Docking and *in vitro* studies on Antioxidant, Antibacterial and Cytotoxic properties of Cinnamon (*Cinnamomummalabathrum*)

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ABSTRACT *Cinnamomummalabathrum is aversatile plantused in foodstuffs, perfumes and medicinal products. In the current study, Cinnamon leaves were assessed for antioxidant, antibacterial, cytotoxic properties andits phytoconstituentswere docked with tyrosinase, an antioxidant receptor. DPPH antioxidant assay with methanol extract of cinnamon leaves showed higher activityat concentrations from 100-400 µg/mL which was even significantly higher than that of the positive control tannic acid($p > 0.05$).Comparative analysis reveals that methanol extract and essential oil of C. malabathrum exhibited the highest antibacterial activity against Streptococcus pyrogenes with inhibition zone diameter of 2cm.C. malabathrumessential oil exhibited77.33+0.629% cytotoxic activity to L929 cell linesat the dose of 400µg/mL.GC-MS analysis revealed that the abundant constituent of essential oil was: 5-Benzyloxy-4-butyl-2-methyl-2-nonene.A total of 9 compounds identified by GC-MS were subjected to molecular docking studies for theinhibition of tyrosinaseenzyme. Docking studies showed that 1-deoxy-D-ribitol was found to be potent inhibitor of tyrosinase receptor since it exhibited minimum free energy (−1.17kcal/ mol) and inhibition constant(139.82mM)and strongly docked using 8 hydrogen bond formationswith 6 amino acid residues at the active site.Hence 1-deoxy-D-ribitolcan be evaluated further for drug development as antioxidant.*

Keywords: *Cinnamomummalabathrum; Molecular docking; Tyrosinase; Antioxidant; Antibacterial; Cytotoxic; 1-deoxy-D-ribitol.*

I. INTRODUCTION

Cinnamomum malabathrum (Batka) commonly known as country cinnamon,belongs to the family Lauraceae andcomprises of about 250 species distributed in India, China, Srilanka and Australia. Cinnamomum species are widely used in herbal therapy in treating colds, sinusitis, bronchitis and fungal infections (Kharwaret al. 2012). Few studies on its essential oils (Wang et al. 2008) which included antioxidant, antimicrobial activity (Singh et al., 2007) and antidiarrheal activity have displayed that the plant extract is effective against pathogens.However, research on *C. malabathrum*has been very limited. Gas chromatography and mass spectroscopy make an effective combination for chemical analysis. Mallavarapu et al. (1995) had identified 53 constituents along with the major component eugenol (84.5%) in *Cinnamomumzeylanicum* leaf oil by GC-MS analysis.

Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (Devasagayam et al. 2004).Antioxidants from plant species which reduce oxidative stress from intrinsic and external sources have huge applications and functions in human health care (Gülçin et al. 2001). Tyrosinase is a copper-containing enzyme present in plant and animal tissues that catalyzes the production of melanin and other pigments from tyrosine by oxidation (Wu et al. 2018). To our knowledge, to date no data are available on molecular docking studies on*C. malabathrum*to tyrosinase enzymes. Thus the current study was designed to computationally screen the antioxidant compounds in *C. malabathrum* leaves and check its inhibitory potential against tyrosinase by molecular docking.

The main objective of this study was therefore to: (1) evaluate the biological potential of *C. malabathrum*leaves for itsantioxidant, antibacterial and cytotoxic properties (2) screen for antioxidant compounds in *C. malabathrum* leaves by *in silico* against tyrosinase.

II. MATERIALS AND METHODS

A. Collection of plant materials

Fresh leaves of *C. malabathrum* were collected from Neyyanttinkara in Trivandrum district in Kerala, India.

B. Bacterial strains

Eight bacterial strains namely *Lactococcuslactis*, *Mycobacterium tuberculosis, Salmonella typhi, Lactobacillus plantarum, Pseudomonas aeruginosa, Enterobacter aerogens, Escherichia coli, Bacillus megaterium*were obtained from the Microbial type Culture Collection (MTCC).

C. Extraction of essential oils from leaves

The leaves of *C. malabathrum* were shade dried. The dried leaves (100 g) were subjected to hydro-distillation using a Clevenger apparatus for 4 hrs for the isolation of essential oils. The essential oil yield was calculated based on the dry weight of plant materials. The volatile oils were stored in sealed vials at 4°C until GC-MS analysis.

D. Preparation of crude extracts

The shade dried leaves of *C. malabathrum* were ground into 60 mesh diameter powder using an electric grinder. The dried powder (5g) was extracted by soaking with 70% methanol, butanol and acetone separately using orbital shaker for 48 hrs at room temperature. The extracts were filtered through Whatman No.1 filter paper. Residues were re-extracted twice with fresh aliquots of the same solvents. Solvents from the combined extracts were evaporated using a vacuum rotary evaporator. These extracts were stored at 4°C.

E.Determination of in vitro antioxidant activity

(i) *In-vitro valuation of antioxidant activity by DPPH method*

Different concentrationslike 25, 50, 100, 200, 400 mcg/ml of solvent extracts of leaf and ascorbic acid (standard) were used in the assay. DPPH (0.002%) in methanol was used as free radical. Equal amount of different concentrations of solvent extracts and DPPH were mixed in clean and labelled test tube separately and incubated in dark for 30 minutes. The optical density was measured at 517nm using UV-visible spectrophotometer. The degree of stable DPPH decolorization (reduced form of DPPH) yellow indicated the scavenging efficiency of extract. The scavenging activity of extract against the stable DPPH was calculated using the following equation

$$\text{Scavenging activity (\%)} = A\text{-}B/A \times 100$$

where A is absorbance of DPPH, B is absorbance of DPPH and extract in combination (Khalaf et al. 2008).

(ii) *In-vitro evaluation of antioxidant activity by reducing power method*

Extracts of leaf at different concentrations were prepared with 1ml of distilled water, phosphate buffer (2.5ml, 2M, pH-6.6) and potassium ferricyanide (2.5 ml) of tricholoroacetic acid (TCA, 10%) was added to the mixture and centrifuged at 1500 rpm for 10 minutes. The upper layer solutions (2.5ml) was mixed with distilled water (2.5ml) and FeCl3 (0.5ml, 0.1%) and the absorbance was measured at 700nm.

F. Assessment of antimicrobial activity

Disc diffusion method was used for the assay. Sterile nutrient agar plates were prepared for 8 bacterial strains and Rose Bengal agar plates for 6 fungal strains. The plates were inoculated by a spread plate method under aseptic conditions. Filter paper discs of 5 mm diameter (Whatman No. 1 filter paper) were prepared and sterilized. 5 µl of each leaf extractor 10 µl of essential oils were added to each disc. The sterile impregnated discs with plant extracts and essential oils were placed on the agar surface with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Filter paper discs soaked in solvent were used as negative controls. The antimicrobial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in cm) produced by the respective extract at the end of incubation period.

G. *In vitro* cytotoxicity

Cytotoxicity of the *C. malabathrum* extracts on L929 cells treated at different concentrations (100-400µg/mL) for 24 hours was determined using the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay to assess cell viability method (Coates et al. 2007).

H. GC-MS analysis

GC-MS analysis of the essential oil from the leaves of *C. malabathrum* was performed using Shimadzu GC 17A QP 5000MS coupled with a mass detector, fitted non- polar DB-5 (Diphenyl Dimethyl Siloxane). The length of the capillary column was 25mX0.25mmId. GC-MS operation conditions with initial temperature set at 60°C, was programmed from 60°C-300°C with the injection temperature at 260°C and detector temperature at 300°C. The injection volume was 0.1µl with helium gas as carrier at flow rate of 0.6 ml per minute. Relative retention times (RRts) of constituents was determined using C5-C30 straight chain alkanes as standards. Individual constituents of the extract were identified by WILEY and NIST database matching by comparison with their RRts.

I. Statistical Analysis

All of the tests were performed in triplicates. Results were represented as mean ± standard deviation (SD). One-way ANOVA was performed using SPSSpackage. The results were considered significant when $p < 0.05$. Pearson correlation test was performed to find correlation between variables.

III. RESULTS AND DISCUSSION

A. Radical Scavenging Activity

DPPH free radical scavenging activities at different concentrations of methanol extract of *C. malabathrum* and ascorbic acid is shown in Fig. 1(A). As the concentration of extracts increased from 25 to400 µg/mL, the DPPH radical scavenging activity increased linearly($R^2 = 0.5317$, $p<0.05$).Strongest DPPH radical scavenging activity was exhibited at a concentration of 400µg/mL, that was not significantly different from that of the positive control ($p > 0.05$).This finding is consistent similarwith the report of Kumarb et al.(2010) whereDPPH radical was scavenged in a concentration dependent manner. Thus *C.malabathrum*could be suggested to be used as a supplementary product to combat the degenerative effects.

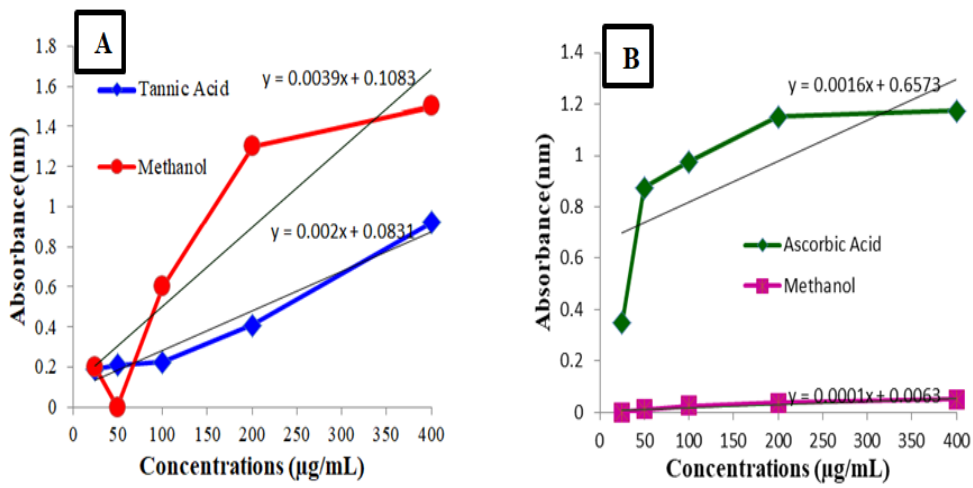


Figure 1: DPPH radical scavenging activity (A) and reducing power activity (B) of methanol extracts of *Cinnamomum malabathrum* and the reference compounds.

B. Ferric reducing power activity

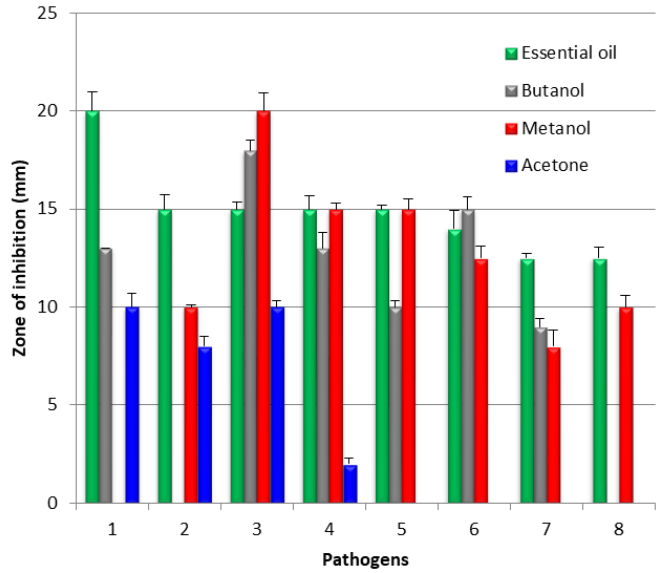
The ferric reducing ability of inulin is presented in Fig 1(B). A dose-dependent reducing power of methanol extract of*C. malabathrum* was observed. The extract showed a good linear connection ($R^2 = 0.871$, $P < 0.05$) to concentration in the range from 25 to 400 µg/mL. However, the extract showed significantly lower ferric reducing power than tannic acid at doses of detected concentrations ($P < 0.05$).

C. Antibacterial Activity

The antibacterial activity of *C. malabathrum*essential oil and crude extracts compared on various bacterial strains is represented in Fig. 2. The maximum inhibitory effect of the essential oil and methanol extract was against *Streptococcus* species and *Lactobacillus lactis* respectively with a zone of 2cm. Acetoneextract exhibited very less inhibition against *Mycobacterium tuberculosis* and no activity against *Salmonella typhi*,*Lactobacillus plantarum*, *Escherichia coli* and*Enterobacter aerogens.* The presentstudy indicated that the essential oil and methnanol extract of leaves of *C. malabathrum*showed effective antibacterial activity. This justifies the traditional use of *C. malabathrum* juice obtained from the slightly heated fresh leaves of this plant against antimicrobial infections like skin infections, wound infection, abscess and gastrointestinal disorder (Gill, 1992). The available literature also states that the essential oils were used widely in pharmaceutical industry (Sahinet al. 2004).

D. Cytotoxicity Analysis

Cytotoxicity of *C. malabathrum* essential oil against L929 cells appeared to be active at the concentrationsfrom 50µg/mL to 400µg/mL (Table 1). Essential oil of Cinnomon leaves showed highest cytotoxic activity (77.33+0.629%)compared to the other extracts.The current observations on the cytotoxic activity of the extracts on L929 cells reveal that theyare dose dependent. Similar report on the cytotoxicity effect of aqueous extractof *C. malabathrum*essential oil was reported against sarcoma 18017 (Nakajima, 1989).Henceforth more toxicity based studies to assess the safety and efficacy of the compounds should be concentrated.



(1)*Bacillus megaterium*; (2)*Pseudomonas aeruginosa*; (3) *Lactococcuslactis*; (4)*Mycobacterium tuberculosis*; (5) *Salmonella typhi*(6)*Lactobacillus plantarum*; (7)*Escherichia coli*; (8)*Enterobacter aerogens*

Figure 2: Antibacterial potential of *C. malabathrum* extracts. Data are represented by mean±SD of 3 independent experiments.

Table 1:Viability of L929 cells upon exposure to different concentrations of the essential oil of *C. malabathrum*. The values are expressed as mean ± SE with 3 sets of experimentation

Concentration of essential oil (µg/ml)	Cytotoxic activity (%)
400	77.33±0.629
200	75.25±0.371
100	72.43±0.184
50	66.92±0.453

E. GC-MS Analysis

The phytochemical active compounds of *C. malabathrum* essential oil werequalitatively analyzed Table 2. Presence of9 compounds were identified, the major constituents of the oil were: 5-Benzyloxy-4-butyl-2-methyl-2-nonene (17.26%) and hexadecanoic acid methyl ester (16.48%) and 1-deoxy-D-ribitol in a least proportion. Fig. 3 represents the chromatogram obtained.A similar study was carried out by Aravindet al. 2014 using the bark of *C. malabathrum* where the major component was Linalool (68.21%).

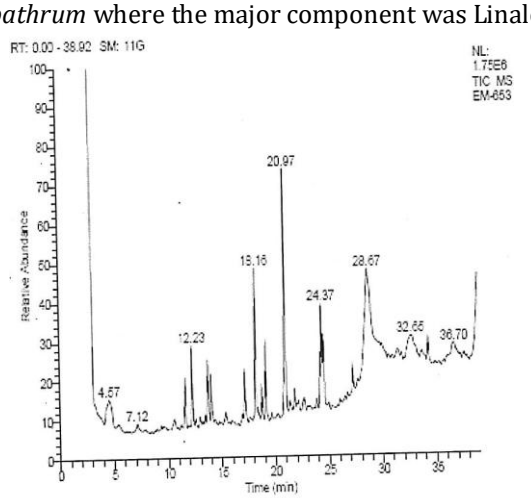


Figure 3: Chromatogram obtained by GC-MS analysis of *C. malabathrum* essential oil.

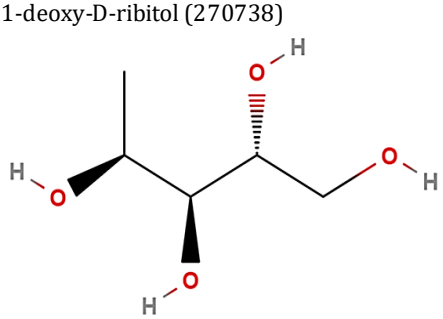
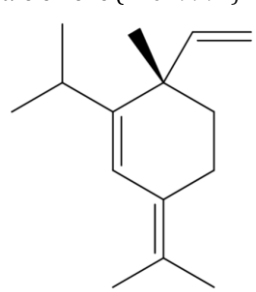
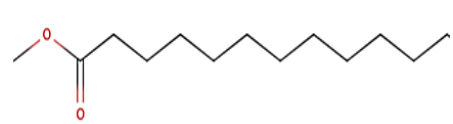
Table 2: Major components in essential oilof *C. malabathrum*identified by GC-MS analysis.

Sl. No.	Compounds identified	Retention time (min)	Compound (%)
1	5-benzyloxy-4-butyl-2-methyl-2-nonene	4.57	17.26
2	1-deoxy-D-ribitol	7.12	0.92
3	α-elemene	12.23	5.26
4	6(R)-2,6-dimethyl-2,17-octadecadien-8-ol	18.16	9.12
5	hexadecanoic acid methyl ester	20.97	16.48
6	Hexadecanoic acid dimethyl ester	24.37	16.06
7	3,3 dichloro-4-(o-chlorophenyl)-4-[trimethyl silyl0oxy]but-1-ene	28.67	6.74
8	Bis(oct-3yl)phthalate	32.65	1.59
9	7-bromodispiro(2.0.2.2)octane	36.70	1.26

F. Molecular Docking

The essential oil of *C. malabathrum* when tested for antioxidant studies gave significant results. Henceforth *in silico* studies of ligands with tyrosinase was planned through molecular docking analysis. Binding energy and H-bond docking parameters of potential ligands in *C. malabathrum* against tyrosinase receptor are represented in Table 2.

Table 2: Energy calculations and H-bond docking parameters of potential ligands in *C. malabathrum*against tyrosinase receptor

PubChem CID & Structure	Binding Energy (kcal/mol)	Inhibition Constant	H bonds	Interactive amino acid residues
1-deoxy-D-ribitol (270738) 	-1.17	139.82mM	8	Asn-243, His251, Ala-246, Val-38, Met-319, Arg-321
α-elemene (11019992) 	-5.84	52.73µM	0	-
hexadecanoic acid methyl ester (8181) 	-0.73	289.38mM	4	Arg-321, Asn-243, Glu-239

Molecular docking study shows that 1-deoxy-D-ribitol bind strongly to tyrosinase by forming 8 H-bond interaction with six amino acids (Asn-243,His-251, Ala-246, Val-38, Met-319, Arg-321) of tyrosinase. Fig 4 shows the interaction between the ligands - 1-deoxy-D-ribitol and hexadecanoic acid methyl ester of *C. malabathrum* and the protein tyrosinase. The compound 1-deoxy-D-ribitol shows good interaction with least binding energy and inhibition constant showing the compound inhibits the protein well. These computational studies show that the ligands 1-deoxy-D-ribitol and hexadecanoic acid could interfere with the target protein. This molecule should be characterized and studied for its toxicity profile which could be a potential drug target.

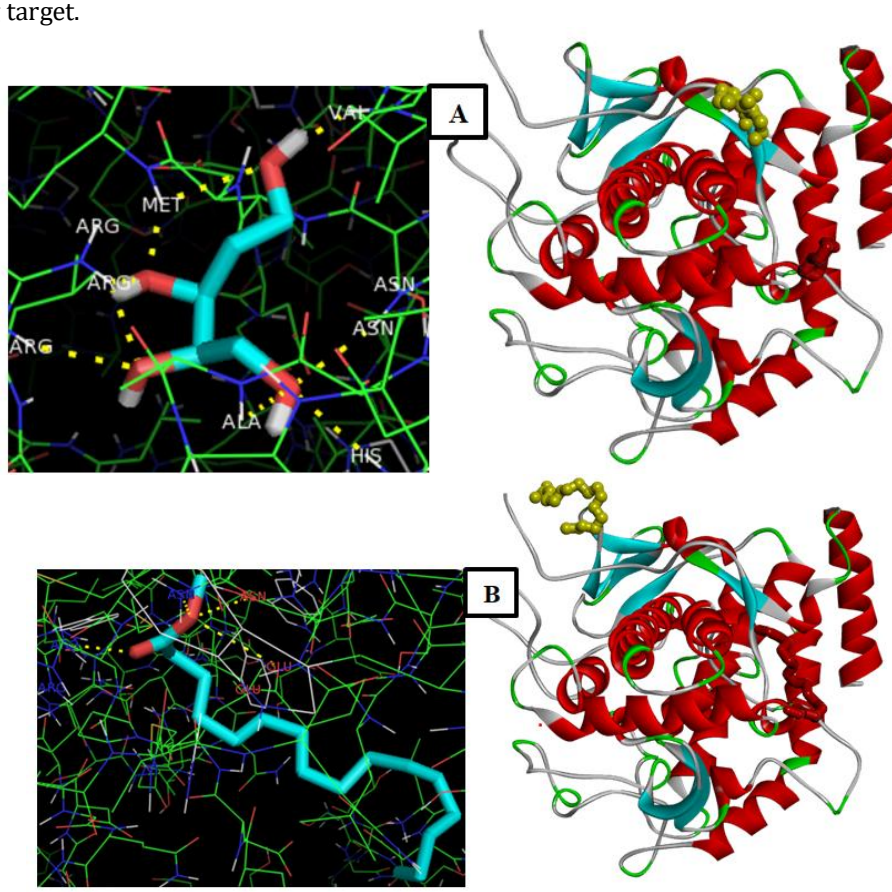


Figure 4: Lowest-energy docked poses of essential oil components 1-deoxy-D-ribitol(A) and hexadecanoic acid methyl ester(B) of *Cinnamomum malabathrum*in the binding site of tyrosinase (PDB ID: 2Y9X). Hydrogen bonds are represented as yellow dotted lines in A and ligands as green and redsticks(A)/yellow stick with balls(B).

IV. CONCLUSION

This study demonstrated that essential oil extracts of*C. malabathrum* had good *in vitro* antioxidant, antibacterial and cytotoxic properties. Our studies revealed that two phytochemical compounds namely 1-deoxy-D-ribitoland hexadecanoic acid methyl ester in*C. malabathrum* could be potential inhibitory sources against the crucial receptor tyrosinase. The finding in our current study concludes that these two compounds could boost anti-tyrosinase activity. Further *in-vitro* and *in-vivo* investigations will be required for its potential use in the pharmaceutical industry.

REFERENCES

1. Aravind, R. A., Bindu, A. R. B., Bindu, K. B. &, Varghese, A. (2014). GC-MS analysis of the bark essential oil of Cinnamomummalabatrum (burman. f) blume. Research Journal of Pharmacy and Technology. Research Journal of Pharmacy and Technology, 7, 754-759.
2. Coates, E. M., Popa, G., Gill, C. I., McCann, M. J.,& McDougall, G. J. (2007). Colon-available raspberry polyphenols exhibit anti-cancer effects on in vitro models of colon cancer. J Carcinog., 6, 4, 1-10.
3. Devasagayam, T. P. A., Tilak, J. C.,&Boloor, K.K. (2004). Review: Free radical and antioxidants in human health. Curr Stat Fut Pros JAPI., 53, 794-804.

4. Gill, L. S. (1992). The ethnomedical uses of plants in Nigeria Benin city, Edo State: Published by University of Benin Press, University of Benin, 46-143.
5. Gülçin, I., Elmastas, M.,&Aboul-Enein, H. Y. (2001). Determination of antioxidant and radical scavenging activity of Basil (Ocimumbasilicum L. Family Lamiaceae). Phytother. Res., 21, 354-361.
6. Khalaf, N. A., Shakya, A. K., Al-Othman, El-Agbar, Z., &Farah, H. Antioxidant activity of some common plants. Turkish Journal of Biology, 32, 51-55.
7. Kharwar, R. N., Maurya, A. L., Verma, V. C., Kumar, A., Gond, S. K.,& Mishra, A. Diversity and antimicrobial activity of endophytic fungal community isolated from medicinal plant Cinnamomumcamphora. National Academy of Sciences India Section B Biological Sciences, 82, 557–565.
8. Kumarb, H., Basheer, S.,&Haseena. (2010). Antioxidant potential and antimicrobial activity of Cinnamomum malabathrum (Batka). Orient. J. Chem., 26(4), 1449-1453.
9. Mallavarapu, G. R., Ramesh, S., Chandrasekhara, R. S., Rao, B. R., Kaul, P. N.,&Battacharya, A. K. (1995). Investigation of the essential oil of cinnamon leaf grown at Bangalore and Hyderabad. Flav.Fragr. J., 10, 239-242.
10. Nakajima, K., Ohtsubo, K., Shirato, K., Takamiya, R., Kitazume, S., Angata, T., & Taniguchi, N. (2013). Mass isotopomer analysis of metabolically labeled nucleotide sugars and N- and O-glycans for tracing nucleotide sugar metabolisms. Mol. Cell Proteomics., 12, 2468-2480.
11. Sahin, F., Gullace, Karaman, I., Oguteu, H., Sengul, M.,&Adiguzel, M. (2004). Antimicrobial activity of aqueous and methanol extracts of Juniperusoxycedrus L. Journal of Ethnopharmacology, 85, 231-235.
12. Singh, G., Maurya, S., deLampasona, M. P.,& Catalan, C. A. N. (2007). Chemical constituents, antimicrobial investigations and antioxidative potential of volatile oil and acetone extract of star anise fruits.Food and Chemical Toxicology, 45, 1650-1657.
13. Wang, R., Wang, R., & Yang, B. (2008). QLoss of has-miR-337-3p expression is associated with lymph node metastasis of human gastric cancer.Innovative Food Science and Emerging Technologies,10, 289.
14. Wu, L., Rathi, B., Chen, Y., Wu, X., Liu, H., Li, J., Mingc, A., &Hana, G. (2018).Characterization of immobilized tyrosinase – an enzyme that is stable in organic solvent at 100o C.RSC Adv.,8, 39529–39535.