and hexane extracts, respectively. Polar extracts had more

total phenolics in them than non-polar extracts. Total phenolics determined in this study were lower than those reported by Kaneria et al. (2012). They indicated that extraction solvent had an influence on total phenolics content of *Ocimum sanctum* leaves grown in India but the hierarchy was acetone (400 mg GAE/g dw) > water (230 mg GAE/g dw) > ethyl acetate (220 mg GAE/g dw) > toluene (25 mg GAE/g dw).

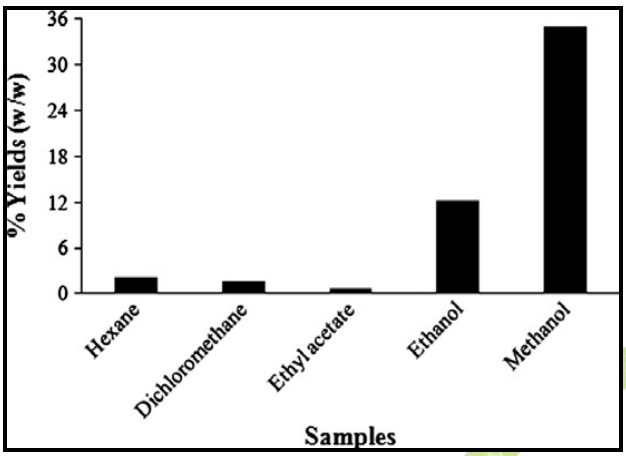

Figure 1: Yeilds of polar fractions (ethanol & methanol) of *Ocimum sanctum* leaves are more important than the non-polar fractions of (hexane & dichloromethane)

Such difference may be because of difference in the order of solvents used. Quantitatively, total phenolics (313 ± 6.4 GAE/g dw) presented in Tulsi leaves in our study are greater than those reported by Zhang et al. (2011) in 70% ethanol extract of leaves of Chinese *Ocimum sanctum* (289.76 ± 1.55 mg GAE/g dw). In addition, in previous study, Tulsi leaves of Gabsi variety harvest in October 2010 were individually extracted by maceration method using two solvents (water and methanol) (Elfalleh et al., 2012). However, in our work, successive extraction using different solvents of increasing polarity were used: hexane, dichloromethane, ethyl acetate, ethanol and methanol. We outlined that the methanol extract in our study contained higher amounts of total phenolics than that obtained by Elfalleh et al. (2012) (14.78 ± 2.10 mg GAE/g dw). Such difference may be due to harvest time. Thus, Zhang et al. (2010) indicated that total phenolics level was highest in April (237.4 mg/g dw). It decreased in the early stages of leaf growth and then increased gradually until the end of September. They reported that Tulsi leaves harvest in May 2007 contain approximately 210 mg/g dw of total phenolics which was lower than that determined in this study.

Flavonoids Content

Results showed that the total flavonoids content varied strongly with the solvent used (Fig 1). The ethanol extract of Tulsi leaves can be consider advantageous since it contains the highest flavonoids level (76.9 ± 2.45 mg QE/g dw). Methanol and ethyl acetate extracts showed the lowest values with 4.5 ± 0.17 and 1.2 ± 0.05 mg QE/g dw, respectively. Dichloromethane and hexane contained no flavonoids. Therefore, polar extracts had more total flavonoids content than non-polar extracts. In the same way, Kaneria et al. (2012) found that flavonoids content of Tulsi leaves dependents on the solvent used however the hierarchy was acetone (30 mg QE/g dw) > ethyl acetate (20 mg QE/g dw) > water (10 mg QE/g dw) > toluene (5 mg QE/g dw). These values are less important than those determined in our study. We outlined that the ethanolic extract contains higher amounts of flavonoids than that obtained by Elfalleh et al. (2012). They reported 26.08 ± 1.24 mg rutin equivalents/g dw in methanolic extract of leaves from Gabsi variety. Such difference may be due to harvest time (Zhang et al., 2010).

Tannins Content

The distribution of total tannins content in the extracts showed that it was concentrated in hexane extract of Tulsi leaves (260.8 ± 11.5 mg CE/kg dw) (Fig 1). The ethyl acetate extract was found to contain high concentration (82.7 ± 4.3 mg CE/kg dw), followed by dichloromethane extract (63.7 ± 1.7 mg CE/kg dw). Ethanol and methanol contained no tannins. We reported that non-polar extracts contains more tannins than polar extracts. In previous study, the amount of hydrolysable tannins reported in Fig. 1. Extraction yields (%) of *Ocimum santum* leaves extracts. methanolic extract was 128.02 ± 4.49

mg tannic acid equivalent/g dw (Elfalleh et al., 2012).

Anti-Oxidant Activity

In this work, we investigated antioxidant activity (DPPH and ABTS assays) of five extracts prepared from *Ocimum santum* leaves.

DPPH Assay

The antioxidant activity of the methanolic extract was superior to that of all extracts tested with the lowest IC_{50} value of 5.62 ± 0.23 mg/l required to scavenge 50% of DPPH radical, followed by the ethanolic extract (9.25 ± 0.72 mg/l) (Fig. 2). We can deduce also that the methanolic extract showed an antioxidant activity comparable with that of quercetin (2.86 ± 0.09 mg/l). Lower antioxidant activity was obtained for methanolic leaves extract of Gabsi variety (11.44 ± 1.04 mg/l) by Elfalleh et al. (2012). This difference may be due to the content of total phenolics and the harvest time. Thus, previous studies reported that the antioxidant activity of tulsi leaves strongly dependents on the total phenolics and flavonoids and the harvest time (Zhang et al. 2010). Dichloromethane extract showed lower antioxidant activity (IC_{50} = 71.57 ± 3.65 mg/l). However, the extract obtained with hexane exerted the lowest DPPH activity with IC_{50} value of 263.44 ± 12.72 mg/l. Our results showed that antioxidant propriety varied according to solvent extraction and the hierarchy was methanol > ethanol > ethyl acetate > dichloromethane > hexane. We outlined that when polarity increases the antioxidant activity of *Ocimum santum* leaves increased. With change in solvent polarity its ability to dissolve a selected group of antioxidant compounds alters and influences the antioxidant activity estimation. Antioxidant activity of our extracts was higher than that reported by Kaneria et al. (2012). Thus, the acetone extract (IC_{50} = 17 mg/l) of *Ocimum santum* leaves exhibited the best free radical scavenging activity, followed by water (IC_{50} = 42 mg/l), ethyl acetate (IC_{50} = 42 mg/l) and toluene extracts (IC_{50} = 215 mg/l). We noticed that this antioxidant activity is less important than that of Tulsi leaf extracts in the current study.

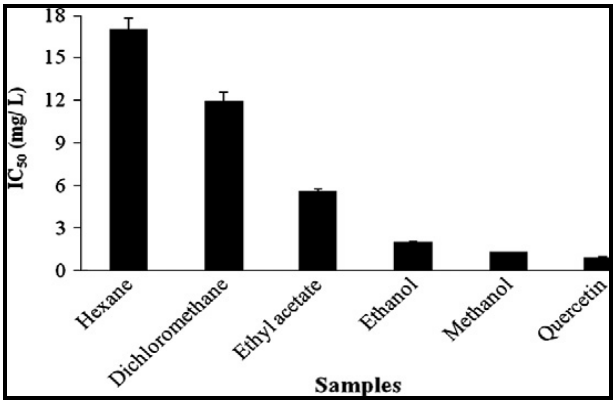

Figure 2: Anti-inflammatory activity of Tulsi leaves varied according to the solvent concentration

ABTS Assay

A high antioxidant activity was presented by the methanolic extract (IC_{50} = 1.31 ± 0.00 mg/l), followed by the ethanolic extract (IC_{50} = 2.01 ± 0.04 mg/l) (Fig. 3). The ABTS activity of the methanol and ethanol extracts are comparable to quercetin (IC_{50} = 0.93 ± 0.03 mg/l). Lower antioxidant activity was obtained for ethyl acetate (IC_{50} = 5.65 ± 0.11 mg/l), dichloromethane (IC_{50} = 11.95 ± 0.61 mg/l) and hexane extracts (IC_{50} = 17.08 ± 0.77 mg/l). We noted that when polarity increases, the antioxidant activity of *Ocimum santum* leaves increased.

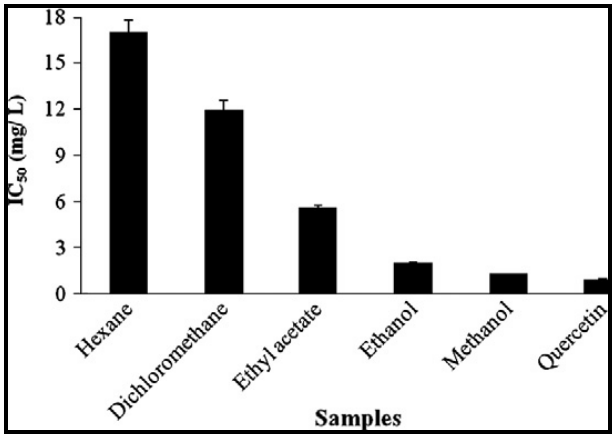

Figure 3: A high anti-oxidant activity was presented by the methanolic extract

Thus, the best antioxidant activities correspond to the polar fractions (methanol and ethanol).

We can deduce also that ABTS assay presents more activity when we compare the results of ABTS assay to those of the DPPH one. According to the results found, the methanol and ethanol extracts exhibited an antioxidant activity higher than that reported in the literature (Zhang et al., 2010) and the component(s) responsible (s) on this activity is (are) unidentified. Thus, the IC_{50} values are encouraging enough to identify the molecules responsible for this activity.

Anti-Inflammatory Activity

According to our knowledge, no studies on 5-LOX inhibition activity of Tulsi leaves have been reported up to date. We outlined that the anti-inflammatory activity of Tulsi leaves varied according to the solvent extraction (Fig 2). Results showed that ethanol and methanol extracts, with IC_{50} values of 6.20 ± 0.17 and 6.83 ± 0.37 mg/l, exhibited the strongest anti-inflammatory activity. These values are comparable with that of the reference drug, NDGA (7.00 ± 0.22 mg/l). Ethyl acetate extract showed also good anti-inflammatory activity (IC_{50} = 16.57 ± 0.50 mg/l). On the other hand, none of dichloromethane and hexane extracts were active (IC_{50} > 200 mg/l). It might be interpreted that the greatest anti-inflammatory activity was exerted by the best antioxidants extracts, which contain the high amounts of total phenolics. Thus, according to the literature, among LOX inhibitors are phenolics. Thus, caffeic acid with an IC_{50} equal to 46 lM (8.28 mg/l) and p-coumaric acid with an IC_{50} value of 2.5 lM (0.41 mg/l) inhibited strongly 5-LOX (Voss et al., 1992). Also, quercetin showed an important anti-LOX activity and exhibited an IC_{50} equal to 25 lM (7.55 mg/l), obtained by Prasad et al. (2004). It is important to note that ethanol and methanol extracts showed anti-inflammatory activity higher than that of caffeic acid and quercetin and this result is encouraging to prompt us to try to identify the molecules responsible for this activity.

Anti-Cholinesterase Activity

Cholinesterase activity of Tulsi leaves extracts was tested against AChE and BuChE (Fig 2). To the best of our knowledge, this is the first report on the anti-cholinesterase activity of Tulsi leaves using AChE and BuChE enzymes. The ethanol extract exhibit a good anti-AChE with an IC_{50} value of 14.83 ± 0.73 mg/l as compared to the reference drug Galanthamine (0.45 ± 0.03 mg/l). Ethyl acetate extract inhibit moderately AChE activity (IC_{50} = 46.00 ± 3.26 mg/l). None of dichloromethane, methanolic and hexane extracts was active (IC_{50} > 250 mg/l). It has been noted that ethanol extract of Tulsi leaves inhibit strongly BuChE. IC_{50} value was found to be 2.65 ± 0.21 mg/l, which is better than that of the reference drug Galanthamine (3.74 ± 0.28 mg/l). The ethyl acetate, dichloromethane, hexane and methanol extracts were found to be inactive against BuChE (IC_{50} > 250 mg/l). The high cholinesterase inhibitory activity of ethanol extract may be resulting from the high amount of flavonoids. Further work is needed to purify the ethanol extract that gave a high anti-cholinesterase activity to identify the molecule(s) responsible for this activity.

Cytotoxic Activity

Cytotoxic activity of the Tulsi leaves extracts against breast cancer cell line MCF-7 was assessed using MTT assay, which is reliable to detect proliferation of cells. In this study, the activity of tulsi leaves against breast cancer cell line MCF-7 varied markedly with solvent of extraction (Fig 2). Among five extracts tested, three Tulsi leaves extracts exhibited promising activity with IC_{50} values of less than 50 mg/l. The methanolic extract presented the best cytotoxic effect with an IC50 value of 31.00 ± 1.02 mg/l, followed by the ethanolic extract (IC_{50} = 33.50 ± 0.54 - mg/l) and ethyl acetate extract (IC_{50} = 45.00 ± 2.34 mg/l). Hexane extract was poor active (IC_{50} value > 100 mg/l). Thus, polar extracts were more cytotoxic than ethyl acetate and dichloromethane ones. The values of cytotoxic activity of methanolic, ethanolic and ethyl acetate are just good but not very high ones compared with the standard substance (Doxorubicin). The cytotoxic activity of these extracts could be the result of a synergistic action of all or some components present in these extracts. We suggested that the

high amounts of total phenolics in polar extracts predominantly contributed to their cytotoxic activity. Prakash and Premkumar (2012) evaluated the cytotoxic activity (MCF7) of three extracts, prepared not successively (ethanol, acetone and ethyl acetate). All extracts were poor active (IC_{50} value > 100 mg/l).

CONCLUSION

Ocimum santum leaves were investigated for their chemical composition. Total phenols, flavonoids, tannins and anthocyanins contents of different extracts were determined. We can conclude that the methanolic extract with the highest antioxidant activity (but only after the confirmation of their good effects on health of experimental animals in investigations, in vivo) could be considered as natural source of strongly antioxidant substances for the use as a natural additive in food and pharmaceutical industries. To the best of our knowledge, we report the first study on 5-Lox, AChE and BuChE inhibitory of *Ocimum santum* leaves. Nevertheless, further work is in progress to identify the components present especially in methanolic and ethanolic extracts responsible for antioxidant, anti-inflammatory, anti-cholinesterase and anti-proliferative activities.

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