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CHEMICAL COMPOSITION AND IN-VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF ESSENTIAL OIL FROM FRESH GRASS OF CYMBOPOGANTRA VANCORENSIS

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Abstract-In the present study, the chemical composition of the essential oil from Cymbopogantravancorensis and its efficacy for its antioxidant and anti-inflammatory activities are reported. The essential oil from fresh grass of Cymbopogantravancorensis (Poaceae), obtained by hydrodistillation method was analysed by Gas Chromatography Mass Spectrometry (GC-MS). Totally sixty nine components, representing more than 98.22 % of the oil with an yield of 2.0 % (v/w) were identified. From this oil 13 components were identified by GC-MS studies. Tricyclene, α-Pinene, Camphene , dl-Limonene , β-Ocimene , Camphor, (-)-Borneol 2-(3-Methoxyphenoxy) propanoicacid, Fenchyl alcohol , Borneol,acetate , α-Cubebene, Elemicin and Caryophyllene oxide are the major constituents of the essential oil extracted from the aerial (grass) part of the plant. The essential oil sample when screened for its possible antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), Hydrogen peroxide (H_2O_2), Reducing power, Nitric oxide (NO) methods showed significant inhibition efficiency. The oil when tested for its anti-inflammatory activity by Human Red Blood Cell membrane stabilization (HRBC) method showed higher inhibition efficiency than standard diclofenac sodium at all the test doses.

Keywords: Hydrodistillation, Essential oil, Cymbopogantravancorensis, Antioxidant and anti-inflammatory.

1. INTRODUCTION

Plants have been the basis of traditional medicines throughout the world for thousands of years and they continue to provide new remedies to humankind; a great deal of effort has therefore been focused on using available experimental techniques to identify biologically active phytoconstituents in plants.

Essential oils are extracted from various parts of plants such as roots, leaves, seeds, barks and flowers. Recent research showed that approximately 3000 plant species contain essential oils, out of which nearly 300 are commercially important [1]. Essential oils and some of their constituents are used in pharmaceutical products, agriculture, in cosmetics and perfumes and other industrial fields [1]. Essential oil can be extracted by a number of methods by using liquid carbon dioxide or microwaves or by distillation employing boiling water or hot steam. As essential oil possesses bactericidal and fungicidal properties, they are useful in pharmaceutical and food industries and more and more widespread as alternatives to synthetic chemical products to protect the ecological equilibrium. Essential oils are extracted by steam distillation, expression, extraction with lipophilic solvents and sometimes with supercritical carbon dioxide and in the extraction of essential oil, factors like the number of extracted molecules and their stereochemical types, the need based type of extraction chosen play a predominant role. The quality and quantity as well as the composition of the oil extracted depend on factors like climate, soil composition, plant organ, age and vegetative cycle stage [2, 3].

Essential oils are moreover used in massages as mixtures with vegetal oil or in baths but most frequently in aromatherapy. Some essential oils appear to exhibit particular medicinal properties that have been claimed to cure one or another organ dysfunction or systemic disorder [4, 5]. Essential oils have been reported to exhibit antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory, insecticidal and antiviral activities [6, 7]. Some constituents of essential oils have also been shown to exert both in-vitro and in-vivo anti-tumour activity against murine leukaemia, hepatoma, human breast adenocarcinoma cell lines, colon, neuroblastoma and melanoma cells. Further, the chemotherapeutic value of some essential oil components against cancer cells is reflected by the fact that they have reached Phase I clinical trials in the past [8].

Antioxidants present in oils are important in the stabilization of free fatty acids [9]. The antioxidant activity of phenols and other compounds present has been well and widely studied by several authors [10, 11]. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), tocopherol (vitamin E), glutathione, carotenoids and flavonoids). This imbalance leads to cell damage [12, 13] and subsequently to health problems [14, 15]

Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease. However, synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely used as antioxidants in food industry and may be responsible for liver damage and carcinogenesis [16, 17] and for such reasons interest in the use of natural antioxidants has been found increasing.

Inflammation is caused by a variety of stimuli including physical damage, ultra violet irradiation, microbial invasion, and immune reactions. The classical key features of inflammation are redness, warmth, swelling, and pain. Inflammation can lead to the development of diseases such as chronic asthma, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis and psoriasis. It is a complex process, which is frequently associated with pain and involves occurrences such as increase in vascular permeability, increase of protein denaturation and membrane alterations [18]. The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes [19, 20].

Most clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation-related diseases. Though these have potent activity, long-term administration is required for treatment of chronic diseases. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally originated agents with very little side effects are desirable to substitute chemical therapeutics [21].

The genus Cymbopogan belonging to the Poaceae family includes 11 species. Cymbopogantravancorensislinn, commonly known as Chukkunari-pillu in Tamil, has large thorny shrubs, growing up to 2.5 meters in height. Grass are arranged as simple, alternate, oblong, entire or crenulated.

Earlier studies on Cymbopogantravancorensis report the presence of three limonoids in the root bark of the petroleum ether extract [22] and the methanolic extract of the plant showed mosquitocidal effects [23], Hexane, chloroform and ethyl acetate extracts of Cymbopogantravancorensis grass have also been studied for their ovicidal activity [24]. No previous study on the chemical composition as well as biological activity of essential oil from Cymbopogantravancorensis from fruits is reported and hence the present investigation has been carried out to identify the chemical composition and also the in-vitro antioxidant efficiency of the essential oil by DPPH, H_2O_2 scavenging, Reducing power assay, NO radical scavenging methods and in-vitro anti-inflammatory activity assay by Human Red Blood Cell stabilisation method.

2. RESEARCH ELABORATIONS

2.1 Chemical and Reagents

2,2-Diphenyl-1-picrylhydrazyl hydrazyl radical (DPPH), anhydrous sodium sulphate, ascorbic acid, Diclofanac sodium, H_2O_2, potassium ferricyanide, Trichloroacetic acid, sodium nitro prusside and Griess reagent were purchased from Sigma-Alrich Chemicals Private Limited.

2.2 Plant Material

Cymbopogantravancorensis fresh grass were collected from Periyamalai Hills, Sankari Taluk Salem district of Tamil Nadu, in September 2015. The plant was identified by Mr. R. Kottaimuthu, Department of Biology, Gandhigram Rural Institute - Deemed University, Gandhigram and a voucher specimen of the sample is preserved in the Department of Chemistry, GRI for future reference.

2.3 Sample Preparation

Essential oil was obtained by hydrodistillation of 1kg fresh grass of Cymbopogantravancorensis in a Cleyenger apparatus for 5 hours. The oily layer separated from the aqueous phase by using a separating funnel was dried over anhydrous sodium sulphate and stored in an amber coloured bottle at 4 ˚C for analysis.

2.4 Chemical Investigation

The quantitative and qualitative analysis of essential oil isolated was carried out by using gas chromatography and gas chromatography-mass spectroscopy (GC-MS).

2.5 Gas Chromatographic Analysis (Gc/Ms) of the Oil

The GC-MS analysis of the essential oil was performed on an Agilent GC 6890N model gas chromatograph-5973N model mass spectrometer equipped with a 7683B series auto-injector (Agilent, USA). The GC was equipped with a HP-5MS Capillary Column (30 m x 0.25 mm x 0.25 µm film thickness). The column temperature program began at 40 ºC and was held for 3 min. It was increased at a rate of 10 ºC min^{-1} to 150 ºC and held for 8 min and then increased at a rate of 10 ºC min^{-1} to 280 ºC and held for 15 min. Injection volume was 1.0 ML and inlet pressure was 7.06 psi. Helium was used as the carrier gas and linear velocity (u) was 36 cm/sec. Injection mode was split (30:1) and MS interface temperature was 250 °C. Mass spectra were recorded in the scan mode at energy was 70 eV and MS spectra were scanned from 50 to 550 m/z at 2.2 scan s^{-1}. Compound identification was based on the comparison of retention indices using a MS library. The NIST 05a and Wiley7a spectrometer data bank was used to determine the percentage composition of the compounds [25].

2.6 Preparation Of Oil Sample For Anti-Oxidant Activity Study

2.6.1 DPPH Radical Scavenging Assay

DPPH solution (0.3 mM) in absolute ethanol was prepared and 1 ml of this solution was added to 3 ml of 0.15 % of the oil sample dissolved in ethanol at different concentrations (10, 20, 30, 40 and 50 µg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance at 517 nm was measured using a spectrophotometer [26]. The percentage scavenging activity at different concentrations was determined and compared with that of ascorbic acid as the standard. Percentage of inhibition (I %) was calculated by using the formula,

$$\text{Percentage of inhibition (I \%)} = [A_c - A_s] / A_c \times 100$$

where A_c is the absorbance of the control, A_s is the absorbance of the sample solution.

2.6.2 Hydrogen Peroxide Scavenging Assay

A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Stock solutions, each containing 1 mg/ml of the sample were prepared [27]. Different amounts (10, 20, 30, 40 and 50 µg/ml) of the stock solutions taken in different test tubes were added 1 ml of H_2O_2 solution (40 mM) and the volume was adjusted to 25 ml with phosphate buffer solution (PBS) and all the reaction mixtures were incubated at 230 nm. A blank solution was prepared in the same way without the sample. The percentage of H_2O_2 scavenging ability was calculated for the oil sample and the positive control by using the equation

Percentage of inhibition (I %) = $[A_c-A_s] / A_c$ X 100

where A_c is the absorbance of the control, A_s is the absorbance of the sample solution.

2.6.3 Reducing Power Assay

The reducing power of the essential oil was determined according to the method suggested by Yen and Chen, 1995 [28]. The essential oil samples of various concentrations (10, 20, 30, 40 and 50 µg/ ml) in absolute ethanol were mixed individually with 2.5 ml of PBS (0.2 M, pH 6.7) and 2.5 ml of potassium ferricyanide (1 %, w/v). The mixture was incubated at 50 ^{0}C for 20 min. Trichloroacetic acid (2 ml of 10 %, w/v) was added to the reaction mixture which was then centrifuged for 15 min. The upper layer of solution (2.5 ml) was mixed with pure water (2.5 ml) and ferric chloride (0.5 ml, 0.1 %, w/v), and the absorbance was measured at 700 nm. The percentage inhibition was calculated by using the formula

Percentage of inhibition (I %) = $[A_c-A_s] / A_c$ X 100

where A_c is the absorbance of the control, A_s is the absorbance of the sample solution.

2.6.4 Nitric Oxide Radical Scavenging Activity Assay

A stock solution of the essential oil sample was prepared [29] to contain 1 mg/ml of it and different amounts (10, 20, 30, 40 and 50 µg/ml) of the stock solution were transferred to different test tubes and the volume was adjusted to 25 ml by the same solvent in each case. 0.2 ml of sodium nitro prusside (20 mM) in 1.8 ml of PBS (pH 7.4) was added and the mixture was incubated at 37 ^{o}C for 3 hrs. 1 ml of each solution was diluted with 1 ml of Griess reagent (1 % sulfanilamide, 2 % H_3PO_4, 0.1 % N-1-naphthyl ethylenediamine). Similarly a blank was performed containing an equivalent amount of reagents but without the test samples and ascorbic acid was used as a positive control. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. The percentage of inhibition was calculated using the formula

Percentage of inhibition (I %) = $[A_c-A_s] / A_c$ X 100

where A_c is the absorbance of the control, A_s is the absorbance of the sample solution.

2.7 IN-VITRO ANTI-INFLAMMATORY ACTIVITY BY HUMAN RED BLOOD CELL MEMBRANE STABILIZATION (HRBC) METHOD

The HRBC membrane stabilization has been used as a method to study the anti-inflammatory activity [30]. Blood collected from healthy volunteers was mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05 % citric acid and 0.42 % sodium chloride in water). It was centrifuged at 3000 rpm and the packed cells were washed with isosaline (0.85 %, pH 7.2) and a 10 % v/v suspension was made with isosaline. The assay mixture contained the drug at various concentrations (50, 100, 150,200 and 250 µg/ml), 1 ml phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36 %) and 0.5 ml of HRBC suspension. Diclofenac sodium was used as the standard drug. Instead of hyposaline, 2 ml of distilled water was used in the control. All the assay mixtures were incubated at 37 °c for 30 min and centrifuged. The hemoglobin content in the supernatant solutions was estimated at 560 nm using a spectrophotometer and the percentage of inhibition was calculated from HRBC membrane stabilization by using the formula

Percentage of Inhibition = $100 \times [OD_{control} - OD_{sample} / OD_{control}]$

where, OD sample is optical density of the oil sample and OD control is that of the control.

3. RESULTS

3.1 Chemical Composition Of The Essential Oil

The volatile constituents identified in the oil from fresh grass of Cymbopogantravancorensis are listed in Table 1. Hydrodistillation of fresh grass of Cymbopogantravancorensis afforded yellowish oil with pleasant aroma. These components are 2.0 % (v/w). From this oil 13 components were identified by GC-MS studies. Tricyclene, α-Pinene, Camphene , dl-Limonene , β-Ocimene , Camphor , (-)-Borneol, 2-(3-Methoxyphenoxy) propanoicacid, Fenchyl alcohol , Borneol,acetate , α-Cubebene, Elemicin and Caryophyllene oxide are the major constituents of the essential oil extracted from the aerial (grass) part of the plant.

Totally 52 phytoconstituents have already been identified from this grass. In the present work, the oil isolated from Cymbopogantravancorensis grass when analysed by GC-MS showed the presence of thirteen major compounds Table 1.

3.2 Determination of in-Vitro Antioxidant Assay

The percentage of efficiency of scavenging of free radicals at different concentrations ranging from 10 µg/ml to 50 µg/ml, was studied for Cymbopogantravancorensis oil by DPPH radical scavenging method using ascorbic acid as the standard. The results of the study are shown in Table 2.

The percentage inhibition was found to increase dose dependently in all the assay methods for the test sample as well as for the standard.

Reactive Oxygen Species (ROS) produced by in-vitro methods include super oxide radical, hydrogen peroxide, hypochlorous acid, etc. Hydrogen peroxide and super oxide can interact in the presence of certain transition metal ions to yield a highly reactive oxidizing species, the hydroxyl radical. The antioxidants react with the stable deep violet colour free radical DPPH and convert it to 1,1-diphenyl 2-2-picrylhydrazine with discoloration.

The essential oils which contain monoterpene hydrocarbons, oxygenated monoterpenes and / or sesquiterpenes have shown greater antioxidant properties [31]. There are many reports indicating the antioxidant potential of the thymol and γ-Terpinene [32-33]. As can be seen from Table 1, Limonene, Benzenemethanol and γ –Terpinene which are the major components of the essential oil isolated by us may possess higher antioxidant activity and hence these compounds could be assumed as major contributors for the total antioxidant activity of Cymbopogantravancorensis essential oil.

The reductive potential measures the ability of a sample to act as electron donor and, therefore, it reacts with free radicals converting them to more stable products and thereby terminating radical chain reactions. Reducing power characteristic of any compound may serve as a significant indicator of its potential antioxidant activity and is a supporting feature for its antioxidant activity [34]. The concentrations used were 10, 20, 30, 40 and 50 µg/ml and absorbance was read at 700 nm. The reductive potential of Cymbopogantravancorensis essential oil increased steadily with its increasing concentration (Table 2), the maximum inhibition of 83.42 % was obtained at 50 µg/ml of Cymbopogantravancorensis.

H_2O_2 scavenging activity is a measure of the antioxidant activity of the essential oil. In this direct method which involved UV spectrophotometry, the determination is based on the intrinsic absorption of H_2O_2 in the UV region at 230 nm. The essential oil showed moderate hydrogen peroxide scavenging activity which may perhaps be attributed to the presence of terpenes and phenolic groups that could donate electrons to hydrogen peroxide there by neutralizing it into water.

Nitric oxide radical generated from nitroprusside at physiological pH was found to be inhibited by the essential oils moderately as shown Fig. 2. The concentrations of oil were 10, 20, 30, 40 and 50 µg/ml and inhibition percentages were found to be 75.65, 76.24, 77.99, 78.77 and 79.47 % and ascorbic acid standard exhibited percentage inhibition of 94.61 – 98.47 % at the test doses.

3.3 Determination of in-Vitro Anti-Inflammatory Activity Study

The in- vitro anti-inflammatory activity of the essential oil from Cymbopogantravancorensis and the standard by HRBC membrane stabilization method are shown in Table 3.

In the present study, the essential oil showed significant stabilization towards HRBC membrane and the percentage protection of oil was found to be higher than that of the standard at all test doses are shown in Fig. 3. The synergic effect of the phytoconstituents like terpenes and oxygenated compounds present may be the responsible for the higher efficiency of the oil.

The present study showed the essential oil of Cymbopogantravancorensis to possess good antioxidant and inflammatory activities. The bioactive components of Cymbopogantravancorensis oil can act as primary and

secondary antioxidants, scavenging free radicals and can therefore inhibit the lipid peroxidation. The flavoring of food products with this essential oil may play an important role in future, because of the high value of fruity aroma insisted in human nutrition (soft drinks, fruit products, flavored teas, flavored milk products, chewing gums, cakes, sweeties, tarts and also in fine perfumery wherein this essential oil may be a valuable raw material for various applications. Further studies using animal models are warranted more in detail to confirm the pharmacological activity of the oil.

CONCLUSIONS

The yield of essential oil of the fresh grass of Cymbopogantravancorensis was 2.0 % (v/w). Among the 52 phtoconstituents identified by GC-MS analysis of the oil sample, 13 major phytoconstituents are Tricyclene, α-Pinene, Camphene , dl-Limonene , β-Ocimene , Camphor , (-)-Borneol, 2-(3-Methoxyphenoxy) propanoicacid, Fenchyl alcohol , Borneol,acetate , α-Cubebene, Elemicin and Caryophyllene oxide are the major constituents of the essential oil extracted from the aerial (grass) part of the plant. The essential oil when tested for its antioxidant activity by various methods showed significant inhibition efficiency and also it showed higher inhibition efficiency than standard diclofenac sodium at the test doses when tested for its anti-inflammatory activity by Human Red Blood Cell membrane stabilization (HRBC) method.

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REFERRENCES

[1] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, “Biological effects of essential oil A review, Food Chem. Toxicol.”, 46, 2 446–475, 2008.

[2] V. Masotti, F. Juteau, J.M. Bessiere, J. Viano, “Seasonal and phenological variations of the essential oil from the narrow endemic species Artemisia molinieri and its biological activities, J. Agric. Food Chem.”, 51, 715–7121, 2003.

a. Angioni, A. Barra, V. Coroneo, S. Dessi, P. Cabras, “Chemical composition, seasonal variability and antifungal activity of Lavandula stoechas, L. ssp. stoechas essential oils from stem/leaves and flowers”, J. Agric. Food Chem., 54, 4364–4370, 2006.

[3] V. Hajhashemi, A. Ghannadi, B. Sharif, “Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of Lavandula angustifolia Mill”, J. Ethnopharmacol., 89, 67–71, 2003.

[4] N.S. Perry, C. Bollen, E.K. Perry, C. Ballard, “Salvia for dementia therapy: Review of pharmacological activity and pilot tolerability clinical trial”, Pharmacol. Biochem. behaviour., 75, 651–659, 2003.

[5] B. Adorjan, G. Buchbauer, “Biological properties of essential oils: An updated review”, Flavour. Frag. J., 25, 407–426, 2010.

[6] P.L. Crowell, “Prevention and therapy of cancer by dietary monoterpenes”, J. Nutr., 129, 775–778, 1999.

[7] M. Baldioli, M. Servili, G. Perretti, G.F. Montedoro, “Antioxidant activity of tocoferols and phenolic compounds of virgin olive oil”, J. Am. Oil Chem. Soc., 73 (1996) 1589–1593.

[8] F. Visioli, G. Bellomo, C. Galli, “Free radical-scavenging properties of olive oil polyphenols”, Biochem. Biophys. Res., 247, 60–64, 1998.

[9] H. Yoshida, S. Takagi, “Antioxidative effects of sesamol and tocopherols at various concentrations in oils during microwave heating”, J. Sci. Food Agr., 79, 220–226, 1999.

[10] S. Bhatia, R. Shukla, S.V. Madhu, J.K. Gambhir, K.M. Prabhu, “Antioxidant status, lipid peroxidation and NO end products in patients of type 2 diabetes mellitus with nephropathy”, J. Clinbiochem., 36, 557–562, 2003.

[11] E. Peuchant, J. Brun, V. Rigalleau, L. Dubourg, M. Thomas, J. Daniel, “Oxidative and antioxidative status in pregnant women with either gestational or type 1 Diabetes”, J. Clinbiochem., 37, 293–298, 2004.

[12] P. Steer, J. Milligard, D.M. Sarabi, B, Wessby, T. Kahan, "Cardiac and vascular structure and function are related to lipid peroxidation and metabolism", Lipids, 37, 231–236, 2002.

[13] K. Uchida, "Role of reactive aldehyde in cardiovascular diseases", Free Radical Biol. Med., 28, 1685–1696, 2000.

[14] H.P. Grice, "Enhanced tumour development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium", Food Chem. Toxicol., 26, 717–723, 1988.

[15] H.C. Wichi, "Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract, Food Chemical Toxicology", 24, 1127–1130, 1986.

[16] E. Umapathy, EJ. Ndebia, A. Meeme, B. Adam, P. Menziura, BN. Nkeh-Chungag, JE. Iputo JE, "An experimental evaluation of Albuca setosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation", J. Med. Plants. Res., 4, 5, 789-795, 2010.

[17] V.R. Winrow, P.G Winyard, C.J. Morris, D.R. Blake, "Free radicals in inflammation: second messengers and mediators of tissue destruction", British Medical Bulletin, 49, 506–522, 1993.

[18] J.M. Gutteridge, "Lipid peroxidation and antioxidants as biomarkers of tissue damage", Clin. Chem., 41, 1819–1828, 1993.

[19] Filomena Conforti, Silvio Sosa, Mariangela Marrelli, Federica Menichini, Giancarlo, Statti, Dimitar Uzunov, "Aurelia Tubaro, Francesco Menichini, Roberto Della Loggia, In-vivo anti-inflammatory and in-vitro antioxidant activities of Mediterranean dietary plants", J. Ethnopharmacol., 116, 144–151, 2008.

[20] D.L. Dreyer, R.D. Bennew, S.C. Basa, "Limonoids From Atalantia Monophylla Isolation and Structure", Tetrahedron., 32, 2367-2373, 1976.

[21] N Sivagnanam, M. Kalyanasundaram, "Laboratory Evaluation of Methanolic Extract of Cymbopogantravancorensis(Family: Rutaceae) against Immature stages of Mosquitoes and Non-target Organisms", Mem Inst oswaldo cruz rio de janeiro, 99, 1, 115-118, 2004.

[22] K. Baskar, S. Ignacimuthu, "Ovicidal activity of Atalantia monophylla (L) Correa against Helicoverpa armigera Hubner (Lepidoptera: Noctuidae", J. Agric. Technol., 8, 3, 861-868, 2012.

[23] P.R. Adams, "Identification of Essential Oil Components By Gas Chromatography/Mass Spectrometry", 4th edition, Allured Publishing Corporation, Carol Stream, Illinois, 2007.

[24] Yagi, A. Kabash, N. Okamura, H. Haraguchi, S.M. Moustafa, T.I. Khalifa, "Antioxidant, free radical scavenging and anti-inflamatory effects of aloes in derivatives in Aloe vera", Planta Med., 68, 957-960, 2002.

[25] R.J. Ruch, S.J. Cheng & J.E. Klainig, "Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea", J. Carcinog., 10, 1003–1008, 1989.

[26] G.C. Yen, H.Y. Chen, "Antioxidant activity of various tea extracts in relation to their antimutagenicity", J. Agric. Food Chem., 43, 27–32, 1995.

[27] N. Sreejayan, M.N.A. Rao, "Free radical scavenging activity of curcuminoids", Drug. Res, 46, 169, 1996.

[28] S. Nivedhitha, M. Gobinath, P. Muthusamy, K. Mallikarjuna Rao, "Pharmacognostical evevaluation on roots of Datura fastuosa Linn.", J. Pharm. Res., 3, 11-15, 2010.

[29] B. Tepe, A. Sihoglu-Tepe, D. Daferera, M. Polissiou, A. Sokmen, "Chemical composition and antioxidant activity of the essential oil of Clinopodium vulgare L.", Food Chem., 103, 3, 766–770, 2007.

[30] G. Ruberto, M.T. Baratta, "Antioxidant activity of selected essential oil components in two lipid model systems", Food Chem., 69, 167–174, 2000.

[31] N.V. Yanishlieva, E.M. Marinova, M.H. Gordon, V.G. Raneva, "Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems", Food Chem., 64, 59–66, 1999.

[32] Reena Lawrence, Kapil Lawrence, "Antioxidant activity of garlic essential oil (Allium sativum) grown in north Indian plains", Asian Pac. J. Trop. Biomed., 1, 1-3, 2011.