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PHYTOCHEMICAL SCREENING, *IN-VITRO* EVALUATION OF ANTIOXIDANT AND ANTIBACTERIAL EFFICACY OF METHANOLIC LEAF EXTRACT OF *CLINOPODIUM NEPETA* (L.) KUNTZE

Mahuya De Ghosh [*1], Dilshad B. Golageri [2], W. K. Srimal Sandaruwan [2], J. Vishnu [1] and Sharon Rachel [2]

Department of Chemistry [1], Department of Biochemistry [2], Indian Academy Degree College Autonomous, Bangalore - 560043, Karnataka, India.

Keywords:

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Correspondence to Author:
Dr. Mahuya De Ghosh

Professor,
Department of Chemistry,
Indian Academy Degree College Autonomous, Bangalore - 560043, Karnataka, India.

E-mail: mahuyadeghosh@indianacademy.edu.in

ABSTRACT: The objective of the present study is to evaluate the phytochemical constituents, antioxidant and antibacterial activities of methanolic leaf extract of *Clinopodium nepeta* (L.) Kuntze. Total phenolic content of leaf extracts was assessed using Folin-Ciocalteu and $AlCl_3$ reagent. Antioxidant assays were evaluated using DPPH, ABTS radical scavenging, and ferric, reducing antioxidant power (FRAP) methods. The leaf extract was screened against four pathogenic bacteria, two gram-positive (*Escherichia coli*, *Bacillus cereus*) and two-gram negative (*Pseudomonas aeruginosa*, *Klebsiellea pneumonia*) using disc diffusion method. The results showed the total phenolic content (378.52mg gallic acid equivalent (GAE)/g) and flavonoid content (189.4 mg quercetin equivalents (QE)/g) of methanolic leaf extracts. DPPH and ABTS radical scavenging activity with an IC_{50} value of 0.49mg/ml and 1.68mg/ml, respectively. It also exhibited good absorbance in the FRAP assay. *The in-vitro* antibacterial assay showed an excellent zone of inhibition against *Pseudomonas aeruginosa* (19.01±0.3mm), *Bacillus cereus* (24.66±0.3mm), *Klebsiellea pneumoniae* (16.74±0.3mm), and *Escherichia coli* (17.56±0.31). *Clinopodium nepeta* (L.) Kuntze showed maximum antioxidant property and maximum inhibition against the test organisms. The high phytochemical constituents coupled with antioxidant and antibacterial activities of the leaves of *Clinopodium nepeta* (L.) Kuntze is indicative of its ability to treat various diseases and therefore supports its use in ethnomedicine.

INTRODUCTION: Presently, there is greater abuse of antibiotics in developing countries, and this resulted in increased antibiotic resistance among disease-causing organisms. This kind of drug resistance of microorganisms has become a global concern. Plants have to adapt to the changing environmental conditions for their sustenance.

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The oxidative environment presents a range of free radicals, including superoxide, hydroxyl radical, nitric oxide, and peroxynitrite, for living organisms to deal with [1-3]. There are a number of concrete evidences about the role of free radicals in the development of various diseases, including cancer, neurodegeneration, and inflammatory diseases. Antioxidants have therefore gained importance for their capacity to neutralize free radicals [4, 5]. In this context, the antibacterial and antioxidant properties of various medicinal plants are being investigated throughout the world because of the toxicological concerns associated with the synthetic antioxidants and preservatives [6, 7].

FIG. 1: CLINOPODIUM NEPETA (L.) KUNTZE

Clinopodium nepeta (L.) Kuntze belongs to the family Lamiaceae is also known as lesser calamint. It is a perennial herb of the mint family. The shrub forms a compact mound of shiny, green, oregano-like leaves commonly used as folk medicine. The plant reaches a height of 18 inches and grows as wild weed in ragi cultivation fields in Karnataka in the summer and become dormant in the winter months. *Clinopodium nepeta* (L.) Kuntze plants have long been used as a source of food and medicine [8]. The beneficial effects of plant products can be attributed to the biological activities of their phytochemical, antioxidant, and antipathogenic constituents such as polyphenols, flavonoids, carotenoids, and saponins. Recently, there has been an increasing interest in the study of natural products through which safe therapeutic results can be achieved.

Clinopodium nepeta (L) Kuntze has not been explored much, although it is used as a tribal traditional medicinal plant. In this present study, we tried to establish the important phytoconstituents, antioxidant properties, and antibacterial activity of this plant. Findings established that the methanolic leaf extract prevented the growth of both Gram-positive and Gram-negative bacteria; the antibacterial and antioxidant activity of the extract was found to be positively associated with the total phenolic and flavonoid content of the extract.

MATERIALS AND METHODS:

Preparation of the Extract: The collected leaves were shade dried for 8 days. The dried leaves were powdered mechanically, and 100gms was extracted with methanol in Soxhlet apparatus and concentrated at room temperature. Extracts were stored in the refrigerator for further analysis.

Preliminary Phytochemical Screening: Chemical tests for screening and identification of bioactive chemical constituents present in the methanol extract of leaves of *Clinopodium nepeta* (L.) Kuntze was carried out using the standard procedures [9, 10].

Test for Alkaloids: The test for alkaloids was performed using Wagner's reagent. The reagent was added to the extract and was observed for the formation of a reddish-brown precipitate.

Tests for Carbohydrates: To 2 ml of leaf extract, a few drops of Molisch's reagent were added, followed by concentrated H_2SO_4 (2ml) and allowed to stand for a few minutes. The formation of red or dull violet color at the junction of the two layers indicate the positive test.

Test for Cardiac Glycosides: To the plant extract, glacial acetic acid was added, followed by a few drops of ferric chloride solution and sulphuric acid. The appearance of an initial brown ring at the junction, which turns greenish, indicates the presence of Cardiac glycosides.

Test for Flavonoids: Dilute ammonia solution was added in portion to crude leaf extract followed by concentrated H_2SO_4. The formation of yellow color indicates the presence of flavonoids.

Test for Phenols: To the leaf extract, 2 ml of distilled water and a few drops of 10% ferric chloride solution was added. The formation of blue or green color indicates the presence of phenols.

Test for Phlobatannins: 2 ml of leaf extract was boiled with 1ml of 1% aqueous hydrochloric acid, the appearance of red precipitate indicates the presence of phlobatannins.

Test for Amino Acids and Proteins: Ninhydrin solution was added to the extract and was heated in boiling water bath for few minutes and was observed for the formation of purple color which establishes presence of amino acids and proteins.

Test for Saponins: The formation of foam on addition of distilled water to leaf extract followed by vigorous shaking with few drops of olive oil indicate the presence of saponins.

Test for Tannins: Formation of blue-black coloration on addition of potassium ferric cyanide

and acidified ferric chloride to leaf extract indicates the presence of tannins.

Test for Terpenoids: On addition of concentrated sulphuric acid and chloroform to the leaf extract, the appearance of reddish-brown precipitate shows the positive test.

Test for Quinones: Formation of yellow precipitate on the addition of concentrated hydrochloric acid to extract indicates the presence of quinone.

Quantitative Phytochemical Estimation:

Estimation of Total Phenol by Folin-Ciocalteu Reagent Method: Using modified Folin-Ciocalteu method[11], total phenol content in the extract was determined. 50 µL aliquots of 25, 50, 100, 200, and 400 µg/ml methanolic gallic acid solutions were mixed with 100 µL Folin–Ciocalteu reagent and 100 µL (75 g/L) sodium carbonate. The mixture was incubated at 25 ºC for 30 min; the quantitative phenolic estimation was performed using a UV-VIS spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (mg/g of dry mass), which is used as a common reference standard.

Estimation of Total Flavonoids by $AlCl_3$ Method: Aluminium chloride colorimetric method was used to determine the total flavonoids [12]. Methanolic leaf extract of *Clinopodium nepeta* (L.) Kuntze was mixed with 5% aluminium chloride solution, 1 M potassium acetate and little distilled water. It was incubated at room temperature for 30 min and evaluated at a final concentration of 1 mg/ml. The absorbance was measured by a spectrophotometer at 420 nm. The total flavonoid content was expressed in terms of quercetin equivalent, which is a common reference standard.

Antioxidant Assay: The antioxidant activity of leaf extract was determined by different methods using DPPH, ABTS free radical scavenging, and Fe^{3+} reducing power assays.

DPPH Radical Scavenging Assay: The assay was performed using a modified method [13] in which DPPH was dissolved in methanol to 0.025 g/L concentration. The leaf extract at various concentrations was diluted with dimethyl sulfoxide (DMSO) to get a sample solution. 195µL DPPH working solution was added to the various leaf extract. The absorbance was measured spectrophotometrically at 515nm after 20 min at room temperature. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of reference standard Ascorbic acid. The ability to scavenge the DPPH radical was expressed as percentage inhibition and calculated using the following equation:

% of DPPH radical inhibition = Control – Sample × 100 / Control

ABTS Radical Cation Scavenging Assay: The ABTS radical cation was produced by the reaction between 5 ml of ABTS stock solution and 5 ml of 2.45 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution was diluted with water to get an absorbance of 0.700 ± 0.020 at 734 nm and equilibrated at 30 ºC. The leaf extract at various concentrations was diluted with dimethyl sulfoxide (DMSO) to get a sample solution. 5 µL of sample solution was homogenized with 195 µL ABTS solution, the mixture was incubated at room temperature for 10 min, and its absorbance was recorded at 730 nm [14]. Ascorbic acid was used as a reference standard, and the inhibition percentage calculated using the following formula:

% of $ABTS^{\bullet+}$ radical inhibition = Control – Sample × 100 / Control

Ferric (Fe^{3+}) Reducing Power (FRAP) Assay: The ability to reduce ferric ions was measured using the method described by Benzie *et al*. [15] The FRAP reagent was produced by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM (tripyridyl triazine) solution and 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Samples at different concentrations were added to the FRAP reagent, and the reaction mixture was incubated at 37 ºC for 30 min. The optical density was measured at 593 nm. The antioxidant capacity based on the ability to reduce ferric ions of the sample was calculated from the linear calibration curve and expressed as mmol equivalents per gram of sample.

Antibacterial Activity and Inoculums Preparation: The antibacterial potency of the leaf extract was evaluated using four pathogenic bacterial strains. Two strains of gram-positive

[*Escherichia coli* (MTCC-739), *Pseudomonas aeruginosa* (MTCC-2453), and two strains of gram-negative [*Bacillus cereus* (MTCC-1369), *Klebsiellea pneumoniae* (MTCC 3378)] bacteria.

The antimicrobial activity of the leaf extract was evaluated using the disk diffusion method. The leaf extract residue (50 mg) was dissolved in 2.5ml of methanol, sterilized through Millipore filter (0.22 µm) then loaded over sterile filter paper discs to obtain a final concentration of 10 mg/disc. Ten ml of Mueller-Hilton agar medium was poured into sterile Petri dishes followed by 15 ml of previously inoculated seeded medium with bacterial suspension (100 ml of medium/1 ml of 10^7 CFU) to attain 10^5 CFU/ml of medium. Sterile filter paper discs loaded with leaf extract of varying concentrations were placed on the top of Mueller-Hilton agar plates. Gentamicin was used as a positive control. Different concentrations of the leaf extract (2.5, 5.0, 10.0, 12.5, and 15 mg/ml) were prepared separately, and the most effective leaf extract which exhibited a strong antibacterial activity at 12.5 mg/ml was manipulated to determine their MIC using disk diffusion method. The plates were kept in the fridge at 5 °C for 2 h. to permit the diffusion of leaf extracts then incubated at 35 °C for 24 h [16]. The presence of inhibition zones was measured and considered as an indication for antibacterial activity. Results were noted as a mean of triplicate readings.

RESULTS AND DISCUSSION:

Phytochemical Screening: The methanolic leaf extracts of *Clinopodium nepeta* (L) Kuntze was tested for various qualitative phytochemical tests. It showed the presence of various phytoconstituents such as alkaloids, flavonoids, phenolic compounds, saponins, and flavonoids depicted in **Table 1**.

TABLE 1: QUALITATIVE PHYTOCHEMICAL SCREENING OF METHANOL EXTRACT OF LEAVES OF *CLINOPODIUM NEPETA* (L.) KUNTZE

S. no.	Phytochemicals constituents	Methanol extract
1	Alkaloids	+
2	Cardiac glycosides	-
3	Carbohydrates	+
4	Phlobatannins	-
5	Flavonoids	+
6	Phenols	+
7	Saponins	+
8	Tannin	-
9	Terpenoids	+
10	Quinones	+

Phytochemical Estimations: The total phenolic content (estimated by Folin-Ciocalteu method) in the methanol extract of leaves of *Clinopodium nepeta* (L) Kuntze was 378.52 mg/g, and the total flavonoid content (estimated by $AlCl_3$ method) was 189.4 mg/g **Table 2**. The phenolic compounds and flavonoids present in the extract of leaves inhibit the formation of free radicals, which otherwise can lead to oxidative damage.

TABLE 2: QUANTITATIVE PHYTOCHEMICAL ESTIMATION OF PHENOLS AND FLAVONOIDS OF METHANOL EXTRACT OF LEAVES OF *CLINOPODIUM NEPETA* (L.) KUNTZE

S. no.	Contents	Amount (mg/g) in Methanol Extract
1	Total Phenol[a]	378.52
2	Total Flavonoid[b]	189.4

[a] Expressed as mg of gallic acid equivalent (GAE)/g of dry leaf material.

[b] Expressed as mg quercetin/g of dry leaf material

DPPH Radical Scavenging Assay: The DPPH method revealed that the scavenging of the free radicals was found to be 38.76, 51.63, 67.85, 78.91 and 88.52% at 0.2, 0.4, 0.6, 0.8 and 1 mg/ml respectively for the methanol extract of *Clinopodium nepeta* (L) Kuntze leaf **Table 3, Fig 2**. In the DPPH assay, the IC_{50} of ascorbic acid was 0.18 mg/ml while that of the leaf extract was 0.69 mg/ml, respectively.

In the present study, there was increased scavenging activity of the DPPH radicals with increasing concentration of the leaf extract, which indicates an increased ability to donate hydrogen ions resulting in a lighter solution proportional to the number of electrons gained [17]. Therefore, it may be postulated that *Clinopodium nepeta* (L) Kuntze exhibit significant DPPH scavenging activity by reducing the radical to corresponding hydrazine as a result of its hydrogen ion-donating ability.

TABLE 3: DPPH RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT OF LEAVES OF *CLINOPODIUM NEPETA* (L.) KUNTZE

S. no.	Concentration (mg/ml)	% of inhibition Methanol Extract	% of inhibition Ascorbic Acid
1	0.2	38.76	45.01
2	0.4	51.63	58.00
3	0.6	67.85	75.54
4	0.8	78.91	86.11
5	1.0	88.52	96.07

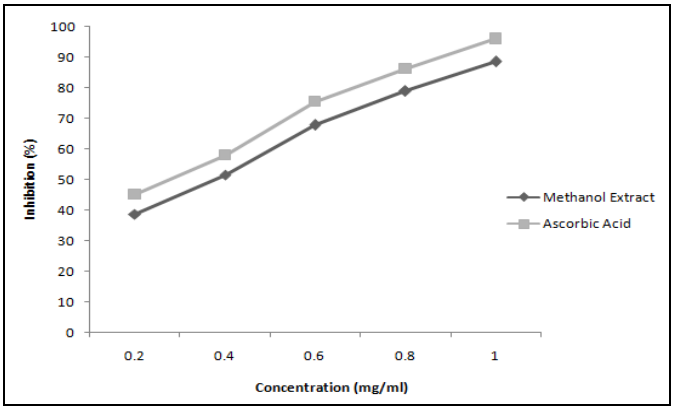

FIG. 2: DPPH RADICAL SCAVENGING ACTIVITY

ABTS Radical Cation Scavenging Assay: In the ABTS radical cation scavenging assay, ABTS reacts with potassium persulfate producing a blue chromophore in the presence of methanol extract of leaves of *Clinopodium nepeta* (L.) Kuntze. The preformed cation radical gets reduced, and the remaining radical cation concentration after reaction with the antioxidant compound was then measured [18].

The maximum ABTS radical cation scavenging activity was 72.30% at 1 mg/ml concentration **Table 4**, **Fig. 3**. The ABTS assay has been employed as an index that reveals the antioxidant activity of the test sample. The IC_{50} (concentration required for 50% inhibition) of ascorbic acid was 0.40 mg/ml while that of the leaf extract was 0.71 mg/ml, respectively. This shows that extract presents a good ability to scavenge the ABTS radical and establishes the capability of *Clinopodium nepeta* (L.) Kuntze to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical related pathological damage.

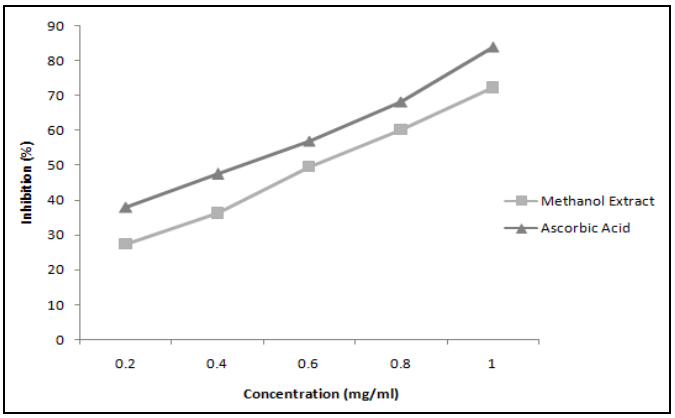

FIG. 3: ABTS RADICAL SCAVENGING ACTIVITY

TABLE 4: ABTS RADICAL CATION SCAVENGING ACTIVITY OF METHANOL EXTRACT OF LEAVES OF *CLINOPODIUM NEPETA* (L.) KUNTZE

S. no.	Concentration (mg/ml)	% of inhibition Methanol Extract	% of inhibition Ascorbic Acid
1	0.2	27.45	38.02
2	0.4	36.21	47.54
3	0.6	49.50	57.00
4	0.8	60.18	68.33
5	1.0	72.30	84.09

Ferric (Fe^{3+}) Reducing Power (FRAP) Assay: The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+} TPTZ) complex and produces a colored complex of ferrous tripyridyltriazine (Fe^{2+} TPTZ). Generally, the reducing properties are linked with the presence of compounds that exert their action by breaking the free radical chain by donating a hydrogen atom [19]. In the present study, as shown in **Table 5, Fig. 4,** the absorbance of *Clinopodium nepeta* (L.) Kuntze clearly increased due to the formation of the Fe^{2+} TPTZ complex with increasing concentration, as seen. The reference ascorbic acid had an absorbance of 4.00 at the maximum dosage of 1 mg/ml. Hence the plant should be able to donate electrons to free radicals stable in the actual biological systems.

TABLE 5: FERRIC (FE^{3+}) REDUCING POWER OF LEAF EXTRACTS OF *CLINOPODIUM NEPETA* (L) KUNTZE

S. no.	Concentration (mg/ml)	Fe^{3+} reducing power (OD)	Ascorbic Acid OD
1	0.2	0.13	0.28
2	0.4	0.19	0.39
3	0.6	0.47	0.69
4	0.8	0.94	1.90
5	1.0	2.83	4.00

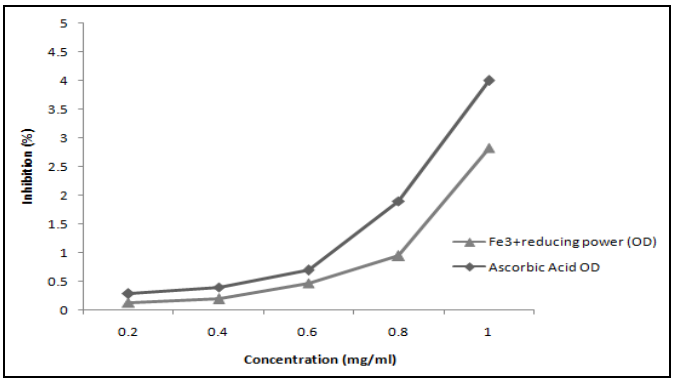

FIG. 4: FRAP ASSAY

***In-vitro* Antibacterial Activity:** With regard to the antimicrobial activity, according to Djeussi *et al.*,

[20-23], the activity is referred to as resistance to bacteria when the inhibition zone is less than 8mm, and it is intermediate when it is greater than 11 mm. The majority of inhibitions are above 11mm, and such bacteria are referred to as susceptible to the plant extracts. The substances present in methanolic leaf extract of *Clinopodium nepeta* (L) Kuntze have the ability to inhibit the growth of bacteria. The microbial inhibition is observed, and all the test organisms are susceptible to methanolic leaf extracts in different concentrations at MIC range of (2.5 to 15 mg/ml). The antibacterial activity of the extracts of the leaves of the plant as a zone of inhibition is presented in **Table 6**.

TABLE 6: ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *CLINOPODIUM NEPETA* (L) KUNTZE AGAINST HUMAN ENTERIC PATHOGEN, ZONE OF INHIBITION

Leaf extract	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Klebsiellea pneumoniae*
Methanol	17.56±0.31mm	19.01±0.3mm	24.66±0.3mm	16.74±0.3mm
Gentamicin	25.15±0.25mm	27.42±0.25	29.05±0.25mm	26.34±0.25mm

*****Values are mean of triplicate readings

The extract showed maximum inhibition to *Bacillus cereus* and moderate inhibition to *Klebsiellea pneumoniae*. The antioxidant and antibacterial activity relationship have been observed in our study of leaf extracts of *Clinopodium nepeta* (L) Kuntze. The DPPH scavenging activity, ABTS radical decolorization, Ferric (Fe^{3+}) reducing power assay, and antibacterial activity have a lot of similarities in their functioning. The antioxidant associated with antibacterial activity is relatively appreciable [24-27].

CONCLUSION: The plant-based antibacterials have enormous therapeutic potential, and more so, they have lesser side effects that are often seen with synthetic antimicrobials. Despite progress in the pharmacological field, therapeutic uses of medicinal plants is still important in many developing countries. The present study has revealed that the methanolic leaf extract of *Clinopodium nepeta* (L.) Kuntze contains a substantial amount of phenolics, and thus can be inferred that these phenolics are responsible for their marked antioxidant and antibacterial activities as assayed through various *in-vitro* models used in this study.

Literature reports on the said studies of *Clinopodium nepeta* (L.) Kuntze is scarce, and probably this is the first elaborate research work carried out to establish the findings. Leaves of this plant is particularly used against respiratory and gastric diseases in folk medicine. However, the present study of *in-vitro* antibacterial and antioxidant evaluation of the plant extract form a base for further phytochemical and pharmaco-logical studies leading to new natural substances designed as a phytomedicine.

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REFERENCES:

1. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW and Feng Y: The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci 2015; 16: 26087-124.
2. Van Dam RM, Naidoo N and Landberg R: Dietary flavonoids and the development of type 2 diabetes and cardiovascular diseases. Review of recent findings. Curr Opin Lipidol 2013; 24: 25-33.
3. Kay CD, Hooper L, Kroon PA, Rimm EB and Cassidy A: Relative impact of flavonoid composition, dose and structure on vascular function: A systematic review of randomised controlled trials of flavonoid-rich food products. Mol Nutr Food Res 2012; 1-12.
4. Ahmad I and Aqil F: *In-vitro* efficacy of bioactive extracts of 15 medicinal plants against ESbL-producing multi drug resistant enteric bacteria. Microb Res 2017; 162: 264-75.
5. Carović-Stanko K, Petek M, Grdiša M, Pintar J, Bedeković D, Herak Ćustić M and Satovic Z: Medicinal plants of the family Lamiaceae as functional foods-A review. Czech J Food Sci 2016; 34: 377-90.
6. Manikandan A, Rajendran R, Abirami M and Kongarasi K: Antimicrobial activity and phytochemical analysis of *Impatiens balsamina* Seed (Kaci-T-Tumpai) collected from Coimbatore District, Tamil Nadu, India. Int J of Pharmaceutical Sciences and Research 2016; 7: 5039-43.
7. Quintans, JSS, Antoniolli AR, Almeida JRGS, Santana-Filho VJ and Quintans-Junior LJ: Natural products evaluated in neuropathic pain models - A systematic review. BCPT 2014; 114: 442-50.
8. Castilho P, Liu K, Rodrigues AI, Feio S, Tomi F and Casanova J: Composition and antimicrobial activity of the essential oil of *Clinopodium ascendens* (Jordan) Sampaio from Madeira. Flavour Fragr J 2007; 22: 139-44.
9. Prashant T, Bimlesh K, Mandeep K, Gurpreet K and Harleen K: Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia 2011; 1(1): 23-31.

10. Farhat MB, Landoulsi A, Chaouch HR, Sotomayor JA and María JJ: Characterization and quantification of phenolic compounds and antioxidant properties of Salvia species growing in different habitats. Industrial Crops and Products 2013; 49: 904-14.
11. Armijos CP, Meneses MA, Guamán-Balcazar MC, Cuenca M and Suárez AI: Antioxidant properties of medicinal plants used in the Southern Ecuador. Journal of Pharmacognosy and Phytochemistry 2018; 7(1): 2803-12.
12. Srikanth M, Devi B, Kotirataiah K, Ramanjaneyulu M, Sulthana PN and Suma RR: Phytochemical Screening and In-vitro antioxidant activity of Peristrophe paniculata. Herbal Medicine: Open Access 2018; 4(11): 898-906.
13. Prevc T, Segatin N, Poklar Ulrih N and Cigi B: DPPH assay of vegetable oils and model antioxidants in protic and aprotic solvents. Talanta 2013; 109: 13-19.
14. Apak R, Mustafa Ozy¨urek M, Kubilay Guçlu K and Esra Çapanoglu E: Antioxidant activity/capacity measurement. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. Journal of Agricultural and Food Chemistry 2016; 64(5): 997-1027.
15. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L and Byrne DH: Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 2006; 19(6-7): 669-75.
16. Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Potala S and Verma RS: Antibacterial activity of the plant used in Indian herbal medicine. Int J of Green Pharma 2010; 10: 22-28.
17. Marco P, Carballo-Sánchez, Gimeno M, Hall G, Ríos-Duránan MG and Shirai K: DPPH Radical-scavenging activity of a purified and sequenced peptide from lactic acid fermentation of *Thunnus albacares* by-products. Appled Biochem and Biotechnol 2019; 189(4): 1084-95.
18. Dong JW, Cai L, Xing Y, Yu J and Ding ZT: Re-evaluation of ABTS assay for total antioxidant capacity of Natural Products. Nat Prod Commun 2015; 12: 1496-1547.
19. Luqman S, Srivastava S, Kumar R, Maurya AK and Chanda D: Experimental assessment of *Moringa oleifera* leaf and fruit for its antistress, antioxidant, and scavenging potential using *in-vitro* and *in-vivo* assays. Evidence-Based Complementary and Alter Med 2012; 6(12): 1619-24.
20. Djeussi DE, Noumedem JAK and Seukep JA: Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC Complementary and Alternative Medicine 2013; 13: 164.
21. Meda A, Lamien CE, Romito M, Millogo J and Nacoulma OG: Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Che 2005; 91(3): 571-77.
22. Alagesaboopathi C: Antimicrobial potential and phyto-chemical screening of *Andrographis affinis* Nees an endemic medicinal plant from India. Int J of Pharma and Pharmaceutical Sci 2011; 3(2): 157-59.
23. Seeram NP, Henning SM, Lee R, Niu Y, Scheuller HS and Heber D: Catechin and caffeine contents of green tea dietary supplements and correlation with antioxidant activity. J Agric Food Chem 2013; 54: 1599-1603.
24. Benzie IFF and Strain JJ: The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. Anal Brioche 1996; 239: 70-76.
25. Kokoska L, Polesny Z, Rada V, Nepovim A and Vanek T: Screening of some Siberian medicinal plants for antimicrobial activity. J Ethnopharmacol 2012; 82: 51-53.
26. Silva CG, Herdeiro RS, Mathias CJ, Panek AD, Silveira CS, Rodrigues VP and Rennó MN: Evaluation of antioxidant activity of Brazilian plants. Pharmacol Res 2014; 52: 229-33.
27. Molla Y, Nedi T, Tadesse G, Alemayehu H and Shebeshi W: Evaluation of the *in-vitro* antibacterial activity of the solvent fractions of the leaves of *Rhamnus prinoides* L'Herit (Rhamnaceae) against pathogenic bacteria. BMC Complementary and Alternative Med 2016; 16: 287-96.

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