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Antioxidant, antimicrobial and cytotoxic activity of Curkolin® (*Curcuma longa* and *Coleus forskohlii* formulation)

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The aim of this study was to screen the *in vitro* antimicrobial, antioxidant and cytotoxicity effect of Curkolin®, a combination of *curcumin and C. forskolin* in the ratio 4:1. *Curcuma longa L.* is the source of curcumin while *C. forskohlii* has been used in traditional ayurvedic medicine for curing various disorders and therefore the forskolin is the source of the diterpenoids. The medicinal properties of plants are investigated throughout the world for scientific advancement for their important pharmacological activities, user convenient, economically viable with low toxicity. The antioxidant property of Curkolin® was determined to be 55.42 µg/ml using (Diphenyl picryl hydrazine) DPPH assay and was then compared with standard Butylated hydroxytoluene (BHT). The antimicrobial activity was assessed by calculating the MIC and MBC using microplate serial dilution technique. It exhibited 250 µg/ml, the least MIC value of *Bacillus cereus* as compared to the rest Gram-positive (*Streptococcus mutans* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Salmonella typhi*) bacteria. Further, it was analyzed for cytotoxic screening on MCF-7 breast cancer cell line by MTT assay, which has shown a strong anti-proliferative activity. The IC_{50} values of Curkolin® were found 135.8 µg/ml. Together these results suggest the combination to be an effective antioxidant, antimicrobial and cytotoxic agent.

Keywords: Antioxidant, Antimicrobial, Curkolin®, HPLC, MTT assay.

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India has a rich history in the use of plants and their derivatives for medicinal purpose. A large number of medicinal plants are used in traditional medicine since ancient times[1]. Medicinal plants continue to provide new remedies to the mankind. Medicinal herbs are rich in active phytochemical compounds with different biological activities[2].

In Ayurveda, Unani, Siddha and Chinese traditional medicine, the turmeric (*Curcuma longa* L.) leaf and roots are extensively used as home remedy for various diseases. This plant belongs to Zingiberaceae family, is a rhizomatous herbaceous perennial and native of the Indian subcontinent and Southeast Asia[3]. The most active compound curcumin, which is found in turmeric, has many health benefits such as the ability to prevent Alzheimer's, heart disease and cancer. It is extensively used as spice in various food preparations of India. It is considered as auspicious and used in all religious rituals. In customary Indian medicine, turmeric powder is used for the treatment of disorders like Cory, cough, biliary obstruction, anorexia, diabetic wounds, hepatic etc. The active principle phytochemical curcumin is antibacterial, antiprotozoan, antiviral, hypolipemic, hypoglycemic, anticoagulant, antitumor and anticarcinogenic in nature. It is recorded in ayurveda under the Sanskrit name 'Makandi' and 'Mayani[4]. In ayurvedic medicine, *C. forskohlii* species were used under the name *pashanabhedi* for lung diseases, organal spasms, insomnia, and also in convulsions[5]. The diterpenoid forskolin is derived from the root of the plant, is one of the main components of clinical interest in *C. forskohlii*. Since then, along with coleonols and diterpenoids, the forskolin is responsible for all the pharmacological activities attributed to *C. forskohlii* [6].

At present, there is a demand for ayurvedic products, especially for anticancer, anti-inflammatory, antidiabetic and antioxidant activity, which can

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overcome the effects of free radicals, and low toxicities comparing to synthetic antioxidants that are used in food products, cosmetics drugs etc[7]. The phytochemical are more demanded antioxidants all over the world. Due to the extensive usage of antibiotics, the microbes have acquired resistance to them. This resulted in various clinical problems, especially treating the infectious diseases. So, there is a call to develop alternative strategies; it is necessary to screen phytochemicals for antimicrobial activities. The phytochemical remain important resources to combat serious diseases. As for WHO (1993) guidelines, approximately 80% of the population is dependent on the herbal medicine. This study concentrates on the antimicrobial, antioxidant and cytotoxic activity on Curkolin® and looking forward to its pharmacological application.

Materials and Methods

Collection of samples

The plant samples (*Coleus forskohlii root* and *turmeric rhizome*) were collected and identified by the R&D Department of Star hi herbs Pvt. Ltd Jigani, Bengaluru and Karnataka, India. Then they were shade dried and powdered.

Extraction of plant materials

Coleus forskohlii extraction

The dry root powder was extracted with toluene for 6 hours. The extract was filtered, concentrated and dried under reduced pressure in a rotary evaporator. The extract was mixed with hexane and kept for crystallization for 72 hours. After that the extract was filtered and dried at 80ºC temperature.

Curcumin extraction

The 250 g of turmeric powder was subjected to extraction using ethyl acetate for 6 hours. The extract were filtered and concentrated by rotary evaporator. The extract was mixed with isopropyl alcohol. Then it was kept for crystallization for 5 days. After, the extract was filtered and dried at 80ºC temperature.

Sample preparation

Curkolin® was prepared by mixing 80 g of curcumin (95%) and 20 g of *C. forskohlii.* The powder was blended properly and assayed for various biological activities.

Antioxidant activity of Curkolin®

The antioxidant screening of the samples were measured according to the Blois method by using UV- visible spectrophotometer at 517 nm[8]. The 0.1 mm DPPH radical was prepared in methanol and 1 ml of this solution was mixed with 3 ml of sample at different concentrations ranging from 50, 100, 150, 200 and 250 µg/ml respectively. The control was maintained with 1 ml of DPPH solution and 3 ml of methanol without sample. The reaction mixtures were incubated at ambient temperature for 30 minutes. The absorbance was measured at 517 nm against the blank solution. Butylated hydroxytoluene (BHT) with different concentrations (20, 40, 60, 80 and 100 µg/ml) was used as positive control. The radical scavenging activity of DPPH was calculated as mentioned below.

% Inhibition = (Control ABS – Sample ABS)/ Control ABS) × 100.

Antimicrobial activity of Curkolin®

Test organisms

The Curkolin® was tested against *Bacillus cereus (*MTCC *492), Staphylococcus aureus* (MTCC 737), *Streptococcus mutans* (MTCC 497), *E-coli (MTCC 1610) and Salmonella typhi (MTCC 424).* The microbes mentioned above were collected from IMTECH, Chandigarh, India.

Resazurin solution preparation

The 50 ml standard solution of resazurin was prepared with 337.5 mg resazurin powder using double distilled water. Solution was mixed for 1 hour in a cyclomixer. The preparation was performed in dark room and the resazurin solution was stored in a brown bottle.

Sample preparation

The sample was prepared by adding 330 µL of sample and 170 µL of DMSO in sterile eppendorf. 100 µL (1000 µg) of the dissolved sample was tested against each pathogen.

Media preparation

Luria Bertani (LB) broth (tryptone 10 g, sodium chloride 10 g, yeast extract 6 g and distilled water 1000 ml) was prepared and autoclaved at 121°C for 15 min.

Plate preparation

About 300 µL sterile distilled water was added in the outermost wells of microtiter plates (A_1-A_{12}, B_{12}-H_{12}, H_{11}-H_1, and G_1-B_1) to prevent the sample from drying. 100 µL sterilized LB broth was added to

all the remaining wells. 30 µL of 0.1% resazurin dye in wells B_2- F_2 in one plate and B_2- E_2 in another plate was added as color blank. In wells B_3- F_3 the test organisms and 30 µL of 0.1% resazurin dye were added as culture control in respective plates. 100 µL of the formulated extract was added in respective plate wells and serially diluted by transferring 100 µL of the mixture to subsequent wells up to outermost wells (11^{th} well) and 100 µL of the excess sample was discarded from 11^{th} well respectively, 100 µL of the test organisms and 30 µL of 0.1% of resazurin dye was added to the diluted samples respectively. The plates were incubated at 37° C for 24 hours. The presence of blue color indicates absence of microbial growth whereas pink colour indicates growth of the organism (not inhibited). The lowest concentration of resazurin in which colour change was measured and it is taken as the MIC value.

Cell line and culture

The MCF-7 (human carcinoma cell lines) was obtained from NCCS Pune. The cell was maintained at 37°C during a humidified atmosphere 95% containing 5% CO_2. All cell line was cultured in RPMI-1640 with 10% v/v FBS, 100 U/ml penicillin and 100 mg/ml streptomycin, as previously described[9].

MTT assay

The cytotoxic study was performed with the usage of MTT assay, as formerly described. The cells had been placed in the course of a 96-nicely lifestyle plate with various concentrations (50, 100, 150, 200 and 250 µg/ml) of the Curkolin®. The culture plates had been incubated for twenty-four, forty eight and seventy two h at 37°C and 5% CO_2. Following incubation, 20 µl MTT solutions in PBS was delivered to every well at a final concentration of 0.5 mg/ml observed by in addition incubation for 3 h at 37°C. MTT reagent is removed and then a 100µl DMSO solubilization solution is added. The absorbance was measured at 570 nm with the usage of an ELISA reader (Start Fax 2100; Awareness Technology Inc., Fisher Bioblock Scientific, Tournai, Belgium). The awareness of the Curkolin® which - during a 50% reduction of cellular viability, the 1/2 maximal inhibitory attention, changed into calculated using the following formula:

% Inhibition (control ABS - sample ABS) / (control ABS) ×100.

HPLC Analysis

Content of curcuminoids

Quantitative analysis of Curcuminoids was performed by HPLC with Shimadzu LC20AT series system with UV-Vis detector (operated at 420 nm) with auto injector. Compounds were separated on a C 18, 4.6 X 250 mm, i.e., 5 µm pore size Phenomenex Kinetex column with a flow rate of 0.8 ml per minute. The mobile phase was prepared from 0.1% orthophosporic acid in HPLC grade water and acetonitrile in the ratio 50:50 (v/v). Before use, the mobile phase was filtered through 0.45 µm filter and degassed. The standard was prepared by weighing about 25 mg of the curcuminoids standardized and dissolving it in Tetra hydrofuran (THF) and made up the volume in a 50 ml volumetric flask. 5 ml of this solution was pipetted out into a 50 ml volumetric flask. Diluted to volume with methanol and sonicated. Similarly the sample was prepared. Injected 20 µl of standard and sample separately and data were integrated with Shimadzu LC solution software and results were obtained by comparison with a standard.

Content of Forskolin

Quantitative analysis of forskolin was performed by HPLC with Shimadzu LC20AT series system with UV-Vis detector (operated at 210 nm) with auto injector. Compounds were separated on a C 18, 4.6 X 250mm, i.d., 5 µm pore size YMC Pack ODS column with a flow rate 1ml per minute. The mobile phase was prepared from HPLC grade water and Acetonitrile in the ratio 40:60 (v/v). Before use, the mobile phase was filtered through 0.45 µm filter and degassed. 1mg per ml forskolin standard solution was prepared in methanol. The sample was prepared by weighing about 100 mg of the sample and transfer into a 50 ml volumetric flask. Dissolved it in methanol and made up the volume. Diluted to volume with methanol and sonicated. Injected 20 µl of standard and sample separately and data were integrated with Shimadzu LC solution software and results were obtained by comparison with a standard.

Results and Discussion

Antioxidant activity

In the presence of an antioxidant, the free radical pairs up with the hydrogen donor of the antioxidant molecule and gets reduced to Diphenyl picryl hydrazine (DPPH). On reduction there occurs a color change from purple (DPPH) to yellow (DPPH) with respect to the total number of electrons captured.

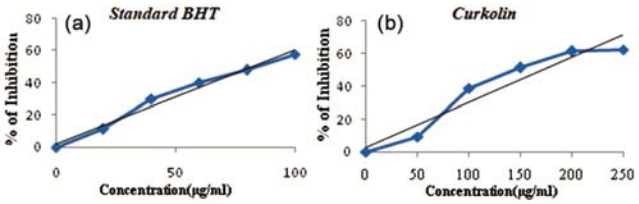

Fig. 1 — Antioxidant activity (IC_{50}µg/ml) of Curkolin® and BHT standard

Table 1 — Minimum Inhibition Concentration (MIC) standard *Tetracycline* and Curkolin® against pathogens

Test Organism	MIC in *Tetracycline* µg/ml	MIC in Curkolin® µg/ml
Bacillus cereus	62.5	250
E-coli	125	500
Salmonella typhi	15.62	500
Staphylococcus aureus	31.25	500
Streptococcus mutans	125	500

Higher the antioxidant activity higher will be the reduction and resultant discoloration[10]. The antioxidant activity of the Curkolin® is presented in Figure1. The Curkolin® activity was compared with standard butylated hydroxyanisole (BHA). The free radical scavenging activity of BHT and Curkolin® were found to be 82.65 and 55.42 µg/ml respectively. So the Curkolin® is more potent antioxidant than the standard compound BHT. Further, its antioxidant activity was good when compared with curcumin and forskolin separately. This study reveals that a combination of *Curcumin* 95% and *Forskolin* 29.46% in the ratio 4:1 forms an excellent antioxidant source due to their synergistic effect.

Antimicrobial activity

Various standard methods were used to evaluate the antimicrobial activities in crude extracts of plant. However, dilution methods have been preferred over other methods, for the determination of MIC which includes broth, agar and MBC broth dilution respectively[11-]. The dilution technique used in this study is an efficient antibacterial technique to determine MIC. The microdilution (resazurin broth) technique was more sensitive compare to the other agar dilution methods; this was clearly observed in the extract of Curkolin® (*Bacillus cereus*, *E-coli*, *Salmonella typhi*, *Staphylococcus aureus, Streptococcus mutans*) in the antibacterial assay (Table 1 and Figure 2 and 3). Resazurin is a redox indicator that is used for the evaluation process of cell growth in cytotoxicity assays, which is a blue non-

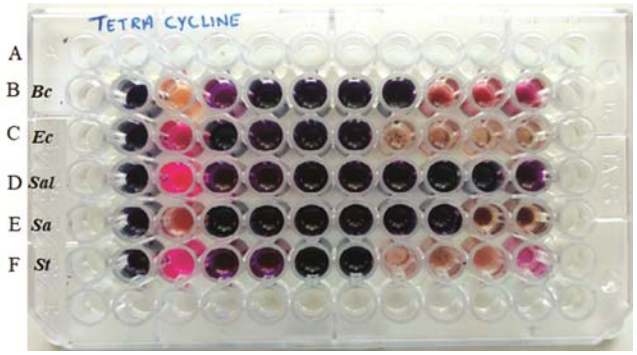

Fig. 2 — Minimum Inhibition Concentration (MIC) Standard *Tetracycline* against pathogens. (*Bc*) *Bacillus cereus,* (*Ec*) - *E-coli,* (*Sal*)-*Salmonella typhi,* (*Sa*)-*Staphylococcus aureus,* and (*St*)-*Streptococcus mutans*

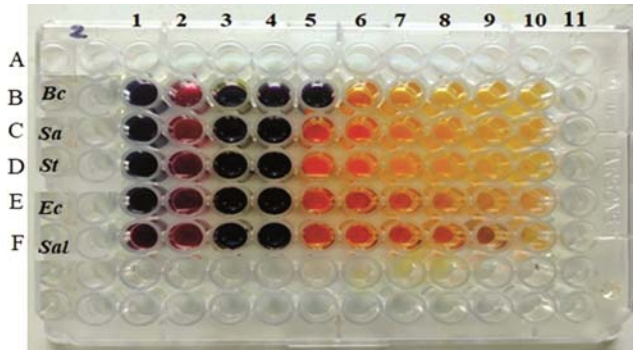

Fig. 3 — Minimum Inhibition Concentration (MIC) Curkolin® against pathogens. (*Bc*) *Bacillus cereus,* (*Sa*)-*Staphylococcus aureus,* (*St*)-*Streptococcus mutans,* (*Ec*) - *E-coli* and (*Sal*)-*Salmonella typhi*

fluorescent and non-toxic dye that changes to pink (fluorescent). The resorufin was reduced to hydroresorufin[12-13]. In the research conducted by using Curkolin®, the sample expressed the least MIC value for *Bacillus cereus* (Bc) that is 250 µg/ ml and MIC was 500 µg/ml for the pathogens *Staphylococcus aureus* (Sa), *Streptococcus mutans*, *E. coli* (Ec) and *Salmonella typhi* (Sal). Further, its antibacterial activity was good when compared with curcumin and Forskolin separately.

Cytotoxic activity

The cytotoxic activity of the Curkolin® on MCF-7 cell line was investigated in vitro using 3-(4) 5-Dimethyl-thiazol-Zyl) - 2, 5 biphenyl tetrazolium bromide (MTT) assay are shown in Figure 4. The results showed decreased cell viability and cell growth inhibition during a dose dependent manner. The IC_{50} value of doxorubicin 33.7 µg/ml and Curkolin®135.8 µg/ml was recorded, respectively (Fig. 4). Curkolin® demonstrated strong anti-proliferative activities. Further, its cytotoxic

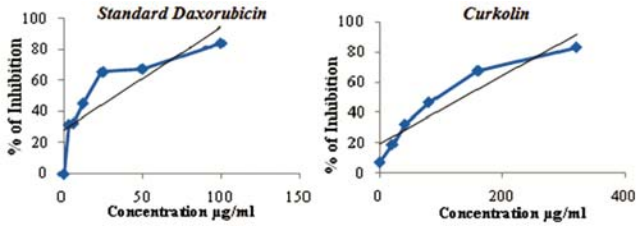

Fig. 4 — Cytotoxic activity (IC_{50} µg/ml) of Curkolin® and *Doxorubicin* standard

activity was good as compared with the cytotoxicity activity of curcumin and forskolin separately.

The secondary metabolites in plants are answerable for the principle cytotoxic effect which has the metabolites like alkaloids, glycosides, steroids, tannins, phlobatannins, terpenoids and flavonoids in their extract[14]. The MTT enters the cells and passes into the mitochondria where it's reduced to an insoluble, coloured (dark purple) formazan product. Since reduction of MTT can only occur to metabolically active cells the extent of activity could also be a measure of the viability of the cells[15-16]. Curkolin® demonstrated strong anti-proliferative activities. Further, its cytotoxic activity was good as compared with the cytotoxicity activity of curcumin and forskolin separately.

HPLC analysis

HPLC chromatogram of curcuminoids and forskolin peaks are shown in Figure 5. From the figure, it's evident that the retention times for demethoxycurcumin, bisdemethoxycurcumin, and curcumin were found to be 9.47, 10.09 and 10.75, respectively. Separation of curcuminoids by HPLC was conducted employing a C18 column (column conditions of isocratic elution, 50 ml 0.1% orthophosporic acid in HPLC grade water and 50 ml acetonitrile solution, constant flow rate at 0.8 ml/min, at temperature 25±1° with detector was set at 420 nm). From the detection wavelength, it's clear that no interference from diluents, impurities, excipients present within the curcuminoids sample. Sharp and symmetrical peaks were obtained for curcuminoids when analyzed under standard defined conditions. The curcuminoids content in Curkolin® is 76.4%.

Chromatograms of forskolin peaks are shown in Figure 5. The RT (retention time) for forskolin were found 9.50. The HPLC analysis for forskolin was performed using a C18 column by an elution with HPLC grade water and acetonitrile in the ratio 40:60

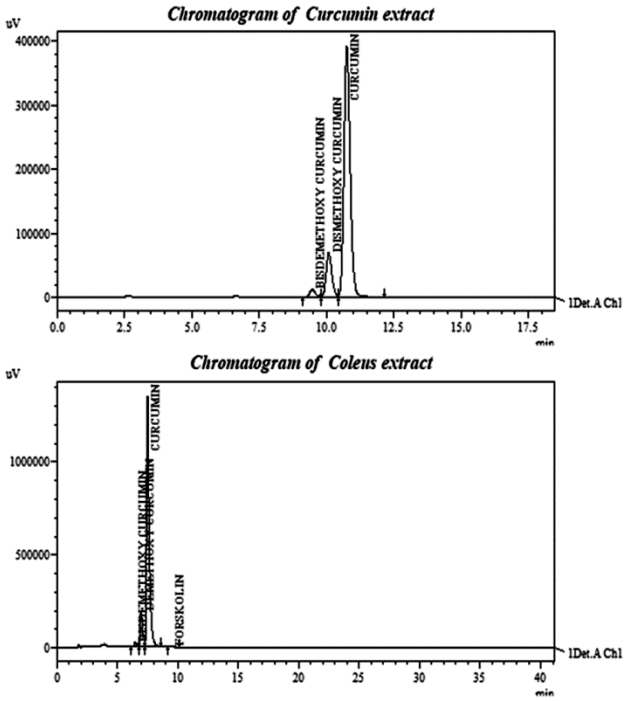

Fig. 5 — HPLC finger prints of Curcuminoids and Forskolin in Curkolin®

with constant flow rate at 1 ml/min at temperature 25°C and detector were set at 210 nm. No interference was faced from diluents, impurities, or excipients present in the *C. forskohlii* sample. When forskolin was anaylsed under these conditions sharp and symmetrical peaks were obtained. The content of forskolin in Curkolin® is found to be 5.83%.

Conclusion

The Curkolin® revealed strong antioxidant and antimicrobial agent. Curkolin® in the present investigation decreased cell viability and exhibited cell growth inhibition in a dose dependent manner. The referred study concludes: a combination of curcumin and forskolin in the ratio 4:1 forms an excellent antioxidant source, and possesses antimicrobial and cytotoxic activity due to their synergetic effect. Although there are some reports on the antioxidant, antimicrobial and cytotoxic effects of *C. longa*[17-19] and *C. forskohlii*, the activities of formulated *C* longa L. and C. forskohlii were similar to previous findings. Thus, the present findings support the previous findings which indicate the potent antioxidant, antimicrobial and cytotoxic activities of *C. longa* and *C. forskohlii* extract. This study serves as a scientific data for the usage of Curkolin® for treatment of microbial infections as a

traditional medicine and also for its use as a rich antioxidant source.

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Conflicts of Interest

Authors declare that there are no conflicts of interest

Author Contributions Statement

All the authors have contributed equally.

References

1 Chattopadhyay I, Biswas K, Bandyopadhyay U, *et al*., Turmeric and curcumin: Biological actions and medicinal applications, Review articles, *Curr Sci*, 87 (1) (2004).

2 Nemudzivhadi V & Masoko P, In Vitro Assessment of Cytotoxicity, Antioxidant, and Anti-Inflammatory Activities of *Ricinus communis* (Euphorbiaceae) Leaf Extracts, *Evid Based Complement Alternat Med*, (2014) Article ID 625961.

3 Valdes L J, Mislankar S G & Paul A G, *Coleus barbatus* (*C. forskohlii*) (Lamiaceae) and the potential new drug forskolin (Coleonol), *Econ Bot*, 44 (1987) 474-483.

4 Shah V & Kalakoti B S, Development of *Coleus forskohlii* as a medicinal crop. In: International Conference on Domestication and commercialization of non-timber forest products in agro forestry systems, Food and Agriculture Organization of the Unaed Nations corporate document repository, (1996).

5 Patel M B, Forskolin: A successful therapeutic phytomolecule, *East and Central African J Pharmac Sci*, 13 (2010) 25- 32.

6 Patel T & Saraf M, A review on the pharmacology of *Coleus Forskohlii* Briq:-A threatened medicinal plant, *Research Gate*, (2016).

7 Santos U P, Campos J F, Torquato H F, *et al*., Antioxidant, antimicrobial and cytotoxic properties as well as the phenolic content of the extract from *Hancornia Speciosa Gomes*, *PLOS ONE*, 11 (12) (2016) doi:10.1371/journal. pone.0167531.

8 Blois M S, Antioxidant determinations by the use of a stable free radical, *Nature*, (1958) 1199-1200.

9 Toktam M, Toktam H, Hossein K, *et al*., Evaluation of the cytotoxic effects of *Cyperus longus* extract, fractions and it's on the PC3 and MCF7 lines, *Oncology Letters*, 11(2016) 1353- 1360.

10 Ghasemzadeh1 A, Azarifar M, Soroodi O, *et al*., Flavonoid compounds and their antioxidant activity in extract of some tropical plants, *Journal of Medicinal Plants Research,* 6 (13) (2012) 2639-2643.

11 Wiegand I, Hilpert K, & Hancock R E W, Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances, *Nature Protocols*, 3 (2) (2008) 163–175.

12 Sarker S D, Nahar L & Kumarasamy Y, Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth and its application in the in vitro antibacterial screening of phytochemicals, *Methods* (*San Diego, Calif*), 42 (2007) 321–324.

13 Rahman M A & Islam M S, Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole *Leucas aspera* extract, *Asian Pacific J Trop Biomed*, (2013) doi: 10.1016/S2221-1691(13)60062-3.

14 Gini E J, Sivakumar T & Kuppuswamy S, Preliminary phytochemical screening, evaluation and comparison of anticancer activity of *Ficus gibbosa* blume by MTT assay method, *Int J Res Pharmac Sci*, 7 (3) (2016) 190-194.

15 Yallapu M, Othman M, Curtis S, *et al*., Curcumin-loaded magnetic nanoparticles for breast cancer therapeutics and imaging applications, *Int J Nanomed*, 7 (2012) 1761-1779.

16 Islam A, Rebello L & Chepyala S, Primary evaluation of anticancer activity of Nanocurcumin® against human breast cancer cell line, SkBr3, *Int J Nat Life Sci*, 3 (2019) 13-19.

17 Lin W, Cooper C & Camarillo I, The effectiveness of electroporation-based nanocurcumin and curcumin treatments on human breast cancer cells, In: Proceedings of ESA Annual Meeting on Electrostatics, University of Notre Dame, Notre Dame, Indiana., USA, *Electrostatics Society of America*, (2014) 1-7.

18 Islam A, Rebello L & Chepyala S, Review on nanoformulations of Curcumin (*Curcuma longa* Linn.): Special Emphasis on Nanocurcumin®, *Int J Nat Life Sci,* 3(1) (2019) 1-12.

19 Khatun S, Çakılcıoğlu U & Chatterjee N C, Phytochemical constituents vis-a-vis histochemical localization of forskolin in a medicinal plant Coleus forskohlii Briq,- *J Med Plants Res*, 5 (5) (2010) 711-718.