International Journal of Advance Research in Science and Engineering
Volume No. 11, Issue No. 08, August 2022
www.ijarse.com
IJARSE
ISSN 2319 - 8354

Qualitative and quantitative phytochemical analysis and in vitro antimicrobial activity of *Calycopteris floribunda* plant extract.

Yogesh P. Thawari1*, Ashish C. Kavale2, Kishor S. Itankar3

1 (Department of Chemistry, N.H. College Bramhapuri, Gondwana university, Gadhchiroli, India).

2 (Department of Chemistry, S.S. Jaiswal college Arjuni/Morgaon, R.T.M. Nagpur university, Nagpur, India).

3 (Department of Chemistry, Yashwantrao Chawhan arts, comm. and science college lakhandur, R.T.M. Nagpur university, Nagpur, India).

ABSTRACT

In order to maintain health and to prevent, diagnose, or to treat physical and mental illness, traditional medicine plays key role. These traditional medicine practices can include plants and herbs. The current study focused on the preliminary quantitative and qualitative analysis with antimicrobial activity of various extract of different bits of wild *Calycopteries Floribunda* plants. plant parts include leaves, flowers, stem etc. *Calycopteris Floribunda* is a rich source of secondary metabolites as well as this study is also includes the investigation of antimicrobial potential of n-hexane, Methanolic, acetone and aqueous extract of given plant species. The various extract of *C. Floribunda*was tasted against pathogenic bacteria such as *E. coli* and *P. Vulgaris*. The maximum zone of inhibition was shown by the Methanolic and aqueous extract. As *Calycopteries Floribunda* is rich source of biochemical constituents and therefor to be considered as an important medicinal plant with antimicrobial property.

Keywords-Antimicrobial activity, Calycopteris Floribunda, Phytochemical analysis, Qualitative and Quantitative analysis, Secondary metabolites

1. INTRODUCTION

Our biological ecosystem composed of number of known and unknown variety of plant species includes poisonous, medicinal and non-poisonous. [1] The plant has been an important source of medicine used by man from prehistoric time for relieving, diagnosis, treatment and ailments. [2,3] Medicinal plants play vital role in disease prevention [4,5].

C. Floribunda is a climbing shrub and found in low laying tropical region forest of western ghat and rarely in the eastern ghat of India. This plant species having various name in distinct region. It is commonly known as Ukshi, kokkarai in Hindi, minnarakoti in Tamil, adivijama in Telugu. *C. Floribunda* is also grown in central and southern parts of india also in the vidharbha region of Maharashtra. Plant synthesize diverse range of bioactive molecule make them rich source of different type of medicine. Higher medicinal plant consists of rich source of natural product play a dominant role in drug development program in pharmaceutical studies. [6,7].

Methods of treatments and effect of medicinal plants on human health were known till 18th century, but the presence of bioactive molecules was unknown. [8,9]. The use of medicine was gradually expanded until today, in modern medicine, medicinal plants displaced from direct used and therefor they are used as raw material in many cases. [10]. The large area of world continues to use traditional medicine based on direct used of medicinal plant due to their low cost [11].

C. Indicum belonging to same family as *C. Floribunda*. Phytochemical study of *C. Indicum* reveals the occurrence of proteins, carbohydrate, tannins, steroids, etc. as well as quantitative analysis determined ash percentage in it. [12]. Similarly,*T. Arjuna* is one of the important medicinal plant of *combretaceae* family also exhibit bioactive molecule therefor it makes this plant as potential medicinal species. [13,14]. *Terminalia Catappa* is native to southern Asia which used traditionally by villagers due to its strong antimicrobial activity against *P. aeruginosa*, *P. testosterone*, *P. Vulgaris*, etc. The further investigation also indicates its wound healing and antidiabetic activity [15].

There are several species are found in combretacea family, most of the species exhibit numerous secondary metabolites and shows antimicrobial properties against pathogenic bacteria. [16,17] Among these species C.Floribunda is unrevealed species in combretacea family. The current research investigation is based on C. Floribunda, that reveals the presence of natural product present in it with the help of various solvent extract as well as its antimicrobial activity.

2.MATERIAL AND METHOD

2.1. COLLECTION OF PLANT

Healthy and disease free various segments of C. *Floribunda* were collected from forest region of Bramhapuri taluka dist. Chandrapur and the identification of collected species were doing through professors of department of botany N.H. college Bramhapuri. The accumulated plants bits i.e. leave, flowers and stem was thoroughly washed 2 to 3 times with deionized water and kept in shaded area for drying purpose at room temperature for 10 to 15 days.

2.1. PREPARATION OF LEAVE, FLOWER AND STEM EXTRACT

Different solvent like n-hexane, acetone, chloroform, methanol, and water used for extract preparation. Dried plant segments were separately pulverized into medium fine powder using grinder. 10 to 15 g powder of each segments of selected plant were taken in different thimbles made up of filter paper and was put into soxhlet extractor. All material was extracted using soxhlet extraction apparatus for approximately 10 to 12 hours using following solvents.[18,19,20]

i. n-hexane – 10 to 12 hrs. (leaves, flowers and stem)
ii. Acetone – 6 to 8 hours (leaves, flowers and stem).
iii. Chloroform - 10 to 12 hours. (leaves, flowers and stem).
iv. Methanol – 10 to 12 hours. (leaves, flowers and stem).
v. Water - 10 to 12 hours. (leaves, flowers and stem).

Leaf, flower and stem extract in different solvent appears different colour as shown in fig.1.

International Journal of Advance Research in Science and Engineering
Volume No. 11, Issue No. 08, August 2022
www.ijarse.com
IJARSE
ISSN 2319 - 8354

leave extract **flower extract** **stem extract**

Fig. 1: Different solvent extract of leaf, flower and stem

3.PRELIMINARY PHYTOCHEMICAL SCREENING:

3.1. QUALITATIVE ANALYSIS

In order to perform qualitative analysis, miniature portion of extract was used for phytochemical test which shows the presence of anthocyanin, Saponins, carbohydrates, terpenoids, proteins, flavonoids, in various extract. The test of presence of these bioactive molecule was done by method given by Rahul S. Patil et.at. [2015] [21]. The result analysis of above study is given in the table no 1.

Table no 1: Observation table of phytochemical test showing presence of secondary metabolites in *C. Floribunda* plant extract

Solvent Extract	Phytochemical Constituent	Leaf	Flower	Stem
n-hexane Extract	Flavonoid	+	+	-
	Tannin	+	-	-
	Carotenoid	+	+	-
	Saponins	+	+	+
	Alkaloid	+	-	+
	Cardiac glycoside	+	-	-
	Protein	+	-	-
	Fatty acid	+	-	-
	Volatile oil	+	+	+
	terpenoids	-	+	+
	Tannin	+	+	-
	Carotenoid	+	+	+
	Saponins	-	+	+
	Alkaloid	-	+	+
	Cardiac glycoside	+	-	-

Acetone Extract	Protein	-	-	+
	Fatty acid	+	-	-
	Volatile oil	+	+	+
	terpenoids	+	+	+
Methanol Extract	Flavonoid	-	+	-
	Saponins	-	+	-
	Alkaloid	+	+	+
	Cardiac glycoside	+	+	-
	Volatile oil	+	+	+
	terpenoids	+	+	+
	Phenolic	-	+	+
Chloroform Extract	Flavonoid	+	-	-
	Carotenoid	+	-	+
	Saponins	+	-	+
	Alkaloid	+	+	+
	Volatile oil	+	+	+
	terpenoids	+	-	+
Water Extract	Flavonoid	+	+	+
	Tannin	+	+	+
	Carotenoid	+	+	-
	Saponins	+	+	+
	Alkaloid	+	+	+
	Cardiac glycoside	+	-	-
	Phenolic	+	-	+
	terpenoids	+	+	-

3.2 QUANTITATIVE ESTIMATION

3.2.1 ALKALOIDS**: -**

Alkaloids estimation was performed by taking 1gram of plant material was weighed into 250ml beaker and 40ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, As shown in fig.2. Which

was transferred into a pre-weighed beaker and weight the alkaloid present in material. And it was found that 1 gm of leaf contained 0.013 g of alkaloids.

Leaves Flower Stem

Fig.2 : The quantitative estimation of alkaloid observes green solid precipitate.

3.2.2FLAVONOIDS: -

1 gm of leaf powder was taken in a soxhlet extractor and the compounds were extracted with methanol for 48 hrs. till it becomes colourless. The Methanolic extract was concentrated and filtered. 5-10 ml of water was added to the filtrate and lead acetate was added in the solution. The flavonoids get precipitate as lead phenolate. The precipitate was taken and suspended in ethyl alcohol. Through this alcohol filtrate, H_2S was passed for 5-10 minutes. The lead Sulphide gets precipitate out as black solid as shown in fig.3. The solution was filtered through filter paper and filtrate was concentrated in after transferring into a pre-weighed beaker. The beaker and its constituents were dried and the increase in weight was noted. After weighing the amount of flavonoid contained found to be 0.0203 g.

LeaveFlower Stem

Fig 3: The quantitative estimation of flavonoid observes black solid precipitate

4. ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT

Along with phytochemical study the aim of the current study was also focused on antimicrobial activity of *C. Floribunda* plant bits' extract. Antimicrobial activity of n-hexane. acetone, methanol, chloroform and water extract of leave, flower and stem of *C. floribunda* were determining by using well diffusion method against gram negative bacteria i.e. *E. coli* and *P. Vulgaris.*

Antimicrobial test was carried out by using nutrient broth and Muller-Hinton agar media. Nutrient broth was prepared using method given by P. Poovendram et.al. [2011] [22] spread on Petri plates, after solidify

bacterial culture was spread by using spread plate techniques. The agar was carefully punched using cork-borer of 5 mm in diameter. 0.5 ml of prepared extract was dispensed into the well of agar using micropipette. The positive antibacterial activity was established by the presence of assessable zone of inhibition after the 24 hours of incubation at 36 ºC temperature.

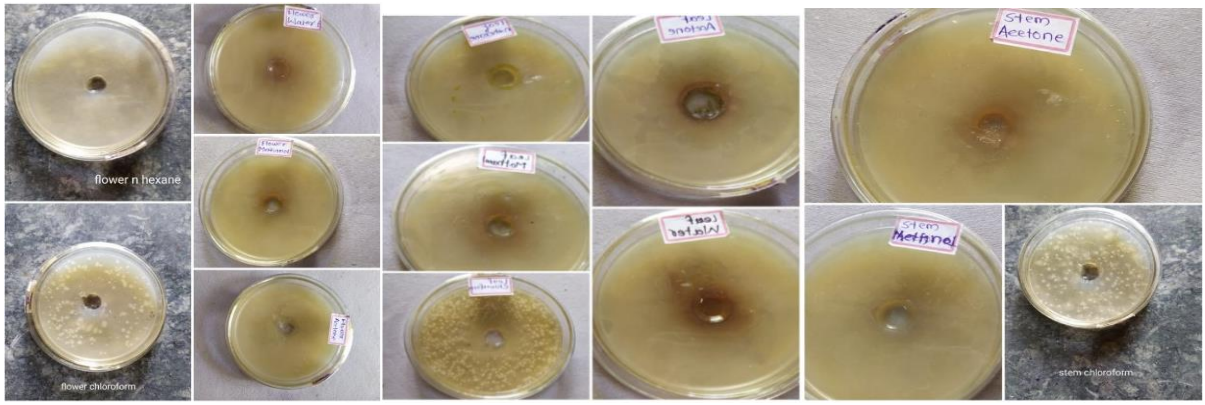

Fig. 4. Antibacterial activity against *P. Vulgaris* of n-hexane, chloroform, methanol, acetone and water extracts of *C. Floribunda* plant extract.

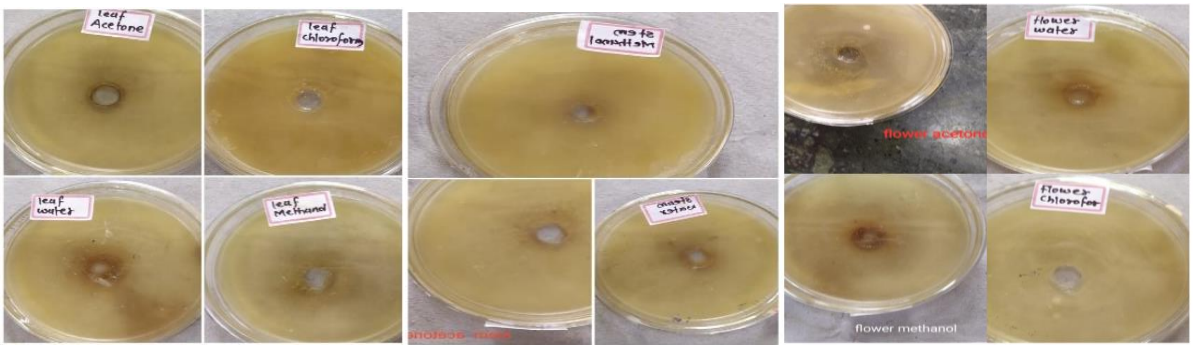

Fig. 5. Antibacterial activity against *E. coli* of n-hexane, chloroform, methanol, acetone and water extracts of *C. Floribunda* plant extract.

Solvent extract	Plant section	Zone of inhibition (indiameter)	
		E.coli	***P.Vulgaris***
n-Hexane	Leaf	-	0.5 mm
	Flower	-	-
	Stem	-	-
Methanol	Leaf	1.5cm	1.6 cm
	Flower	1.3 cm	1.2 cm
	Stem	0.2 cm	1 cm
Acetone	Leaf	0.5 mm	0.7 mm
	Flower	0.6 mm	0.9 mm
	Stem	0.8 mm	0.1 mm
Chloroform	Leaf	-	1 cm
	Flower	-	-
	Stem	-	-
Water	Leaf	0.6 mm	1.5 cm
	Flower	0.3 mm	0.7 mm
	Stem	0.2 mm	-

Table no 2: Observation table indicates zone of inhibition by various solvent plant extract against gram negative bacteria. **[(-) – Show no zone of inhibition].**

Antimicrobial activity of each extract against given gram negative microorganism was detected by calculating their zone of inhibition in mm and cm. The obtained analysis shows that n-hexane extract of leaf, flower, and stem does not show any activity against *E. Coli*, while n-hexane extract of leaf show little inhibition of 0.5mm diameter against *P. Vulgaris*. as well as chloroform extract leaf also gives the zone of inhibition against the same microorganism.

Concerning solvent of extraction,it was observed that Methanolic, acetone, and aqueous extract had the maximum significant antimicrobial activity and while of chloroform and n-hexane extract had least antimicrobial property against *E. coli* and *P.Vulgaris*.

5. CONCLUSION

Result of phytochemical test of different extract of *C. Floribunda* plant were summarized in table no. 1. In natural product screening n-hexane extract of leaf, flower, and stem yielded flavonoids, tannins, carotenoids, Saponins, alkaloids, proteins, terpenoids etc. During the analysis of Methanolic extract and aqueous extract detect flavonoids, volatile oil, cardiac glycosides, phenolic compound, tannin, terpenoids, Saponin in all sections

of *C. Floribunda*, moreover detection of acetone and chloroform extract shows least results compared to other. The presence of such active ingredients makes *C. Floribunda* as one of the essential medicinal species in *combretaceae* family. This plant was observed to be rich source of alkaloids and flavonoids as major component in quantitative analysis. 0.013 g and 0.0203 g of alkaloids and flavonoids was extracted from 1 g leaf extract using quantitative analysis.

The scientist in recent year has made attempt to reveal the effectiveness of better known plant having certain medicinal value, mainly to determine their antibacterial phenomenon against different pathogenic microbes [23]. The objectives of current study focused on phytochemical evaluation for presence of natural product and antimicrobial activity of given plant and has disclose the capability higher plant look as new anti-epidemic agent as serving drug discovered from natural product. The study prefers *E. Coli* and *P. Vulgaris* a gram negative microbes for its antimicrobial activity were used against n-hexane, acetone, Methanolic, chloroform and aqueous extract. The inhibitory action was observed in terms of inhibition zone. the antimicrobial activity was maximum of Methanolic and acetone extract as they show highest zone of inhibition while that of others extract i.e. n-hexane and chloroform show minimum activity. Amongs this aqueous extract of *Calycopteris Floribunda* shows moderate activity against given microorganism.

Now a day'speoples irrespective of the region are in search of the herbal are to avoid the obnoxious effect of the commonly available treatment modalities. *Calycopteries floribunda* is one of the unexplored plant with various phytochemical constituents and antimicrobial potential so as to derived novel antimicrobial agents for the treatment of various infection for developing new medicine.

6. ACKNOWLEDGEMENT

We are thankful to Mr. Vishnu Jadhav, assistant professor, Department of microbiology Y. C. College lakhandur and Gahane madam, department of microbiology, N.H. College Bramhapuri for providing helpful facilities and valuable support for this research work.

REFERENCES:

[1]Tesfahuneygn, Gebrehiwet & Gebreegziabher, GebremichaelMedicinal Plants Used in Traditional Medicine by Ethiopians: *A Review Article*.(2019). *2 (4);*18-21.

[2]Harney N.V. Ethnomedicinal Plants Diversity of Bhadrawati Tahsil of Chandrapur District, Maharashtra, India. (2013).*3(8).*

[3]Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide Research Trends on Medicinal Plants. *Int J Environ Res Public Health.*(2020) *.12;17(10):*3376.

[4]Sofowora A, Ogunbodede E, Onayade A. Aug The role and place of medicinal plants in the strategies for disease prevention. *Afr J Tradit Complement Altern Med.*(2013) *12;10(5):*210-29 .

[5]KC, Kiran & Dhanush, C. & C V, Gajendra & Reddy B, Madhusudhana. Diversity and Seasonal Availability of Potential Wild Edible Plants from Vidarbha Region of Maharashtra State, India. *International Journal of Current Microbiology and Applied Sciences.* (2019).*8.* 1434-1446.

[6]D.A. Dhale1 and S.K. Markandeya. Antimicrobial and Phytochemical Screening of Plumbago zeylanica Linn. (Plumbaginaceae) Leaf. *Journal of Experimental Sciences.*(2011).*2 (3)* Pages 04-06.

[7]Om, Ahmad & Azizan, Azlan & Baharum, Syarul. Phytochemical Screening of Ubi Gadong (Dioscorea hispida) by using GC-MS.(2016).

[8]Aiyegoro, O.A., Okoh, A.I. Preliminary phytochemical screening and *In vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complement Altern Med.* (2010) .*10, 21.*

[9]Najafi, Shahla & Sanadgol, Nima & Sadeghi-Nejad, Batool & Beiragi, Maryam & Sanadgol, Ehsan. Phytochemical screening and antibacterial activity of Citrullus colocynthis (Linn.) Schrad against Staphylococcus aureus. *Journal of Medicinal Plants.* (2010).*4*. 2321-2325.

[10]Khatun, Shahanaz & Pervin, Farzana & Karim, Md. Rezaul & Ashraduzzaman, Mohammad & Rosma, Ahmad. Phytochemical screening and antimicrobial activity of Coccinia cordifolia L. plant. *Pakistan journal of pharmaceutical sciences.*(2012). *25*. 757-61.

[11] Eman Abdullah M. Ali.Antimicrobial activity, cytotoxicity and phytochemicals screenings of Epipremnum aureum (Linden and Andre) G. S. Bunting extracts.*Egypt. J. Exp. Biol. (Bot.).*(2018).*14(2):* 219-255.

[12] Jyothi Jose , Subrahmanya P , Ravi Rao S . pharmacognostical and phytochemical study on leaf of combretum indicum (l.). *Jyothi Jose et al / Int. J. Res. Ayurveda Pharm.*(2021). *12 (5).*

[13] N.Tensingh Baliah, andA. Astalakshmi. Phytochemical analysis and antibacterial activity of extracts from Terminalia chebulaRetz. *Int.J.Curr.Microbiol.App.Sci.*(2014). 3(3): 992-999.

[14] Devi, P. & Kaleeswari, S. & Mani, Poonkothai. Antimicrobial activity and phytochemical analysis of fruit extracts of Terminalia Bellerica. *International Journal of Pharmacy and Pharmaceutical Sciences.*(2014).*6*. 639-642.

[15] Anand, Vijay. An updated review of Terminalia catappa. *Pharmacognosy Reviews*.(2015).*9.* 93-8.

[16] Vemuri P. K, Dronavalli L, Nayakudugari P, Kunta A, Challagulla R. Phytochemical Analysis and Biochemical Characterization of Terminalia Chebula Extracts for Its Medicinal Use. *Biomed Pharmacol J;*(2019).*2(3).*

[17] Yahaya, O. & Yabefa, J.A. & Usman, Bukar. Phytochemical Screening and Antibacterial Activity of Combretum glutinosum Extract against Some Human Pathogens. *British Journal of Pharmacology and Toxicology*.(2012). *3*. 233-236.

[18] Mr. Akshay G. Deshmukh, Dr. S. P. Rothe. Phytochemical Analysis of Cissus RepandaVahl: an Uncommon Medicinal Plan. *International Journal of Interdisciplinary Innovative Research & Development (IJIIRD).*(2017).*02 (01).*

[19] Singh, Manju & Kumari, Rajesh & Nandini, Durgesh & Kotecha, Mita. Preliminary phytochemical screening of Basella rubra Linn. *Journal of Pharmacognosy and Phytochemistry*.(2016). *224*. 224-226.

[20] Khatua, Souti & Pandey, Akhil & Biswas, Surjyo & Surjyo, Correspondence & Biswas, Jyoti. Phytochemical evaluation and antimicrobial properties of Trichosanthes dioica root extract.(2016). *410*. 410-413.

[21] Patil, Rahul & Harale, Pooja & Shivangekar, Kiran & Kumbhar, Pooja & Desai, Ranjeet. Phytochemical potential and in vitro antimicrobial activity of Piper betle Linn. leaf extracts. *Journal of Chemical and Pharmaceutical Research.*(2015).*7*. 1095-1101.

[22] Mandal S, Patra A, Samanta A, Roy S, Mandal A, Mahapatra TD, Pradhan S, Das K, Nandi DK. Analysis of phytochemical profile of Terminalia arjuna bark extract with antioxidative and antimicrobial properties. *Asian Pac J Trop Biomed.*(2013).*3(12):*960-6.

[23] Sathish, Sahaya & Vijayakanth, Periyasamy & Palani, Ar & T, Thamizharasi & Vimala, A.. Antimicrobial And phytochemical screening of Tragia Involucrata L. using UV-VIS and FTIR. (2013).*1(1).*