ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Phytoconstituents Profiling and Evaluation of Antimicrobial and Antioxidant Attributes of Methanolic Extract of *Centella asiatica.*

Manisha K Byakodi, Zabin K Bagewadi*, and Uday M Muddapur.

Department of Biotechnology, KLE Technological University, Vidyanagar, Hubballi, Karnataka 580031, India

ABSTRACT

The present study aims at evaluation and characterization of phytoconstituents of medicinally important plant *Centella asiatia* and screening for antimicrobial and antioxidant activities of the plant extracts. Methanolic extract of the whole plant was analysed for various phytoconstituents. Preliminary phytochemical screening and quantitative analysis of alkaloids, phenols, tannins, flavonoids, terpenoids and saponins was carried out. Antibacterial activity of the extract was tested by agar-well and disc diffusion method against pathogenic bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa, Zymomonas mobilis, Micrococcus luteus, Staphylococcus aureus, Bacillus Subtilis* and *Bacillus cereus*. Antifungal activity was tested against fungal strains like *Aspergillus niger, Aspergillus sydouri* and *Trichoderma reesei* by fungal disc method. Antioxidant potential was determined by $ABTS^{\bullet+}$ [2,2´-azinobis(3-ethyl-benzothiazoline-6-sulfonate)] and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Structural elucidation of the extract was carried out by (FTIR) analysis. The methanolic extract of *Centella asiatia* constituted of alkaloids (15.66µg/mg extract), flavonoids (13.2 µg/mg extract), phenols (43.7 µg/mg extract), tannins (30.09 µg/mg extract), Terpenoids (3.08 µg/mg extract) and saponnins (52.1 µg/mg extract). Extract were found to be rich in phenolic compounds. *Centella asiatia* exhibited antimicrobial activities and showed highest zone of inhibition for *Pseudomonas aeruginosa* (15 mm) and *Micrococcus luteus* (15 mm) as well as ABTS and DPPH radical scavenging activity of 31.76% and 27.33% which is greater than the standards ascorbic acid and butylated hydroxytoluene (BHT) antioxidant activities. FTIR results revealed the presence of carboxylic, hydroxyl, aromatic rings, alkyl and amine groups. The present study reveals that the potential phytochemicals can prove to be an alternative source of medicine and could be boon for the treatment of human health problems.

Keywords: Phytoconstituents, antimicrobial, antioxidant, FTIR.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Centella asiatica is a medicinal herb and its use is dated back to the ancient medicinal systems such as Ayurveda. Its use has not only been stated in Indian medicinal system but also in Chinese medicinal system. Its use has also been found in countries like Srilanka, Nepal and Madagascar. In the ancient Indian medicinal system (ayurveda) *Centella asiatica* is termed as "Brain Tonic", as it has been known to revitalize the brain cells and nerve cells [1]. It is commonly known as Gotukola [2] and Indian Pennywort [1]. Formerly it was confused with *Baccopa monneri* as both the plants were known as brahmi [1]. *Centella asiatica* belongs to Apiaceae family. It grows well in warm-climate countries [2]. The plant morphology is displayed in figure 1.

Centella asiatica is a traditional medicinal herb with high pharmaceutical importance. Number of biochemical compounds are obtained from aquaeous and alcoholic extracts of the whole plant or parts of plants especially leaves. Most of the compounds obtained are proven to be of pharmaceutically important. The components of extract show high antioxidant activity, which decreases amyloid-β (Aβ) deposition in the brain. It was also found that it has excellent radical scavenging activities, antmicrobial and antifungal activities [3]. The *Centella asiatica* leaves showed vast range for micronutrients like Mn (25.2–90.1 mg), Cu (2.9–46.8 mg), Na (23.6–697.7 mg), Zn (16.6–122.9 mg), Ca (1354.9–2870.8 mg), Fe (112.4–247.2 mg), and Mg (398.0–757.4 mg) [4], this makes it as excellent source of micronutrients that can be added in dietary supplements and also in medicinal use. Its stated that *Centella asiatica* extract is also rich in phenols and terpenoids. [3]

The present study focuses on the evaluation of phytoconstituents of medicinally important plant *Centella asiatica,* and screening for antimicrobial and antioxidant activities of the plant extracts. Further research targets on the structural elucidation of chemical compounds by FTIR analysis.

MATERIALS AND METHODS

Chemicals and standards

In the present study, all the chemicals and standards atropine, gallic acid, quercetine, linalool and diosgenin used were purchased from Hi-Media, Sigma-Aldrich. All the reagents and solvents used were of analytical grade.

Plant material and extraction

The plant *Centella asiatica* was collected from the nursery, maintained by Saidapur farms, Agricultural University, Dharwad, Karnataka. The plant was uprooted early in the morning, washed thrice with distilled water and shade dried in room temperature for 15 days. Then it was powdered with electric grinder into coarse powder and maintained at 4 °C for further use. The phytochemicals from the powdered plant sample was extracted in with 200 ml methanol using Soxhlet apparatus operating at 60 ºC for 6 hours. The extract obtained was concentrated using rotary evaporator (Bibbly Sterlin RE2022C). The extract thus obtained was stored in brown glass bottle in the refrigerator at 4 ºC.

Phytochemical profiling

The preliminary phytochemical profiling was carried out to confirm the presence of the bioactive secondary metabolites present in the methanolic extract of the plant *Centella asiatica.* Various phytochemicals were analyzed for their occurrence by standard biochemical tests according to previously published methods [5].

Quantitative estimation of phytochemical constituency

Alkaloid determination

Alkaloids were determined by using bromocresol green solution (BCG) as described by Shamsa et al. [6]. BCG solution was prepared by adding 6.98 mg BCG to 0.3 ml of 2 N NaOH and 5 ml distilled water until it was completely dissolved. This solution was further diluted to 100 ml by adding distilled water. Phosphate buffer (pH 4.7) was prepared by adjusting 2 M sodium phosphate solution to pH 4.7 by adding 0.2 M citric acid.

For the analysis, 0.1 ml extract (50 mg/ml) was taken and 4.9 ml phosphate buffer was added to it. To all these test tubes 5 ml of BCG reagent was added, followed by 4 ml chloroform. The mixture was vortexed and allowed to stand for 5 min for the layers to distinctly separate. The absorbance of the yellowish complex formed in the chloroform layer was measured at 470 nm against blank. Atropine was used as the standard in the range of 50-500 µg/ml. The assay was carried out in triplicates and the total alkaloids were expressed in Atropine Equivalents.

Flavonoid determination

Flavonoids were estimated by aluminium chloride assay as described by Pourmorad et al. [7]. The sample (10 µl) was taken and diluted to 1 ml by adding methanol. To it 0.3 ml $NaNO_2$ (5% w/v) was added followed by 5 min of incubation. Further, 0.3ml of 10% $AlCl_3$ was added to it and incubated for 6 min and then 2 ml of 1 M NaOH solution was added to it. The total volume was made up to 10 ml by adding distilled water and this final solution was vortexed. The absorbance was read at 510 nm. Quercetin was used as the standard in the range of 20-100 µg/ml. The assay was carried out in triplicates and total flavonoids were expressed in Quercetin Equivalents.

Determination of total phenols

The total phenolics content of *Centella asiatia* was estimated using Folin-Ciocalteau reagent as described by Senguttuvan et al. [8]. 10 µl of sample was taken and diluted to 1 ml by methanol. To it 500 µl of FCR (1:2) reagent was added followed by 2.5 ml of 20 % Na_2CO_3. This was incubated in dark conditions for 40 min. The absorbance was measured at 750 nm. Gallic acid was taken as the standard in the range 10-50 µg/ml. The assay was carried out in triplicates and total phenols were expressed in Gallic acid Equivalents.

Tannin estimation [8]

For the analysis, 500 µl of sample was taken and to it 100 mg polyvinyl polyprrolidone and 500 µl distilled water was added. This was kept at 4 ºC for 4 h. Further this was centrifuged at 8,000 rpm for 5 min and 20 µl of the supernatant was used for analysis. This supernatant consists of only simple phenolics free of tannins as tannins would have been settled down as precipitate along with polyvinyl polyprrolidone. The simple phenolic content of supernatant was analysed by FCR method and the absorbance was measured at 725 nm and expressed as free phenolic content. Thus the tannins were estimated by the following equation,

Tannin content = Total phenolic content– Free phenolic content.

The assay was carried out in triplicates and total tannins were expressed in Gallic acid Equivalents.

Terpenoid estimation[9]

In this method, 50 µl of sample was considered and it was diluted to 200 µl by addition of methanol. To this sample, 1.5 ml chloroform was added and was subjected to vortex and then allowed to rest for 3 min. then 100 µl of concentrated H_2SO_4 was added to it and this mixture was incubated for 1.5 – 2 h under dark. After incubation the supernatant is discarded carefully and the brown precipitate is retained. This brown precipitate is further dissolved in 1.5 ml of 95% methanol and mixed thoroughly. The absorbance was recorded at 538 nm. Linalool was taken as the standard for the assay in the range of 4-20 mg/200 µl of methanol. The assay was carried out in triplicates and total Terpenoids were expressed in Linalool Equivalents.

Saponins estimation[8]

This was carried out by Vanillin-sulphuric acid colorimetric reaction. 10 µl of sample was taken and to it 250 µl distilled water was added followed by Vanillin reagent (0.4 g in 5 ml of 99.5 % ethanol). Further 2.5 ml of 72 % H_2SO_4 was added and heated at 60 ºC for 10 min in water bath. The mixture was allowed to cool and the absorbance was recorded at 544 nm. Diosgenin was taken as the standard for the assay in the range of 10-50 µg/ml of ethanol. The assay was carried out in triplicates and total Saponins were expressed in Diosgenin Equivalents.

Antibacterial activity

The antibacterial property of the methanolic extracts were assessed against various gram positive bacterial strains such as *Micrococcus luteus, Staphylococcus aureus, Bacillus Subtilis* and *Bacillus cereus* and gram negative strains like *Escherichia coli, Pseudomonas aeruginosa* and *Zymomonas mobilis,* These strains were procured from National Collection of Industrial Microorganisms (NCIM), Pune and Microbial Type Culture Collection (MTCC), Chandigarah.Two assays were carried out:

Agar well method

Nutrient agar plates were prepared. Nutrient broth containing 24 hour old bacterial cultures was used for analysis. About 100-200 µl of extract was loaded in the 6-7 mm diameter agar well in the agar plate. 0.1 ml bacterial culture was spread. Methanol was used as blank (negative control). The plates were incubated (37 °C) for 24-36 h and zone of clearance (diameter) was measured in mm [10].

Disc Diffusion method

Filter paper discs were prepared and autoclaved. Nutrient agar plates were spread with 0.1 ml of 24 hours old bacterial cultures. The filter paper discs impregnated with 10-20 µl of extract were placed on the agar plates. These plates were kept for incubation at 37°C for 36 h and zone of clearance (diameter) was measured in mm [11]

Antifungal activity

The antifungal activity was assessed by disc method. Potato dextrose agar plates were prepared with addition of 1 ml of extract for 100 ml of media, after autoclaving the media. Then the fungal discs 6-7 mm in diameter were placed on to the agar plates and the plates were incubated for 5 days at room temperature. After incubation the zone of fungal growth was compared with the control agar plate (without extract) which consisted only of the potato dextrose media. Results were analyzed based on comparative growth on control and test plates. The fungal strains tested were *Aspergillus niger, Aspergillus sydouri* and *Trichoderma reesei* [12].

Antioxidant Activity

The radical scavenging activity was tested for two radicals namely ABTS and DPPH

$ABTS^{\bullet+}$ radical scavenging assay

$ABTS^{\bullet+}$ [2, 2′-azinobis (3-ethyl-benzothiazoline-6-sulfonate)] radical scavenging assay was carried out according to methods described earlier [8, 13]. 5 ml of $ABTS^{\bullet+}$ solution (7 mM $ABTS^{\bullet+}$ and 2.45 mM Potassium persulphate) was prepared was kept for incubation in dark conditions for 12 hours at room temperature so as to form a dark blue-green complex. Later, this solution was diluted with ethanol (1:50) and the absorbance was adjusted to OD equal to 0.7±0.001 at 734 nm. 2 ml of this solution was taken and 20 µl of sample was added to it. This was kept for incubation in dark conditions at room temperature for 30 min. The absorbance was recorded immediately after 30 min at 734 nm. The total antioxidant activity unit was defined by the following equation:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where Abs control is the absorbance of ABTS radical in methanol, Abs sample is the absorbance of an ABTS radical solution mixed with sample. All determinations were performed in triplicates (n=3). 0.1 mM Ascorbic acid in methanol and 0.1 mM butylated hydroxytoluene (BHT) in methanol was used as reference.

DPPH radical scavenging assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was executed according to previously described methods [13,14] . 2.7 ml of purple colour DPPH radical solution (6×10^{-5} mol in 1 litre of methanol) was added to 0.1 ml of sample and mixed thoroughly. This was incubated in at room temperature in dark conditions for 1 hour. The absorbance was recorded at 517 nm. The radical-scavenging activity was calculated as a percentage of DPPH discoloration using the equation:

$$\text{DPPH radical scavenging activity}(\%) = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where Abs control is the absorbance of DPPH radical in methanol; Abs sample is the absorbance of a DPPH radical solution mixed with sample. All determinations were performed in triplicates (n=3). 0.1 mM Ascorbic acid in methanol and 0.1 mM butylated hydroxytoluene (BHT) in methanol was used as reference.

FTIR analysis of *Centella asiatia* extract

The FTIR analysis of the crude extract was carried out by KBr (Potassium bromide) method using FTIR (Perkin Elmer, FTIR1760) instrument. The extract was dried and prepared in powdered form for analysis. KBr extract pellets were prepared for analysis by applying pressure. The infrared transmittance spectral data was obtained with scanning range of 500-4000 cm^{-1} [15]

RESULT AND DISCUSSION

Preliminary profiling of phytoconstituents

The biochemical tests were carried out to detect the presence of various phytoconstituents in the crude extract and the results are as listed in the Table 1. The tests showed the presence of major phytoconstituents like alkaloids, flavonoids, phenols, tannins, terpenoids and saponins.

Quantitative analysis

The results of the estimation of major phytoconstituents i.e. alkaloids, flavonoids, phenols, tannins, terpenoids and saponins is as stated in Table 2. The results showed that *Centella asiatica* is rich in Total Phenols and saponins. The methanolic extract of *Centella asiatica* constituted of alkaloids (15.66 µg/mg extract), flavonoids (13.2 µg/mg extract), phenols (43.7 µg/mg extract), tannins (30.09 µg/mg extract), Terpenoids (3.08 µg/mg extract) and saponnins (52.1 µg/mg extract). According to the quantitative analysis done by Biradar and Rachetti, the phytochemicals in the ethanolic extract of *Centella asiatica* was found to be as follows: alkaloid (62 µg/ mg extract), flavonoid (180 µg/ mg extract), terpenoid (90 µg/ mg extract) and saponin content (50 µg/ mg extract). When the present study is compared to his research it was found that, alkaloid, flavanoid and terpenoid content was higher in the ethanolic extract than in the methanolic extract. But the sapponin content was found to be more in ethanolic extract than in methanolic extract [16]. In another report, it was found that ethanolic extract gave phenolics (120.0–318.0 mg) and tannin (206.6–856.6 mg) per 100g of fresh leaves [4]. When these results are compared to the present study, it also stated that Tannins and phenols content was more in ethanolic extract. The results showed a good content of phenolic compounds in methanolic extract of *Centella asiatica*. Overall it can be stated that ethanol is a better solvent that can be used for extraction.

Antibacterial Activity

The antibacterial results of *Centella asiatica* crude extract against tested bacterial strains are shown in Table 3. The result showed that the trend followed for zone of inhibition is almost the same for both the tested methods. The average zone of inhibition showed by the plant extract for gram positive strains is 12.5 mm and for gram negative nature is 12.0 mm. *Centella asiatica* exhibited antimicrobial activities and showed highest zone of inhibition for *Pseudomonas aeruginosa* (15 mm) and *Micrococcus luteus* (15 mm). This states that the extract inhibits the growth of both gram nature bacteria almost to the same extent. The results are different when compared to the research carried out by Valsaraj *et al* [17]. In his research it was found that *Centella*

asiatica didn't inhibit the growth of *P. aeruginosa* and *E. coli*. It is also stated in the same research that *Centella asiatica* inhibited B. subtilis and S. aureus growth. In another research published by Kumar *et al* [18], it was stated that *Micrococcus luteus* showed 12 mm zone of inhibition. Thus it can be concluded that methanolic extract of *Centella asiatica* is a rich source of antibacterial agents.

Antifungal activity

The antifungal activity tested against the fungal strains showed a positive growth inhibition result as shown in Table 4. The extract showed maximum activity for *A. niger* which is in correspondence to the results of research done by Kumar *et al* [18]. The plant extract inhibits the fungal reproductive as well as vegetative growth significantly.

Antioxidant assay

The crude extract of *Centella asiatica* was tested for antioxidant activities. The extract showed potential antioxidant properties as shown in Table 5. *Centella asiatica* proved to be better than the reference antioxidant chemicals such as ascorbic acid and BHT. In the present research it is seen that *Centella asiatica* showed 27% DPPH radical scavenging activity and 31% ABTS radical scavenging activity at 50mg/ml plant extract. But, the results vary when compared to the study published by Subhasree *et al, Centella asiatica* showed 65% DPPH radical scavenging activity and 45% ABTS radical scavenging activity at 250µg/ml plant extract. In the research the extraction was done at 37 ºC and in 10% methanol for 36 hours [19]. Thus it states that the extraction method adopted by B. Subhasree *et al,* is better when compared to the extraction method in the present study, for the analysis of antioxidant activities of *Centella asiatica*.

FTIR analysis of *Centella asiatica*

The FTIR analysis showed a total of 13 peaks with various types of functional groups present in the extract as shown in Figure 2. The analysis showed a number of bonds including aliphatic and aromatic chains and rings. As illustrated in Table 6, the most probable compounds that may be predicted to be present in the extract were : Baicalein which is antivirus, anti-bacterial, protecting the liver function, anti-inflammation, diuresis, anti-tumorous compound [20, 30], Ursolic Acid has protective effect on lungs, kidneys, liver and brain, exhibits anti-inflammatory properties, anticancerous properties, anabolic effects on skeletal muscles, ability to suppress bone density loss leading to osteoporosis, anti-microbial features against numerous strains of bacteria, HIV and HCV [21, 31], 4,6-dimethoxy-2,5-quinodihydrochalcone which is found to be cytotoxic [22, 32], 2,3-dihyro-3,3'4'5,7-pentahydroxy flavones, protocatechuic acid which shows antioxidant properties [23, 33], **3-hydroxy-4 methoxybenzaldehyde [24],** p-coumaric acid which is anti-inflammatory and shows protective effects on cardiac apoptosis [25, 34, 35], Allouzarin, Xysmalorin are found in medicinally important plant *Xysmalobium undulatum* [26, 36], Peperotetraphin is anticancerous compound [27, 37], gastrol A [28] and β-sitosterol is usually used for cardiac disease, hypercholesterolemia, immunomoduation, cancer and rheumatoid arthritis and also in treatment of tuberculosis, cervical cancer, hair loss and benign prostatic hyperplasia [29, 38].

CONCLUSION

The methanolic extract of *Centella asiatica* was proved to be rich in pharmacologically important compound like alkaloids, phenols, flavonoids, tannins, terpenoids and saponins. These compounds play the major role in the antibacterial, antifungal and antioxidant activities. The FTIR analysis stated the presence of functional groups like carboxylic, hydroxyl and aromatic. The compounds baicalein, ursolic Acid, 4,6-dimethoxy-2,5-quinodihydrochalcone, protocatechuic acid, p-coumaric, β-sitosterol that may to be present are stated to be antibacterial, antifungal, anticancerous compounds. Thus *Centella asiatica* is a pharmacologically important plant and can be used in herbal medicine preparation which is a better alternative when compared to the chemically synthesised drugs.

AKNOWLEDGEMENT

The authors thank KLE Technological University, Hubballi for partially supporting and funding this research work through capacity building projects.

REFERENCES

[1] Sakshi Singh, Asmita Gautam, Abhimanyu Sharma and Amla Batra, *Int J Pharm Sci Rev Res*. 2009, 4(2), article 003.

[2] Chong, Nyuk Jet & Aziz, Zoriah. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* 2011, 2, 445 - 459.

[3] Chien-Li Chen, Wen-Hao Tsai, Chun-Jen Chen, Tzu-Ming Pan., *Journal of Traditional and Complementary Medicine.* 2016, 6(4), 362-369

[4] Shrawan Singh, D.R. Singh, V. Shajeeda Banu, Avinash N, *Industrial Crops and Products, 2014,* 61, 115–119

[5] Harborne, A.J., *Phytochemical methods a guide to modern techniques of plant analysis*. Chapman and Hall. New York. 1998

[6] Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi and Mohammadreza Verdian-rizi , *Thai J. Pharm. Sci*., 2008, 32, 17-20

[7] F. Pourmorad, S. J. Hosseinimehr, N. Shahabimajd, *Afr. J. Biotechnol*, 2006, 5 (11), 1142-1145

[8] Jamuna Senguttuvan, Subramaniam Paulsamy, Krishnamoorthy Karthika, *Asian Pacific Journal of Tropical Biomedicine,* 2014, 4, 359-367

[9] Narayan Ghorai, Sondipon Chakraborty, Shamik Gucchait, Samir Kumar Saha & Suman Biswas, *Protocol Exchange*, 2012.

[10] Hammer KA, Carson CF, Riley TV. *J. Appl. Microbiol*. 1999, 86, 985-990.

[11] Karaman I, Sahin F, Güllüce M, Ogütçü H, Sengül M, Adigüzel A., *J Ethnopharmacol*. 2003, 85(2-3), 231-5.

[12] Kwiatek, Dorota & Kubicki, Maciej & Belter, Jolanta & Jastrzab, Renata & Wiśniewska, Halina & Lis, Stefan & Hnatejko, Zbigniew, *Polyhedron*, 2017,133

[13] Magdalena Kondeva-Burdina, Dimitrina Zheleva-Dimitrova, Paraskev Nedialkov, Ulrich Girreser, Mitka Mitcheva, *Phcog Mag*, 2013, 9, 294-301

[14] Lillian Barros, Maria-Joao Ferreira, Bruno Queiro´ s, Isabel C.F.R. Ferreira, Paula Baptista, *Food Chemistry*, 2007, 103, 413–419

[15] Zabin K. Bagewadi, Sikandar I. Mulla, Harichandra Z. Ninnekar, *3 Biotech*, 2016, 6, 101

[16] Sanjay R. Biradar, Bhagyashri D. Rachetti, *American Journal of Life Sciences*,2013, 1(6), 243-247

[17] R. Valsaraj , P. Pushpangadan , U.W. Smitt, A. Adsersen , U. *Nyman Journal of Ethnopharmacology,* 1997, 58, 75-83

[18] Aniel Kumar Owk and Mutyala Naidu Lagudu, *Asian Journal of Clincal Research*, 2016, 2, 24-29

[19] B. Subhasree, R. Baskar, R. Laxmi Keerthana, R. Lijina Susan, P. Rajasekaran, *Food Chemistry*, 2009, 115, 1213–1220

[20] Biswanath Dinda, Bikas Chandra Mohanta, Shio Arima, Nariko Sato, and Yoshihiro Harigaya, *Natural Product Sciences*, 2007, 13(3), 190-194

[21] G. Arunachalam, Paromita Ba2 and D.Chattopadhyay, *J. Pharmacognosy Phytother*, 2009, 1, 001-013

[22] K. M. Hazra, R. N. Roy, S. K. Sen and S. Laskar, *Afr. J. Biotechnol.,* 2007, 6 (12), 1446-1449

[23] Abdul Wajid Khan, Saleem Jan, Shaista Parveen, Rahmat Ali Khan, Asma Saeed, Abdul Jabbar Tanveer, Anwar Ali Shad, *Chemistry Central Journal*, 2018

[24] Sanghyun Lee , Bak-Kwang Kim , Seon Haeng Cho, Kuk Hyun Shin, *Arch Pharm Res*, 2002, 25, No 3, 280-284

[25] Rengasamy Ragupathi Raja Kannan, Rajasekaran Arumugam and Perumal Anantharaman, *Current Bioactive Compounds,* 2011, 7, 118-125

[26] K N Sunil Kumar, R Shakila, K Balakrishna, S Amerjothy, *Indian Journal of Chemistry*, 2015, 54B, 924-929

[27] Majid Ghorbani, Macki Kaloga, Hans-Hasso Frey, Gisela Mayer, and Eckart Eich, *Planta Med.* 1997, 63, 343-346

[28] N. Li, K.-J. Wang, J.-J. Chen, J. Zhou*, Journal of Asian Natural Products Research*, 2007, 9 (4), 373–377

[29] Mohd Zulkhairi, S. Mohd Aspollah, E. Gwendoline Cheng Lian and A. Ahmad Bustamam, *J. Trop. Agric. and Fd. Sc.,* 2017, 45(2), 187-197

[30] Zhang, X.-P & Li, Z.-F & Liu, X.-G., *Chinese Pharmacological Bulletin*, 2001, 17, 711-713.

[31] Łukasz Wo´zniak , Sylwia Sk ¸apska and Krystian Marszałek, *Molecules* 2015, 20, 20614–20641

[32] Andivelu Ilangovan , Shanmugasundar Saravanakumar and Subramani Malayappasamy, *Org. Lett.*, 2013, 15 (19), 4968–4971

[33] Sahil Kakkar and Souravh Bais, *ISRN Pharmacology,* 2014, Article ID 952943

[34] Ya Zhao, Jianxing Liu , Chunping Liu , Xing Zeng , Xiong Li and Jin Zhao, *Med chem* (Los Angeles), 2016, 6 (5), 327-330
[35] Stanely Mainzen Prince P, Roy AJ, *Int J Cardiol*., 2013, 168(4), 3259-3266
[36] Majid Ghorbani , Macki Kaloga , Hans-Hasso Frey, Gisela Mayer, Eckhart Eich, *Planta Med,* 1997, 63(4), 343-346
[37] Li, Y., He, N. & Zhai, C. *Med Oncol*, 2015, 32,(2)
[38] Saeidnia, Soodabeh & Manayi, Azadeh & Gohari, Ahmad & Abdollahi, Mohammad, *European Journal of Medicinal Plants*, 2014, 4, 590-609.