Indian J. Applied & Pure Bio. Vol. 36(2), 443-453 (2021). **ISSN: 0970-2091**
A web of Science Journal

Phytochemical screening and antimicrobial potential of the flower extracts of *Hibiscus rosa sinensis* L., *Moringa oleifera* Lam. and *Catharanthus roseus* (L.) G.Don

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Abstract

The use of preservatives is an essential part of food industry as majority of the food comes with a limited shelf life naturally . In the last few years, there is a trend to explore the antimicrobial properties of plants to control the spoilage and harmful pathogenic bacteria present in food. The reason is not only the limited use of chemical preservatives but also growing concerns about the toxicological ,health and environmental issues. This study explores the potential use of theplant extracts due to their inherent antimicrobial properties as natural preservatives. The flower extracts some commonly growing plant species of *Hibiscus rosa sinensis, Moringa oleifera* and *Catharanthus roseus* were phytochemically profiled and their antimicrobial ability against four major bacterial strains, *Bacillus subtilis* (MTCC 9003), *Escherichia coli* (MTCC10312), *Pseudomonas aeruginosa* (MTCC 104) and *Staphylococcus aureus* (MTCC 106) was studied. Phytochemical profiling revealed phlobatannins as the major constituent of the flower extracts, and effective antimicrobial activity was observed as indicated by the zones of inhibition.

Food intake is an integral process for sustaining life. Access to safe food which is free from physical, chemical and microbiological hazards is important. The basic principles of food preservation should be adeptly applied to eradicate or control incidences of food-borne illnesses and diseases. Preservation of food can be achieved by employing various techniques such as canning, freezing, pasteurization, fermentation, irradiation etc. Among these one of the sustainable methods for extending shelf life is the use of chemical preservatives.

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Many chemical preservatives like benzoate, sulphate, sorbate, hydrogen peroxide and nitrites are widely used to preserve the food. However, various health hazards were reported with the consumption of food containing such type of synthetic preservatives. So, researchers are now turning their focus to isolate medicinal plant-based preservatives which are safe for human consumption, easily degradable, readily available and economical. Because of this demand shift, plants with known traditional therapeutic uses are being closely studied and analyzed to identify potential alternatives to chemical preservatives[5,25].

Because there is a major challenge to preserve food without any chemical/synthetic preservatives, therefore use of such types of extract can be a good alternative and also helps in the reduction of health risk.

Humans have been benefitting from medicinal plants since time immemorial due to the innumerous health benefits which they possess. In accordance to a World Health Organization report, about 80% of the global population resorts to these plants for their basic health care. India is home to approximately 7000 species of medicinal plants which justify the global recognition of the dependence of Indians on ayurvedic practices in their day to day lives. These medicinal plants are abundant in biologically active substances which showcase anti-oxidant, anti-microbial and anti-inflammatory properties[13,31].

Flower extracts of many medicinal plants have been studied and known to possess anti-oxidant and antibacterial properties, such as *Hibiscus rosa sinensis, Moringa oleifera* and *Catharanthus roseus* (L.) G.Don. Some of these flowers also contain pigments that impart a beautiful color, and are used as natural coloring agents in the food industry[16].

Moringa oleifera is popularly called as “Drumstick tree”. This plant belongs to Moringaceae family. This is a deciduous tree which grows very fast when provided with sufficient sunlight. These are extensively cultivated in tropical and sub-tropical regions. This plant has a splendid range of uses ranging from health to nutritional. A report has stated about the presence of active chemical compounds like nitrile and glycosides in the stem, leaves, roots and seed infusions of *Moringa*. These phytochemicals are found to be responsible for lowering cholesterol and blood pressure and protecting hepatic and cardiovascular tissues[2,34]. Anti-microbial property of *Moringa* flower extract is attributed to the presence of pterygospermin. The presence of quercetin in *Moringa* flower has the ability to protect hepatic cells[6,17]. Studies have proven the ability of leaves and flower extracts of *Moringa* to have microbial resistance against *Escherichia coli, Klebsiella pneumoniae, Enterobacter* spp., *Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Candida albicans*[18]. The flower and root extracts were found to be effective cures to various diseases[10,21].

Hibiscus rosa sinensis L. is an evergreen shrub which is grown in tropical and sub-tropical regions for its beauty. This plant belongs to the Malvaceae family. This plant is blessed with alluring flowers which have ornamental and medicinal uses. Flower extract of *Hibiscus* is used to soothe inflammation and other skin

allergies. This is also used as a cure for tumors, ulcers, diarrhea, asthma and fertility issues[27]. The chemical profile of flower extracts revealed the presence of hentriacontans, cyanidin, tannins, terpenoids, and quercetin, which are responsible for the above-mentioned health benefits[16]. Flower extract is proven to have antimicrobial properties against *E. coli* and *B. subtilis*[1].

Catharanthus roseus belongs to Apocynaceae family. This is an evergreen subshrub which was endemic to Madagascar, but also grown in most tropical areas for ornamental purpose. It is commonly called Rose Periwinkle or Madagascar Periwinkle. Apart from being aesthetic, their flowers have been identified to have cancer fighting properties attributed to the presence of alkaloids[3,19]. The flower is of prime importance to various industries like pharmaceutical, perfume, agrochemical and food industry due to the presence of 400 alkaloids[34]. The principal alkaloids present in this flower are Vindolicine, Ajmalicine, Serpentine, Vincristine, Vinblastine and Yohimbine which have been proven to cure diseases such as diabetes, cancer, hypertension, asthma and menstrual disorders[14,22]. The flower extract is found to possess antibacterial properties and is efficient against both Gram positive (*B. subtilis*) and Gram-negative microorganisms like *E. coli*[6].

It is a known fact that most of the severe food-borne diseases are caused by *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa.*

In this study, flower extracts of *Hibiscus rosa sinensis, Moringa oleifera* and *Catharanthus roseus* were screened for various phytochemicals and further it was examined for their antibacterial activities against the four bacterial species *i.e., Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*. The selection of these four bacterial strains was carried out because of the known fact that most of the severe food-borne diseases are caused by *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa.*

Culture and media used :

Strains of *Escherichia coli* (MTCC 10312), *Bacillus subtilis* (MTCC 9003), *Pseudomonas aeruginosa* (MTCC 104) and *Staphylococcus aureus* (MTCC 106) were obtained from Microbial Type Culture Collection (MTCC) situated at Institute of Microbial Technology (IMTECH), Chandigarh, India. Nutrient broth and Standard Plate Count Agar were bought from Merck, India.

Sample procurement :

Flowers of *Hibiscus rosa sinensis*, *Moringa oleifera* and *Catharanthus roseus* were acquired from surroundings of the Bhaskaracharya College of Applied Sciences, New Delhi, and the species of collected samples were confirmed from the Department of Botany, North campus, University of Delhi, India.

Preparation of extract :

Flowers were dried separately in a domestic microwave at a temperature of 110^{o}C generated by the frequency 2450MHz. The dried samples were grinded, pulverized and stored into air tight containers with proper

labeling for further use. For extraction, the dried powder was dispersed in ethanol (90%) and three different concentrations, 35mg/ml, 45mg/ml and 55mg/ml were prepared. These suspensions were left in dark for 24 hours for efficient extraction. After 24 hours, the samples were filtered using Whatman filter paper No. 1.

Phytochemical screening :

The phytochemical profiles of all the flower extracts were screened by using standard methods[20,32].

Tannins : In 10ml of distilled water, with the help of pipette 0.5ml of extract was added and the mixture was allowed to boil. After boiling, few drops of 0.2% ferric chloride solution were added to the test tube. The presence of tannin was indicated by the evolution of blue-black precipitate.

Saponins : In 5ml of distilled water, 0.5ml of extract was added followed by proper mixing for about 2 minutes. If persistent froth appears, then presence of saponins was confirmed. This test is also known as the frothing test.

Phlobatannins : In 2ml of 1% HCl, 0.5 ml of extract was added and slightly heated in the test tube. Appearance of red precipitate confirmed the presence of phlobatannins. This test is also known as precipitate test.

Carbohydrates : In a test tube, 10ml of water and 0.5ml of extract were taken. A few drops of 20% Molisch's reagent were added followed by 2ml of concentrated sulphuric acid along the sides of the test tube. Appearance of red-violet precipitate at the juncture indicated the presence of carbohydrates in the sample.

Alkaloids : Extract (2 ml) was taken and few drops of Wanger's reagent were added. Appearance of reddish-brown precipitate confirmed the presence of alkaloids.

Inoculation development :

Strains of four bacterial species were activated in nutrient broth for 72 hours at 35°C. A loopful of culture was inoculated into the nutrient broth from the primary stock solution, following which the culture was incubated for 24 hours at 37°C.

Antibacterial activity :

Anti-bacterial activity was measured using Disc Diffusion method. In this method, 6 mm discs of Whatman filter paper were prepared and dipped into the flower extract. Later, these discs were placed in PCA plates inoculated with chosen bacterial strains. One plate was divided into 5 quadrants to accommodate five discs having three concentrations viz. 35mg/ml, 45mg/ml, 55mg/ml of flower extract,a positive control of a blank sterile disc and a negative control of disc loaded with 90% ethanol. All the twelve plates (three flower samples for four bacterial strains) were incubated for 24 hours at 37°C. After 24 hours, the zones of inhibition were measured.

Estimation of Minimum Inhibitory Concentration (MIC) :

The Minimum Inhibitory Concentration (MIC) was determined for each of the samples

by noting down the least tested concentration of the sample at which the extract exhibits inhibitory activity, detected by presence of zone of inhibition of a slightly lighter color with respect to that of the surrounding inoculated media. Similarly, the MIC was estimated for each of the flower extracts against each of the tested bacterial strains, to find out which of the flowers possess better inhibitory activities, both against particular strains as well as on an overall basis.

Statistical analysis :

The statistical analysis was performed by using SPSS 25. For each parameter, three independent determinations were made (n= 3), and the results were expressed as mean ± standard deviation (SD) at $p < 0.05$.

Phytochemical screening :

Phytochemical profile analysis for three of the chosen flower extracts was conducted to check the presence of tannins, saponins, phlobatannins, alkaloids and carbohydrates. Table-1 summarizes the phytochemical profiles of the flower extracts. After analysis, carbohydrates, phlobatannins and tannins were the major phytochemical compounds observed in all three extracts. The concentration of these constituents was more abundant in *Hibiscus rosa sinensis* and *Catharanthus roseus*, whereas in *Moringa oleifera* the quantity of all constituents expect saponins was found to be medium. Among all the flower extracts, maximum concentration and variety of phytochemical compounds was found in *Catharanthus roseus*.

Antimicrobial activity :

Anti-bacterial activities of all flower extracts are depicted in Tables 2-4. Each flower extract showed varying resistance against the chosen gram- positive and gram-negative bacteria.

In spite of various concentrations, the *Moringa oleifera* flower exhibited the widest zone of inhibition against *Staphylococcus aureus* and *Bacillus subtilis*, whereas lowest anti-microbial activity was noted against *E. coli* ($p \leq 0.05$). Irrespective of types of microorganism tested the highest zone of inhibition (12.29mm) was measured at a concentration of 55mg/ml.

Table-1. Phytochemical screening of flower extracts of *Hibiscus rosa sinensis, Moringa oleifera* and *Catharanthus roseus*

Flower	Tannins	Saponins	Phlobatannins	Carbohydrates	Alkaloids
Moringa oleifera (Drumstick)	+	-	+	+	+
Hibiscus rosa sinensis (China rose)	-	-	++	++	-
Catharanthus roseus (Sadabahar)	++	-	++	+	+

+ Present moderately, ++ Present strongly, “ Absent

In *Hibiscus rosa sinensis*, the maximum (11.04 mm) zone of inhibition was measured for *Staphylococcus aureus* and minimum (9.58 mm) was observed for *Pseudomonas aeruginosa* regardless of their different concentration level. Irrespective of microbial strain, the highest zone of inhibition was observed at concentration of 55 mg/ml, whereas it was measured lowest (9.08 mm) for control.

In *Catharanthus roseus* extract without taking concentration into consideration, maximum zone of inhibition was observed in *S. aureus*, followed by *B. subtilis*, *P. aeruginosa* and *E. coli*. Moreover, irrespective of tested bacterial strain a significantly ($p<0.05$) highest zone of inhibition was calculated at concentration level of 55 mg/ml and similar to all tested samples the lowest zone of inhibition was observed for control.

Table-2. Antimicrobial activity of flower extracts of *Moringa oleifera*

Concentration	BS	EC	PA	SA	Total
Zone of Inhibition (mm)					
35 mg/ml	9.17 ± 0.29	8.67 ± 0.29	9.67 ± 0.58	10.00 ± 0.50	9.38 ± 0.64^{a}
45 mg/ml	11.50 ± 0.50	9.00 ± 0.50	12.83 ± 0.29	12.17 ± 0.76	11.38 ± 1.58^{b}
55 mg/ml	15.00 ± 0.50	10.33 ± 0.58	11.17 ± 0.29	12.67 ± 0.58	12.29 ± 1.90^{c}
Control	9.33 ± 0.58	8.83 ± 0.76	9.00 ± 0.50	10.17 ± 0.29	9.33 ± 0.72^{a}
Total	11.25 ± 2.49^{C}	9.21 ± 0.84^{A}	10.67 ± 1.59^{B}	11.25 ± 1.32^{C}	

All data are reported as mean ± SD (n=3)
Small and capital alphabets in superscripts indicate significant differences ($p<0.05$) among concentration and tested microbial strain respectively
BS= *Bacillus subtilis* MTCC 9003; EC= *Escherichia coli* MTCC 10312; PA= *Pseudomonas aeruginosa* MTCC 104; SA= *Staphylococcus aureus* MTCC 106
F-Statistics for tested microbial strain = 42.79 at df = 3, p=0.000
F-Statistics for concentrations = 100.87 at df = 3, p=0.000
F-Statistics for tested microbial strain and concentrations = 15.25 at df = 9, p=0.000

Table-3. Antimicrobial activity of flower extracts of *Hibiscus rosa sinensis*

Concentration	BS	EC	PA	SA	Total
Zone of Inhibition (mm)					
35 mg/ml	8.83 ± 0.29	9.00 ± 0.50	8.33 ± 0.58	9.83 ± 0.29	9.00 ± 0.67^{a}
45 mg/ml	11.83 ± 0.29	10.17 ± 0.29	11.17 ± 0.29	11.17 ± 0.76	11.08 ± 0.73^{b}
55 mg/ml	13.83 ± 0.29	12.00 ± 1.00	10.33 ± 0.29	12.83 ± 0.76	12.25 ± 1.45^{c}
Control	9.33 ± 0.29	8.17 ± 0.76	8.50 ± 0.50	10.33 ± 0.58	9.08 ± 1.00^{a}
Total	10.96 ± 2.12^{B}	9.83 ± 1.61^{A}	9.58 ± 1.31^{A}	11.04 ± 1.30^{B}	

All data are reported as mean ± SD (n=3)
Small and capital alphabets in superscripts indicate significant differences ($p<0.05$) among concentration and tested microbial strain respectively
BS= *Bacillus subtilis* MTCC 9003; EC= *Escherichia coli* MTCC 10312; PA= *Pseudomonas aeruginosa*

MTCC 104; SA= *Staphylococcus aureus* MTCC 106
F-Statistics for tested microbial strain = 23.78 at df = 3, p=0.000
F-Statistics for concentrations = 105.77 at df = 3, p=0.000
F-Statistics for tested microbial strain and concentrations = 6.00 at df = 9, p=0.000

Table-4. Antimicrobial activity of flower extracts of *Catharanthusroseus roseus*

Concentration	BS	EC	PA	SA	Total
Zone of Inhibition (mm)					
35 mg/ml	8.83 ± 0.29	9.00 ± 0.50	8.67 ± 0.58	11.17 ± 0.29	9.42 ± 1.12^{a}
45 mg/ml	13.33 ± 0.29	9.33 ± 0.58	10.33 ± 1.04	12.17 ± 0.29	11.29 ± 1.71^{b}
55 mg/ml	16.83 ± 0.76	10.50 ± 0.50	13.00 ± 0.50	14.17 ± 0.76	13.63 ± 2.44^{c}
Control	9.83 ± 0.76	8.33 ± 0.29	7.67 ± 0.58	11.83 ± 0.29	9.42 ± 1.73^{a}
Total	12.21 ± 3.33^{C}	9.29 ± 0.92^{A}	9.92 ± 2.19^{B}	12.33 ± 1.23^{C}	

All data are reported as mean ± SD (n=3)
Small and capital alphabets in superscripts indicate significant differences ($p<0.05$) among concentration and tested microbial strain respectively
BS= *Bacillus subtilis* MTCC 9003; EC= *Escherichia coli* MTCC 10312; PA= *Pseudomonas aeruginosa* MTCC 104; SA= *Staphylococcus aureus* MTCC 106
F-Statistics for tested microbial strain = 92.08 at df = 3, p=0.000
F-Statistics for concentrations = 150.75 at df = 3, p=0.000
F-Statistics for tested microbial strain and concentrations = 16.33 at df = 9, p=0.000

Minimum Inhibitory Concentration (MIC):

MIC is the least concentration of flower extract needed to prohibit the growth of microbes. The MIC for all flower extracts against all the selected microbial strains was at 35mg/ml. Table 5 summarizes the Minimum Inhibitory Concentrations of the flower extracts.

Table-5. Minimum Inhibitory Concentration (MIC) of different flower extracts against different tested microbial strains

Test Microorganisms	*Moringa oleifera*	*Hibiscus rosa sinensis*	*Catharanthus roseus*
Bacillus subtilis MTCC 9003	35 mg/ml	35 mg/ml	35 mg/ml
Escherichia coli MTCC 10312	35 mg/ml	35 mg/ml	35 mg/ml
Pseudomonas aeruginosa MTCC 104	35 mg/ml	35 mg/ml	35 mg/ml
Staphylococcus aureus MTCC 106	35 mg/ml	35 mg/ml	35 mg/ml

Pearson *correlation :*

Table 6 showcases the correlation among the selected microbial strains (*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus)* on their antimicrobial activities. The highest correlation (0.741) between tested bacterial strain i.e., *Bacillus subtilis* and *Pseudomonas aeruginosa* was estimated at a significant level of 0.01.

Table-6. Pearson correlation coefficients (r) among different tested microbial strains

	EC	BS	PA	SA
EC	1			
BS	0.700**	1		
PA	0.492**	0.741**	1	
SA	0.186	0.436**	0.372*	1

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

EC=*Escherichia coli* MTCC 10312; BS= *Bacillus subtilis* MTCC 9003; PA= *Pseudomonas aeruginosa* MTCC 104; SA= *Staphylococcus aureus* MTCC 106

The ethanolic flower extract of *Hibiscus rosa sinensis*, *Moringa oleifera* and *Catharanthus roseus* were tested against four pathogenic microbes viz. *Bacillus subtilis* (MTCC 9003), *Escherichia coli* (MTCC 10312), *Pseudomonas aeruginosa* (MTCC 104) and *Staphylococcus aureus* (MTCC 106). These pathogens infect humans and cause food borne illnesses. *Escherichia coli* is responsiblefor Urinary Tract Infection (UTI), neonatal meningitis and gastrointestinal problems. *Staphylococcus aureus* is the most common microbe which causes diarrhea and septicemia*Pseudomonas aeruginosa* affects pulmonary tract and Urinary tract. Anti-microbial efficacy of these flower extracts was measured in terms of zone of inhibition which is measured as the diameter of the spread[13]. The anti-bacterial properties of these extracts can be attributed to high quantities of biologically active phytochemicals such as tannins, coumarins, flavonoids, alkaloids and aromatic substances which are secondary metabolites produced by plants as a weapon of defense against micro-organisms, insects and herbivores[26,33].

The study specifically points out that all the three extracts were rich in phlobatannins which is a phenolic constituent. Phlobatannin has been studied in various studies and is found to possess great potential to be exploited as an adept anti-microbial agent[24,28]. When closely observing anti-microbial activity of flower extracts, a particular trend of preferentially inhibiting Gram-positive bacteria was observed. This may be due to the presence of an extra protective lipid layer around the cell wall of Gram-negative bacteria[30]. Previous studies have reported that Gram negative bacteria are more resistant to plant based anti-microbials. And majority of Gram-positive bacteria are more sensitive to traditional Indian spices and herbs[8].

Prior studies on *M. oleifera* have showcased the presence of tannins, alkaloids, carbohydrates, polyphenols and flavonoids which were responsible for the anti-bacterial activity offered by this plant[21]. Through this study, it is shown that *M. oleifera* can efficiently resist the growth of *B. subtilis* and *S. aureus* even at low concentrations. Zones of inhibition

for all microbial strains were in almost the same range. The least resistance was offered against *E. coli.*

*Hibiscus rosa sinensis*showed anti-bacterial activity against *E. coli* when compared to the other two flower extracts. This inhibition is attributed to the presence of phlobatannins in abundance. Phlotannins and glucosides may contribute individually or together to fight against micro-organisms. Zone of inhibition increased with the increase in concentration[1].

MIC results of *Catharanthus roseus* indicated that all selected microbes were inhibited by the same concentration of flower extract. *Catharanthus roseus* flower extract exhibited the best performance against *B. subtilis* and *P. aeruginosa*. Anti-microbial activity may be due to the presence of significant amounts of alkaloids[12]. The principal alkaloids present in this flower are Vindolicine, Ajmalicine, Serpentine, Vincristine, Vinblastine and Yohimbine[4]. Flower extracts prepared in different concentration have shown to have proficient anti-bacterial properties against Gram positive and Gram-negative bacteria.

A concentration of 35mg/L has been found effective in this study which is closer to the range of some other studies although with different flower species.[11,15]. This is a one of its kind study where these three species have been explored for their antimicrobial activity for the first time.In some cases, varied observations were observed in anti-microbial activity of plant extracts grown in different regions and studied by different researchers of diverse origins. This variation in results may be because of climatic changes, soil composition, age, quality and quantity of bacterial strain used[9,35]. Even the solvent used for extraction of flower can also have an effect on the resultant resistance against microorganisms[7,23].

The current study demonstrates the ability of *Hibiscus rosa sinensis*, *Moringa oleifera* and *Catharanthus roseus* flower extracts to prevent widespread growth of bacteria and their potential to be used in food industry as bio-preservatives. These bio-preservatives are readily available, economical, enriched with therapeutic goodness and have minimum to no health hazard. All the selected extracts were found to have adeptly offered resistance to microbial infestation. But through the statistical analysis and significant difference, it is prominently indicated that each extract has different capacity to inhibit different strains of bacteria. The plant based anti-microbial agents were found to show efficient performance against *Staphylococcus aureus* and least activity against *Escherichia coli*. These flower extracts can be used for holistic medical and food applications with some further studies by selecting additional microbial strain and quantification of available phytochemicals in the extract.

The authors acknowledge Bhaskaracharya College of Applied Sciences (University of Delhi) for providing facilities to conduct this research work.

Conflict of interest :

Authors declare that there is no conflict of interest.

Authors' contribution :

All authors participated in the

conception or design of the work, data collection, data analysis and interpretation, performing the analysis, drafting the article, critical revision, final approval of the version to be published.

References :

1. Agarwal, S., and R. Prakash (2014). *Biological Forum 6(*2): 194-196.
2. Anwar, F., S. Latif, M. Ashraf, and A. H. Gilani (2007). *Phytotherapy Research, 21*(1): 17-25.
3. Barrales-Cureno, H. J., C. R. Reyes, I.V. García, L. G. L. Valdez, A. G. De Jesus, J.A.C. Ruíz, L.M. Herrera, M.C. Caballero, J.A. Magallón, J.E. Perez and J.M. Montoya (2019). Alkaloids of Pharmacological Importance in *Catharanthus roseus*. In Alkaloids-their Importance in Nature and Human Life. Intech Open.
4. Cortes, J., and M. Vidal (2012). *Breast Cancer Research and Treatment, 133*(3): 821-830.
5. Dapar, M. L. G., G. J. D. Alejandro, U. Meve, and S. Liede-Schumann (2020). *Journal of Ethnobiology and Ethnomedicine, 16*(1)*:* 1-60.
6. Fakurazi, S., S. A. Sharifudin, and P. Arulselvan (2012). *Molecules, 17*(7): 8334-8350.
7. Fayemi, P.O., I. Ozturk, D. Kaan, S. Özcan, M.B. Yerer, A.H. Dokumaci, and H. Yetim (2019). *Biotechnology & Biotechnological Equipment*, 33(1): 764-778.
8. Gottardi, D., D. Bukvicki, S. Prasad, and A.K. Tyagi (2016). *Frontiers in Microbiology, 5:* 1394.
9. Ghavam, M., M. L. Manca, M. Manconi, and G. Bacchetta (2020). *Ex Bentham Scientific Reports, 10*(1): 1-10.
10. Gopalakrishnan, L., K. Doriya, and D. S. Kumar (2016). *Food Science and Human Wellness, 5*(2): 49-56.
11. Jamil, N. and F. Pa'ee (2018) Antimicrobial activity from leaf, flower, stem, and root of *Clitoria ternatea*-A review. Conference proceedings -*Inventing Prosperous Future Through Biological Research and Tropical Biodiversity Management* 2002 (1).
12. Kannathasan, K., A. Senthilkumar, and V. Venkatesalu (2011). *Asian Pacific Journal of Tropical Medicine, 4*(8): 645–648.
13. Kumara Swamy, M., K. M. Sudipta, P. Lokesh, W. Rashmi, R. Vijay, and K. Sen, (2012). *International Journal of Phytomedicine, 4*(3)*:* 375-379.
14. Lahare, R. P., H. S. Yadav, A. Dashhare, and Y. K. Bisen (2020). *Saudi Journal of Medical and Pharmaceutical Sciences, 6*(12): 759-766.
15. Linggam, K., S.M. Mnsor, S. Ramanathan, and S. Sasidharan (2009). *Pharmacologyonline. 1:* 731-738.
16. Mak, Y. W., L. O. Chuah, R. Ahmad, and R. Bhat (2013). *Journal of King Saud University-Science, 25*(4): 275–282.
17. Marrufo, T., F. Nazzaro, E. Mancini, F. Fratianni, R. Coppola, L. De Martino, and V. De Feo (2013). *Molecules, 18*(9): 10989-11000.
18. Nepolean, P., J. Anitha, and R. R. Emilin (2009). *Current Biotica, 3*(1): 33–37.
19. Nejat, N., A. Valdiani, D. Cahill, Y. H. Tan, M. Maziah, and R. Abiri (2015). *The Scientific World Journal,* 19 (2015).
20. Odebiyi, O. O. and E. A. Sofowora (1978). *Lloydia, 41*(3): 234-246.
21. Patil, S.D. and R. Jane (2013). *International*

Journal of Life Sciences, *1*(3): 182-189.

22. Renjini, K. R., G. Gopakumar, and M. S. Latha (2017). *European Journal of Pharmaceutical & Medical Research*, *4*(11): 545-551.
23. Saleem, M., and M. T. Saeed (2020). *Journal of King Saud University-Science*, *32*(1): 805-810.
24. Scalbert, A. (1991). *Phytochemistry*, *30*(12): 3875–3883.
25. Sharifi-Rad, M., E. M. Varoni, B. Salehi, J. Sharifi-Rad, K. R. Matthews, S. A. Ayatollahi, F. Kobarfard, S. A. Ibrahim, D. Mnayer, Z. A. Zakaria, and M. Sharifi-Rad (2017). *Molecules*, *22*(12)*:* 2145-2165.
26. Shimada, T. (2006). *Journal of Chemical Ecology*, *32*(6): 1149–1163.
27. Sikarwar, M. S., and M. B. Patil (2015). *International Journal of Health and Allied Sciences*, *4*(2): 73-78.
28. Sukumaran, S., S. Kiruba, M. Mahesh, S. R. Nisha, P.Z. Miller, C.P. Ben and S. Jeeva (2011). *Asian Pacific Journal of Tropical Medicine*, *4*(9): 735–738.
29. Syeda, A. M., and K. Riazunnisa (2020). *Data in Brief*, Vol. *29:* 105258,1-10.
30. Tajkarimi, M.M., S.A. Ibrahim, and D. O. Cliver (2010). *Food Control*, *21*(9): 1199–1218.
31. Thakur, M. K., and S. Pathak, (2016). *International Journal of Science and Research 5*(9): 400-404.
32. Trease, G. E. Trease and Evans. (1989). Pharmacognosy: a physicians guide to herbal medicine, 13th edn, Bailliere Tindall London.
33 Uritu, C. M., C. T. Mihai, G. D. Stanciu, G. Dodi, T. Alexa-Stratulat, A. Luca, and B. I. Tamba (2018). *Pain Research and Management*, *44:*
34. Vergara-Jimenez, M., M. M. Almatrafi, and M.L. Fernandez (2017). *Antioxidants 6*(4): 91-104.
35. Walia, S., S. Mukhia, V. Bhatt, R. Kumar, and R. Kumar (2020). *Industrial Crops and Products, 150:* 112449.